USE OF THE BIOCONCENTRATION CAPABILITY OF LEECHES TO EVALUATE CHLOROPHENOL POLLUTION

by

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ABSTRACT

The objective of this research was to investigate the use of leeches as *in situ* monitors of the biological availability of chlorophenols and degree of contamination in the North Arm of the Fraser River Estuary, British Columbia, where chlorophenols are used as wood preservatives by several forest industry operations. The objective was accomplished by : 1) an integrated series of grab and continous samples to determine the spatial and temporal variability in chlorophenol contamination, 2) *in situ* and laboratory experiments to compare leech bioconcentration to water levels of pollutants and to determine environmental factors(temperature, pH, leech size) that regulate and affect bioassay interpretation.

Grab samples were not representative of the average level of pollution in the river. High concentrations such as the one reported for Mitchell Island on October 4 (11 ppb TTCP and 2.25 ppb PCP) could give a false impression of the pollution level in the river. Also, plumes of high concentration of chlorophenols could be missed easily if the sampling time does not coincide with the pollutant discharge.

High frequency(every 2 hours) automatic water sampling showed a high variability in chlorophenol contamination(0.278 ppb to 3.678 ppb TTCP for the March 31- April 6 field experiment) which demonstrated the sporadic nature of chlorophenol discharges. Changes in the river flow also affected the level and

the pattern of chlorophenol variation. A method capable of integrating concentration versus time seemed to be the only way to elucidate the irregular pattern of pollutant levels.

Leeches were exposed to the contaminants by submersion in cages at various locations along the North Arm. On the basis of the levels of chlorophenols found in the leech, estimation of the average chlorophenol concentration in the water were made. Concentrations as high as 3.29 ug/g TTCP and 1.11 ug/g PCP were found in leeches exposed in the Mitchell Island area(TTCP and PCP were the only chlorophenols found in the area of study at any time during our sampling program carried out between August 1984 and September 1985). An average concentration higher than 2 ppb TTCP and 2 ppb PCP was estimated in the water for the duration of the leech exposure(7 days) at that location using bioconcentration levels determined in the laboratory.

Laboratory experiments showed that lower pH increased bioconcentration of chlorophenols. Higher bioconcentration factors were achieved at higher temperatures and regression equations(R= 0.96 to 0.99) were calculated for the five chlorophenols used in the experiments(2,4-DCP; 2,4,5-TCP; 2,4,6-TCP; 2,3,4,6-TTCP and PCP). Temperature affected the time needed to achieve steady state which was 4 days at 4 C, 5 days at 12 C and 7+ days at 22 C. Four out of the five chlorophenols tested(2,4-DCP exhibited different bioconcentration characteristics) were bioconcentrated to the same level by leeches, regardless of their Po/w values. This contradicted the linear relationships established by various researchers between the bioconcentration factor and Po/w of various compounds

including chlorinated phenols in other organisms.

Recommendations for setting up a method to use leeches in a biomonitoring program of the chlorophenol pollution in the Fraser River Estuary are proposed from the results of this research. The cost of the analyses using a biomonitoring program could be an order of magnitude lower than an adequate water sampling program to assess the chlorophenol pollution level.

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1. INTRODUCTION

Chlorophenols, namely PCP and TTCP, are used in British Columbia for short and long-term wood protection against sapstain and mould. The media has expressed concern related to the health of workers , and investigations on the use and handling of the chlorophenol containing materials have been carried out. The awareness of people directly involved with the product, of ordinary people, and of the goverment authorities has increased through the years. Chlorophenols have been reclassified in Canada in the Category II of Priority Chemicals and included in the list of Priority Pollutants in the United States.

There have been numerous studies that have shown CPs presence in the forest industries effluents and in the three compartments of the aquatic environment, water, biota, and sediments. An EPS report published in 1979 as a result of an elaborate program of sampling carried in the Fraser River Estuary and on the southeast coast of Vancouver Island(EPS, 1979) revealed the following: " Chlorophenols were found in the aquatic environment at all sampling sites where PCP was used in industrial processes. It is likely, therefore, that chlorophenols may be found in the aquatic environment wherever they have been used in wood preservation or protection." Concentrations as high as 7.3 ppb PCP and 5.2 ppb TTCP were reported at marine sampling sites. It was further concluded that the data indicated a potentially serious environmental problem with respect to the use of CPs(Appendix III contains all the symbols and abbreviations

used in this thesis).

In a survey made on fish tissue and surface sediments from 10 sites in the Fraser Estuary, Hall et al.(1984) reported high levels of chlorinated phenols in starry flounder tissue (0.19-2.52 ug/g TTCP and 0.77-2.77 ug/g PCP on wet weight basis). At lower concentrations, chlorophenols were reported in the tissues of various biota by other researchers(Carey, 1985; Rogers, 1979; Johnston et al., 1975; Bawden et al., 1973). Chlorophenols are not supposed to enter the aquatic environment, but the available data show their presence in the biota, sediments, and the water column. Are the levels found of any immediate danger to the environment, or would they have a long term effect?.

On the basis of the available data on acute and chronic toxicity of chlorophenols, safe concentrations of TTCP and PCP(the main ingredients in the solution formulations) in water could be established. Fox(1980) recommended that PCP in water should not exceed a concentration of 0.04 ppb for the protection of the aquatic life. So far, guidelines exist for various municipal and industrial effluents and for handling of hazardous wastes containing CPs.

The study of the chlorophenol presence in the Fraser River Estuary has two fronts of action: one is to establish the background levels of chlorophenol in the water and the other is to monitor their concentration variation and compliance with regulatory levels which are still to be established. The use of biota to assess and monitor the levels of various pollutants makes very much sense when monitoring is considered. The use of a

bioindicator organism to monitor in situ levels of contaminants is not a new technique. However, leeches have received very little attention as biological monitors. Recent research(Metcalfe et al. ,1984) found that leeches could accumulate high levels of CPs and it was suggested that they could be used as "early warning" indicators of contamination of the aquatic environment by organic pollutants.

Leeches are invertebrates which belong to phylum Annelida, class Hirudinea. In North America there are 5 families of leeches. They are excellent candidates for bioindicator organisms due to their resistance to toxic compounds, natural abundance, reasonable size, long survival in the laboratory, tolerance for brackish water, starvation resistance, bioconcentration capability, and simple correlation established between their body pollutant content and concentration of pollutant in the surrounding water.

The objective of this investigation was to evaluate leeches as a bioindicator organism for chlorinated phenols contamination. Contrary to previous research,the leeches were not collected from the region of study, but they were collected from a pristine environment, implanted in bioassay chambers in the study region, and exposed for a known period of time. The bioconcentration of CPs in the leech was used to provide an estimate of the pollution pattern in a reach of the lower Fraser River. Laboratory studies were conducted to determine the effects of temperature, pH, exposure time, and pollution concentration on the bioconcentration potential.

2.LITERATURE REVIEW

2.1 Introduction

The literature review has been divided into three distinctive sections: the first section deals with the presence of chlorophenol in the Canadian environment with emphasis on the Fraser River estuary ; the second section discusses the enviromental fate of chlorophenols with emphasis on bioaccumulation phenomenon, models and parameters which influence laboratory determined bioconcentration factors; the third section reviews the problems encountered in interpreting results of field surveys using biota as pollution indicators and highlights the potential use of leeches as bioindicators as reflected in some very recent research.

2.2 Chlorophenol Presence in the Canadian Environment

As Jones reported in a technical review which examined the presence of chlorophenol in the Canadian environment(EPS,1981), chlorophenols are ubiquitous in Canada. They have been detected in surface water, rain, snow, landfill leachate, sewage effluent, sediments, aquatic and terrestrial biota. Major sources of chlorophenols to the environment are wood preservation plant sites, agricultural land subjected to the application of phenoxy acid herbicides, and municipal sewage treatment plants. Some other possible sources are improper disposal of contaminated wastes and spills during manufacturing , storage, and use of

, storage, and use of herbicides and wood treatment chemicals which contain chlorophenols. Chlorophenols have a long history in the environment. TTCPs and PCP have been identified in sediments deposited annually since 1949 in the Bay of Quinte area, Lake Ontario(EPS,1983).

Assigned initially to the Category III of Environment Canada's List of Priority Chemicals, chlorophenols are now listed in Category II of the list. Compounds in this category are characterized as "substances which the goverment believes may pose a significant danger to the environment or human health and about which further detailed information is required". On November 28,1980, Agriculture Canada announced in Memorandum T-1-229(EPS,1981) a revision in the use standards for chlorophenols. The major uses still in force for chlorophenols are for long term wood preservation and short term wood protection.

2.2.1 Chlorophenol Related Problems in the Fraser River Estuary

The Fraser River is the largest river system and one of the most important aquatic environments in British Columbia. It is used in a multitude of ways as it constitutes a major fish way, an important means of transportation, and main receiver of industrial, agricultural and municipal discharges. In the past 10 years it became clear that an integrated management plan was necessary to preserve this valuable resource. As a result, provincial and federal goverments undertook a series of studies into various aspects of the Fraser River estuary, such as land

use, recreation, habitat and water quality(Fraser River Estuary Study,1980). A Water Quality Monitoring Program was initiated in late 1985 and studies are currently under way, financial and technical support being provided by both provincial and federal goverments.

In British Columbia, the main source of CPs to the environment is from their use in the wood protection and preservation industry. Approximately 900 tonnes of chlorophenols are used annually in B.C.(Garrett,1980). Due to their environmental persistence(especially the higher chlorinated congeners) and to the fact that some industrial formulations contain highly toxic impurities such as dioxins and dibenzofurans, chlorophenols have been subject to a few thorough studies and investigations. In 1981, a Task Force was formed to respond to worker health and environmental impact concerns regarding the use of chlorophenols at saw mills and lumber export terminals. As a result of their investigations a document on recommendations for design and operation of wood treatment facilities was released(Konasevich et al.,1983). This document outlined recommendations for design features for chlorophenate storage, feed and application facilities, and recommendations for treated lumber storage areas, recommendations on operating practices, transportation of chlorophenate containing materials, disposal of wastes, and spill contingency plans. The Environmental Protection Service(EPS) is presently conducting a survey on the stormwater run-off at various forest industry locations using chlorophenols for wood preservation to determine the effectiveness of current recommendations.

Several commercial formulations are available in B.C. Sodium salts of TTCP and PCP at a ratio ranging from 2:1 to 4:1 are used primarily in B.C.,either by spray application or by submerging the lumber in a dip tank containing the treatment solutions. Table 2.1 presents the most used commercial formulations in British Columbia.

The use, transportation, and disposal of chlorophenols in British Columbia is regulated or "could " be regulated under 6 federal and 4 provincial Legislative Acts(Konasevich et al., 1983).

2.2.2 Chlorophenols Levels in the Fraser River Estuary

One of the most comprehensive studies on chlorophenol evironmental contamination by the wood preservation industry in British Columbia is the EPS Regional Report 79-24(EPS, 1979). Areas covered were the lower east coast of Vancouver Island and the lower mainland of British Columbia(see Fig. 2.1.a and Fig. 2.1.b for the sample sites locations). Analysis of contaminants was done on a variety of samples comprising sediments, surface water, effluent and biota(fish, molluscs and crustaceans). It was found that PCP and TTCP were present in the aquatic environment at all sites where PCP formulations were used. Table 2.2 shows the levels of chlorophenols found in sediments and water at various sites. The sediment levels of PCP varied from 5.0 to 187.9 ppb and the water levels from < 0.05 to 7.3 ppb. The TTCP concentrations ranged from 10.0 to 272.1 ppb in sediments and from 0.06 to 5.2 ppb in water.

TABLE 2.1

COMMERCIAL SAPSTAIN CONTROL FORMULATIONS IN BRITISH COLUMBIA
(from Konasevich et al., 1985)

COMMERCIAL NAME	FORMULATOR	COMPOSITION			
		CHLOROPHENOL ACTIVE INGREDIENTS (Percent by Weight)			ADDITIVES
		TETRA*	PENTA**	OTHER***	
WOODBRITE 24	VAN WATERS & ROGERS LIMITED	16.3	7.7		SODIUM TETRABORATE BUFFER
DIATOX	DIACHEM LTD.	19.4	4.8		UNSPECIFIED BUFFERS
WOODSHEATH	WALKER BROS. LTD.	5.4		1.5	UNSPECIFIED BUFFERS COLOUR WAX
WOODSHEATH (SEABRITE)	WALKER BROS. LTD.	11.35		2.85	UNSPECIFIED BUFFERS COLOUR WAX
ALCHEM 4135	ALCHEM LTD.	27.0			UNSPECIFIED BUFFERS TRIBUTYLTIN OXIDE

SODIUM TETRACHLOROPHENATE
**SODIUM PENTACHLOROPHENATE*
***UNSPECIFIED CHLOROPHENATES*

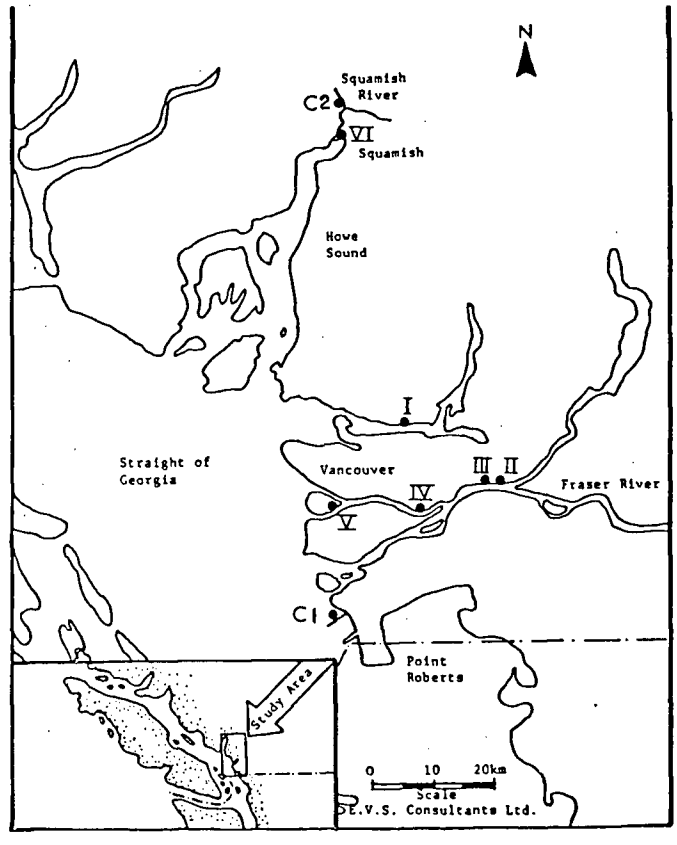

Fig.2.1.a. Locational map of the Lower Mainland, British Columbia, showing sampling sites used for chlorophenol survey.(reproduced from EPS Report 79-24).

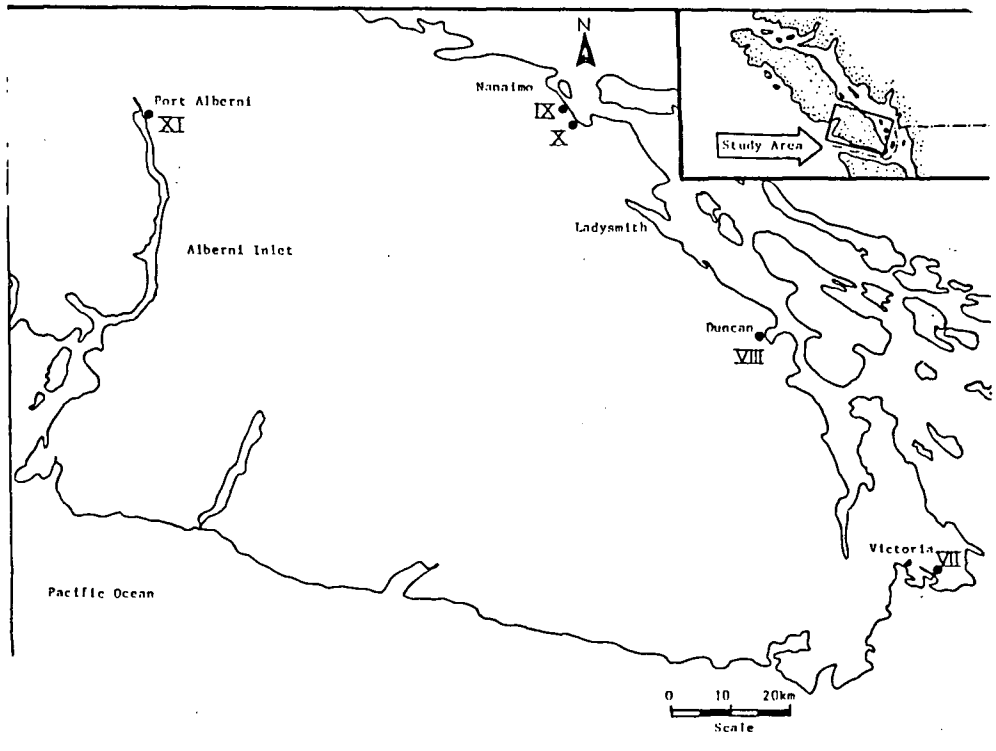

Fig.2.1.b. Locational map of Southern Vancouver Island, British Columbia, showing sampling sites used for chlorophenol survey.(reproduced from EPS Report 79-24).

TABLE 2.2

AVERAGE(1) SEDIMENT AND WATER CONCENTRATIONS FOR CHLOROPHENOLS, CHLORINATED BENZENES, AND PENTACHLOROANISOLES(2)
(reproduced from EPS Report 79-24)

SITE	SEDIMENT LEVELS							Water Levels(3)		
	5CP	4CP	3CP	6CB	5CB	4CB	5CA	5CP	4CP	5CA
CI	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Freshwater										
II	35.0	28.0	N.D.	N.D.	N.D.	N.D.	N.D.	0.28	0.10	N.D.
III	10.8	27.4	2.3	N.D.	N.D.	N.D.	2.6	0.25	1.0	N.D.
IV	18.1	21.9	N.D.	0.73	N.D.	N.D.	31.1	<0.05	0.30	N.D.
V	5.0	10.0	N.D.	N.D.	N.D.	N.D.	N.D.	<0.05	0.20	.006
Saltwater										
I	34.7	39.8	N.D.	N.D.	N.D.	N.D.	0.53	0.75	1.3	N.D.
VI	52.8	98.7	52.1	N.D.	N.D.	N.D.	N.D.	2.4	5.2	.020
VII	106.6	272.1	91.0	3.12	1.9	2.76	3.53	<0.01	0.06	.005
VIII	16.0	19.5	N.D.	N.D.	N.D.	N.D.	0.28	<0.05	0.09	N.D.
IX	42.0	65.4	N.D.	N.D.	0.83	0.71	4.31	<0.05	0.06	N.D.
X	13.1	22.8	N.D.	N.D.	N.D.	N.D.	0.58	3.1	3.3	.005
XI	187.9	157.3	37.3	9.19	9.75	64.9	14.89	7.3	0.22	N.D.

(1) sediment concentrations are expressed on dry weight basis; average of 10 samples is reported.
(2) all concentrations are in ppb, N.D.= not detected.
(3) the water concentrations are the result of only one sample.

5 CP = Pentachlorophenol
4 CP = Tetrachlorophenol
3 CP = Trichlorophenol
6 CB = Hexachlorobenzene
5 CB = Pentachlorobenzene
4 CB = Tetrachlorobenzene
5 CA = Pentachloroanisole

Sculpin liver tissue exhibited preferential uptake with bioaccumulation factors(tissue:sediment ratios) of 7-16 for TTCP and 19-33 for PCP with liver tissue burdens averaging 402 and 448 ppb respectively(dry wt). Livers from prickly sculpins Cottus asper and staghorn sculpins Leptocottus armatus always contained PCP and TTCP at concentrations 1-3 orders of magnitude greater than skeletal muscle from the same individuals.

Cain and Swain(1980) detected 57 trace organic compounds in the wastewaters coming from the three sewage treatment plants(STP) of the Lower Mainland: Annacis Island, Iona Island and Lulu Island(see Fig. 2.1.c for the plant locations). Chlorophenols were identified in all three plant effluents. Initial sampling showed that PCP levels were similar at all plants sampled and that TTCP concentrations were much higher at the Iona Island plant than at the other two plants. Additional monitoring for phenolic compounds conducted by the same researchers, indicated that PCP and TTCP levels were highest at Annacis Island plant and lowest at Iona Island plant. Mean concentrations of PCP in sewage samples from Annacis, Iona and Lulu Islands plants were 2.9-9.9, 1.3-1.7 and 2.1-3.7 ppb respectively, while TTCP levels were 2.9-9.9, 0.9-1.3 and 1.2-5.3 ppb, respectively.

Rogers I. H. et al.(1986) have found chlorophenols in raw sewage at Iona Island STP. Composite samples were taken for a period of two weeks in the summer of 1983. TTCP and PCP reached maximum levels of 7.8 and 13.2 ppb, respectively.

Swain(1980) has reviewed the industrial wastewater effluents that discharge directly to the Fraser River. The survey included only companies which had a permit for effluent

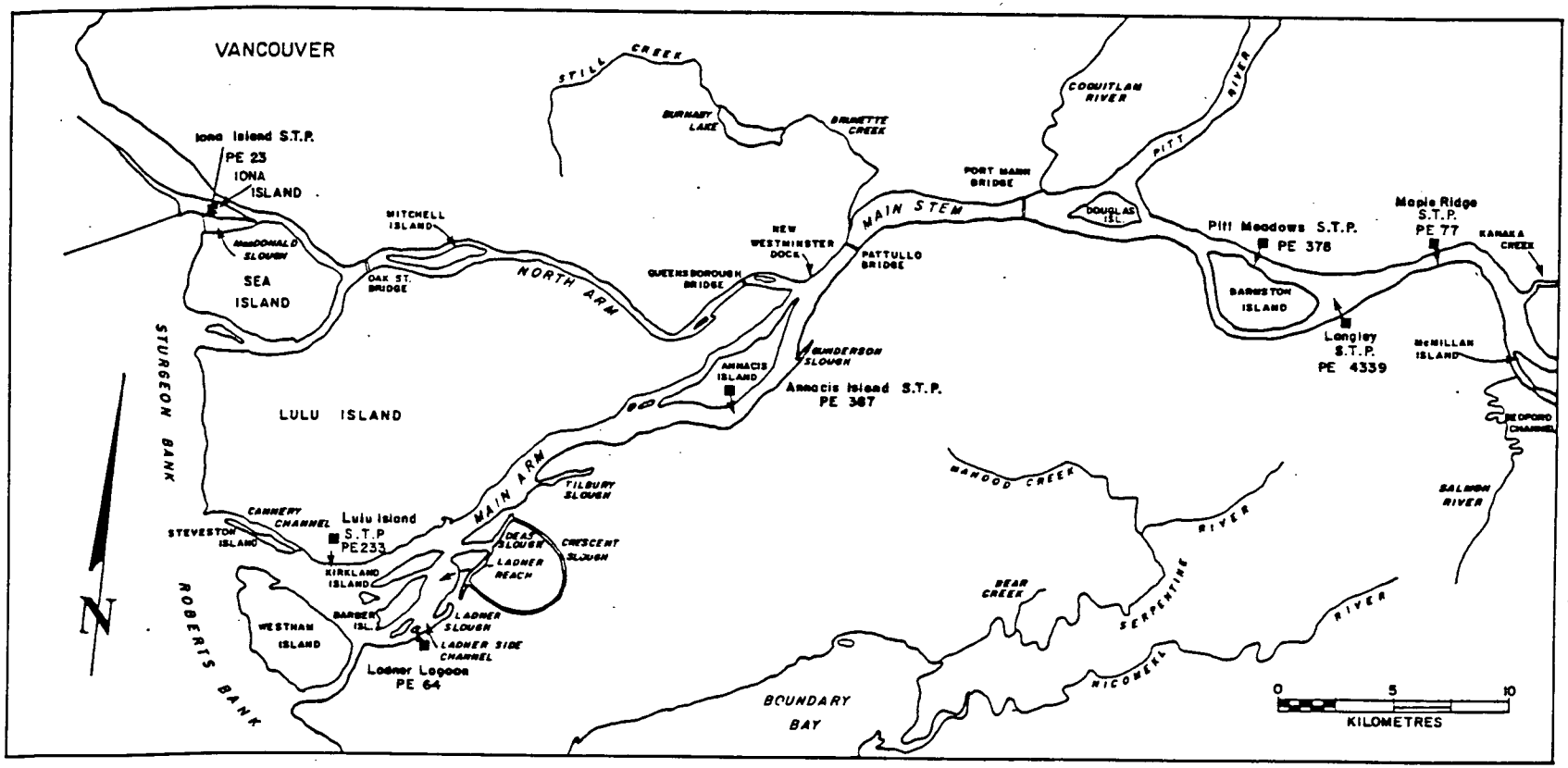

Fig.2.1.c. Location of the sewage treatment plants in the Fraser River Estuary.(from Cain and Swain, 1980).

discharges and the levels of contaminants in the discharges were monitored by the Waste Management Branch whose records were used for the compilation of data. Chlorophenols were detected in effluents coming from Belkin Packaging Ltd., Burnaby; Scott Paper Ltd., New Westminster; MacMillan Bloedell Ltd., New Westminster; MacMillan Bloedel Ltd., and Canadian White Pine Division, Vancouver, all located on the North Arm of the Fraser River. He concluded that for proper monitoring of the effluents from the various industries located in the Fraser River Estuary, bioassay monitoring was the best alternative.

Cain et al.(1980) reported the presence of 2,4,5-TCP, 2,4,6-TCP, 2,3,4,6-TTCP and PCP in wastewaters coming from these industries. The levels were in the low ppb range with the highest values of 7.2 ppb for 2,3,4,6-TTCP and 5.4 ppb for PCP. There are other forest industries along the North Arm of the Fraser River which use chlorophenols for long-term wood preservation and short-term wood protection and they could also contribute to the chlorophenol pollution of the Fraser River. These industries include: British Columbia Forest Products Ltd., Vancouver Sawmill Division, Canadian Forest Products Ltd., Eburne Sawmills Division, Westcoast Cellufibre Industries Ltd., Fraser River Planning Mills Ltd., Terminal Sawmills Ltd., Whonnock Industries Ltd.(all these industries are located in the Mitchell Island area of the North Arm; EPS, personal communication).

Sampling by the Waste Management Branch has indicated high levels of TTCP(up to 90 ppm), PCP(up to 525 ppm) , oils and greases in the sediments off Koppers International pole treating facility. As a result, the Waste Management Branch has ordered

the termination of discharge and the removal of highly contaminated sediments adjacent to the Koppers facility(Garrett,1980).

Most of the available information on organic contaminants levels in Fraser River fish species was obtained from Westwater Research Centre studies. Garrett (1980) has a complete tabulation of results of chlorophenol analysis compiled from the data published by Westwater Research in 1978(the biota samples were collected in 1973 and analysed in 1978); Johnston et al.(1975); Bawden et al.(1973); Rogers (1979). Chlorinated phenols were identified with greater frequency and at higher concentrations in fish from the industrially developed lower part of the Fraser River. PCP and TTCP concentrations did not exceed 125.0 ppb and 62.0 ppb wet weight respectively.

In more recent studies, Hall et al. (1984) reported very high levels of chlorophenols in starry flounder tissue with TTCP from 0.19 to 2.5 ug/g and PCP from 0.77 to 2.77 ug/g wet weight(this gives equivalent concentrations expressed in ppb as high as 2500 ppb and 2770 ppb for TTCP and PCP respectively). All samples analysed by these researchers were collected in November and December 1983. Hall compared the levels of chlorophenols found in starry flounders during his investigation with the levels of chlorophenols found by Singleton(1983). His results were one or two orders of magnitude higher than found in Singleton's investigation. Since a viable explanation for the difference in chlorophenols concentration in the two species of fish was not found more surveys were suggested in order to identify the cause of such a variation.

Carey(1985), sampled fish and water from the Fraser River estuary area in 1984, and he found TTCP and PCP(other chlorophenols were present but at very low levels) . The highest concentration of TTCP in water was 0.133 ppb and of PCP was 0.056 ppb. The highest chlorophenol concentration in biota was found in staghorn sculpin at 49 ppb TTCP and 79 ppb PCP(average of 12 samples; concentration reported on wet weight basis).

2.3 Environmental Fate of Chlorophenols

Callahan et al. (1979) have reviewed the fate and persistence of several chlorophenols in aquatic systems and it was concluded that their fate could not be predicted from system to system. Laboratory studies provide an initial insight into environmental factors which can regulate chlorophenol persistence, but the best way to study the fate of chlorophenols is to study their distribution and persistence in a natural environment. Included in this category are the studies following man created small disasters such as various chlorophenols spills, leaks and disposal malpractices of materials containing these compounds. Pierce et al.(1977) studied the distribution of pentachlorophenol in a fresh water system following a spill which resulted in an extensive fish kill. The results of their investigation showed a short residence time for PCP in the water column, and higher concentrations of PCP in the sediments(up to 1200 ppb)for a period of several months with a large reduction within a year. Fish contained concentrations of PCP from 130 to 2500 ppb(dry weight) up to six months following the spill;

within ten months the concentration in fish dropped to the background levels. High levels of PCP persisted in leaf litter for at least seventeen months. The persistence of chlorophenols in sediments and leaf litter constituted a source of continuous, low level contamination to the water column and facilitated biological magnification via detritus and benthic feeding organisms.

In order to build a predictive model of the distribution of chlorophenols in the environment their chemical and physical characteristics must be thoroughly evaluated. With all the effort put into theoretical interpretations and descriptive equations, the end result may not provide a useful predictive function and can often be rendered useless by the complexity of interactions in the natural ecosystem.

The main part of the environmental analysis is the estimation of the probable distribution of a chemical in the various compartments: air, water, sediment/soil, and biota. There are seven major processes to be considered when one evaluates the environmental fate of a chemical: volatilization, photolysis, oxidation, hydrolysis, sorption, biotransformation/biodegradation, and bioaccumulation(NRCC, 1982).

It must be clearly defined that chlorophenols exist in two forms: the anionic phenolate and the undissociated phenol at acidic pH. For the particular case of PCP the ratio between undissociated and dissociated forms changes dramatically between pH 3 and 8 (NRCC, 1982). At pH 5, the ratio is 1:1 and one can easily see that at regular fresh water pH 6-8 range, most of the

PCP present in the water column would be in the phenolate form. Due to the influence of pH upon the form of chlorophenols, and because of the different reactivities of the two forms, the rates of photolysis, volatilization, solubility and sorptive potential are greatly influenced by changes in pH.

Volatilization affects only the undissociated form, so it can be concluded that for higher chlorinated chlorophenols volatilization in the aquatic environment is not an important process since at the natural occurring pHs they are in the ionic form. Some researchers, Wong(1978) and Kilzer et al.(1979) mentioned volatilization of chlorophenols at higher pH but they did not explain the mechanism of the process. The lower chlorinated chlorophenols are more volatile than PCP.

Photolysis equations developed by various researchers are greatly dependent on latitude, time of day, time of year and light intensity. The absorbtion spectrum varies with pH and the anion absorbs more strongly than does the undissociated form. Wong and Crosby(1978) indicated that the rate of photolysis depended upon pH. The comparative rates at pH 3 and 7 were: $5.2*10^{-7}s^{-1}$ and $2.7*10^{-5}s^{-1}$ respectively, a fifty fold difference consistent with the difference in anion concentrations at the two pHs. The rate constant for primary photolysis is also controlled by depth of water which is the major controlling factor for photolysis in any aquatic system.

Oxidation as a possible route of transformation of chlorophenols in the aquatic environment needs more research. It has been suggested that oxidation makes no contribution to the fate of chlorophenols in the environment(Callahan et al., 1979).

Hydrolysis is also considered unimportant in the fate of chlorophenols in the aquatic environment.

Sediment or soil sorption processes have received a lot of attention from scientists and Ks, the soil partition coefficient, was related to Kow(octanol:water partition coefficient) through Kom(organic matter:water partition coefficient) and Koc(organic carbon:water partition coefficient)(NRCC, 1982). The equations developed for these relationships cannot be applied to the ionic form of chlorophenols. Sorption for the ionic form is estimated to be negligible, and the only processes that would facilitate the sorption of this form would be chelation and specific site binding. In the case of PCP, Kom is about 2200 for the acidic molecular form . The soil partition coefficient Ks can be as low as 22 for a low(1%) organic soil.

Microbial degradation has been observed and the general trend is that the lower chlorinated chlorophenols are more susceptible to microbial attack. At high concentrations the toxic characteristics of chlorophenols inhibit any microbial activity. Baker et al.(1980) demonstrated that PCP was the most resistant compound to biodegradation in the chlorophenol family.

Bioaccumulation is a very important process in assessing the fate and distribution of chlorophenols in a natural environment and it will be discussed separately in the following section.

2.3.1 Bioaccumulation Process

The accumulation of certain chemicals by aquatic biota is a well known process. Many chemicals are potentially dangerous to aquatic life and indirectly to human health, therefore the importance of the reliability of the bioaccumulation data available to the scientific world is very important.

The terms of bioaccumulation and bioconcentration have had a multitude of definitions in the past which has created a sense of confusion. More recent terminology and definitions have been better defined and today it is universally accepted that bioconcentration is represented by the chemical residue obtained directly from water via gill or epithelial tissues(Brungs and Mount, 1978). Bioaccumulation is a broader term which refers to residues obtained from both food and water. Finally, magnification is the total process of bioaccumulation by which tissue residues of toxic material pass through two or more trophic levels. Kenaga (1972) defined the term of "bioconcentration ratio" as the ratio between residue values and ambient water concentration. For consistency in terminology, it implies that the water is the only source of toxicant.

Early studies concerning biomagnification processes seem to favour the food chain as the main contributor to the accumulation of chemicals in biota. More recent studies provide the opposite evidence, namely that the bioconcentration is one of the main processes. In the aquatic environment, direct uptake of a chemical from the ambient water will determine the order of

magnitude of the bioaccumulation factor in a given organism whereas indirect uptake via the food chain will increase this factor only moderately(Esser and Moser,1980).

Kobayashi et al.(1979) studied the relation between the toxicity and the accumulation in goldfish(Carassius auratus), of seven chlorophenols and noticed that the increase of the Cl-atom number in the chlorophenols caused an abrupt increase in toxicity to the fish. This observation is not new to toxicologists and the scientific literature; however, what Kobayashi´s results revealed was that the increase of toxicity from the chlorinated phenols is mostly due to their relative accumulation in fish and not to their intrinsic toxicity. Chlorophenol concentrations in fish achieved a certain lethal level, roughly 100-200 ug/g body weight and only the time to achieve this concentration in the fish tissue differentiated the toxicity of various chlorophenols.

2.3.2 Laboratory Determination of Bioconcentration Factors

Three procedures are used to estimate the bioaccumulation or the bioconcentration factor if only exposure to the contaminant in water is considered. These include:

- deduction from the Po/w, n-octanol:water partition coefficient.

-determination of steady-state partitioning.

-determination of the ratio of uptake and depuration rates. As the bioconcentration is based on partition/sorption phenomena, lipophilicity of the chemicals is expected to play a major role. Consequently, the Po/w, as a measure of lipophilicity, should be

correlated to the bioconcentration factor. There are several methods of experimentally determining Po/w and a list of direct and indirect methods of determination of log Po/w and the problems and limitations existent in each case is presented by Esser and Moser(1980).

Estimation of Po/w from water solubility has a lot of drawbacks. Correlation established between the two parameters seem to be reliable only for a series of structurally related chemicals. Also ,as Todd et al.(1980) reported in their study, solubility data for organic compounds of low solubility in water are very unreliable. They concluded that a given solubility value is strongly dependent on the experimental method used to obtain it. The same observation is valid for the determination of Po/w by other methods. The literature reports a variety of Po/w values for each compound and care must be used when they are considered in bioconcentration factor relationships(Esser and Moser, 1980).

Ionizable compounds such as chlorophenols pose another problem for the determination of Po/w. It is known that the introduction of charges into a molecule decreases lipophilicity. The degree of ionization of the weak acids and bases depend on their pKa values. A model compound with pKa 4.2, is 99.9% ionized at pH 7.2, which results in a concomitant decrease of the original Po/w by three orders of magnitude. For reasons of better standardization, Esser and Moser(1980) recommend that the Po/w of weakly ionized chemicals be measured by using a buffer of physiological pH and sodium chloride concentration as the aqueous phase. The influence of pH upon Po/w may explain the large

variability of this parameter value reported for chlorophenols. Most values are reported without mentioning the experimental pH, which makes the reported values hard to compare.

Some of the regression equations published for the relationship between the bioconcentration factor(BF) and Po/w of various chemical compounds are:

-Log BF=0.542*Log Po/w + 0.124 ; equation was developed by Neely et al.(1974) using the ratio between the rate constants of uptake and depuration in trout.

-Log BF=0.85*Log Po/w - 0.70 ; equation was developed by Veith et al.(1979) using the steady-state approach. Organism of study was fathead minnow.

-Log BF=0.935*LogPo/w - 1.495 ; equation was developed by Kenaga and Goring (1978) using the steady-state approach on various biota.

-Log BF=0.83*LogPo/w -1.71 ; equation was developed by Ellgehausen et al.(1980) using the steady-state and depuration kinetics . Organism of study was catfish.

If one considers a chemical with a Po/w of 1000, the corresponding bioconcentration factors calculated from these equations differ by roughly one order of magnitude. The BF values determined from the above equations are 56, 78, 24, and 6, respectively. These short calculations also show that values of

Po/w above 1000 are necessary before the corresponding BF exceeds 100.

If experimental determination of the BF by the steady-state approach is used, a very strict standardization of the experimental conditions must be imposed. Factors such as variation in species, biomass/volume ratio, temperature, water characteristics, analytical methodology, samples analyzed, and test chemical properties such as weak ionization, high sorptivity, molecular size, volatility, and degradability may contribute to the variation of the measured BF´s by factors of up to 5 and more(Esser and Moser,1980).

If the BF is determined from the ratio of uptake to depuration rate constants, it means that first-order kinetics are assumed for both processes. Some studies have shown that depuration is best described by second-order rate equations(Esser and Moser,1980).Their studies on catfish showed that the half-life of depuration depended upon the initial concentration. The most commonly used kinetic description of the process of bioaccumulation is based on the "one compartment" model, described by the equation(Hamelink,1977):

$$CF=K1*CW*(1-EXP(-K2*T))/K2$$

where: CF,CW = concentration of the chemical in fish and water, respectively

K1,K2 =uptake and excretion rate constants[1/time], respectively.

T = temperature

The bioconcentration factor is given by: BF=K1/K2. The rate constants K1 and K2 have to be determined. A computer program

BIOFAC may be used to fit the data to the equation. This computer program or others that are commercially available require initial estimates of K1 and K2. Often experimental scatter is such that the use of sophisticated fitting routines is not warranted and simpler procedures produce adequate results.

If K1 is expressed as a function of BF and K2 the following equation is obtained:

$$(CF/CW)/BF=1-EXP(-K2*T)$$

It means that if K2 is known the BF can be calculated. The same equation can also be used if the accumulation curve shows signs of reaching equilibrium.

Curtis et al.(1977) used the "two compartment" model and obtained an empirical function to describe the accumulation of methyl mercury in blue gill sunfish(Lepomis macrochirus). The disadvantage of the empirical function is that the parameters used have no physical meaning. The one compartment model is an oversimplification but the application of two or more compartments(Moriarty 1975;Robinson 1975) is difficult to use since the experimental data are seldom detailed and accurate enough to warrant their use.

In all models developed so far it has been assumed that the uptake and excretion rate constants K1 and K2, and consequently BF, are independent on concentration of the chemicals. Experimental data indicate that this is frequently not the case and that the bioconcentration factor usually decreases(but sometimes increases) with increasing concentration of the chemical(Zitko,1980).

Majori and Petronio(1973) demonstrated that the decrease in

BF with increasing CW follows the law of mass action. They also assumed that the concentration in fish(CF) cannot exceed a certain maximum concentration CT("concentration of binding centers") and that the rate of uptake is proportional not only to the concentration of the chemical in water(CW) but to the difference CT-CF("concentration of free binding centers") as well. Using this approach means that the excretion rate constant is higher during the accumulation phase than that during the excretion phase.

According to Streit and Schwoerbel (1976) the equilibrium concentration of atrazine in the leech(Glossiphonia complanata) is given by the following power function for a CW range from 0.001 to 1 mg/L :

CFE=8.690*CW**0.740

The above equation was approximated by the function : CFE=K1*CW*CT/(K1*CW+K2) for CT=6 and K2/K1=0.201. K2 was estimated from excretion data; consequently K1=0.91. CFE is the concentration in fish at equilibrium, therefore, CFE=BF. Comparison between the experimental and calculated data showed a very good agreement.

From the data of Hansen et al.(1974) the CFE´s of Aroclor 1016 in American oysters(Crassostrea virginica) and grass shrimp(Palaemonetes pugio) but not those in the pinfish(Lagodon rhomboides) can be expressed as a function of CW. Attention should be paid to the possibility that the BF values reported in the literature may not be for an equilibrium BF.This can be easily checked, if the excretion rate constant is known, by applying the equation:(CF/CW)/BF=1-EXP(-K2*T) (Zitko,1980). As

also illustrated, the BFs often depend on the concentration of the chemical in water and it is expected that they increase with decreasing concentration.

Generally speaking, compounds that have BFs of more than about 200-300 in laboratory tests, and excretion rate constants of less than 0.02 day-1, are potential suspects for bioaccumulation problems. Even in the absence of demonstrated toxic effects such compounds must be scrutinized carefully,because there is always a potential for subtle chronic effects when compounds reside in an organism for considerable periods of time. Zitko,(1980) presents a very comprehensive list of values for K1 , K2 and log Po/w compiled from the existent literature. The values quoted for pentachlorophenol are the ones reported by Glickman et al.(1977): K1=74 ; K2=2.4 and logPo/w=5.01. One has to bear in mind that the general equations established for BF give only rough estimates. They have been derived from data obtained on a variety of compounds, under different conditions and by various techniques. For a quick orientation, some predicted values of BF are given in Table 2.3.

It appears that accumulation problems are likely in case of compounds with solubilities in water below 1 mg/L and octanol/water partition coefficient higher than $3*10^4$ (log Po/w higher than 4.5). If one needs an accurate value for BF, the experimental method in which the chemical under study is tested on the species of interest appears to be the most appropiate technique.

TABLE 2.3

BIOCONCENTRATION FACTORS CALCULATED FROM WATER SOLUBILITY AND OCTANOL:WATER PARTITION COEFFICIENTS

Water solubility [mg/L]	KOW	LOG(KOW)	BCF from WS	BCF from KOW(18)
0.001	300,000	5.5	30,000	4,400
0.01	110,000	5	8,300	1,500
0.1	40,000	4.6	2,300	640
1	14,000	4.1	600	200
10	5,000	3.7	170	90
100	2,000	3.3	50	40

BCF = bioconcentration factor
KOW = octanol:water partition coefficient
WS = water solubility
BCF values were calculated using relationships established by Kenaga and Goring(1978):

$$LOG(BCF) = -1.495 + 0.935\ LOG(KOW)$$

$$LOG(BCF) = 2.791 - 0.564\ LOG(WS)$$

2.3.2.1 Bioenergetics and Its Influence on the Bioaccumulation Process

The influence of age, body weight, and lipid content on chemical residue levels in biota have been evaluated by many authors. No consistent pattern has emerged and it is not established yet if residue concentrations should be reported on a lipid basis or a whole body weight basis. In numerous cases reporting the residues as a function of the lipid content helped in reducing the variability among samples. As Hamelink and Spacie(1977) remarked, the residue-lipid content correlations do not necessarily prove a cause and effect relationship. That is, the presence of more lipids may not cause greater quantities of residues to be accumulated ;rather the factors that results in lipid deposition may also promote residue storage. Fish must obviously expend energy in order to acquire and store energy. Since lipids have a greater caloric content than muscle, a fat fish must do more eating, swimming and respiring than a lean fish of the same age. As a result a fat fish has more opportunity to take up residues than a lean fish. The fact that a fat fish could retain greater concentrations of residue was probably just related to the lipophilic nature of the chemicals. The fat just happened to be a convenient place to store the chemicals, but the presence of the lipids did not cause the compounds to be taken up and stored.

The factors of age and weight cannot be separated because they normally co-vary in biota. Correlations established between residue concentration and weight of fishes suggest that the

mechanism of residue uptake is ultimately linked to the metabolic activities.

Norstrom et al.(1976) reasoned that the uptake of pollutants should fall within the limits set by those factors that control metabolism and growth, as modified by environmental factors such as temperature and food availability. To evaluate this concept they developed a pollutant accumulation model based on fish bioenergetics combined with some data on pollutant biokinetics. In essence they devised an equation that stated that pollutant body burden changes with respect to time were equal to the uptake from the water plus the uptake from the food minus the depuration rate, wherein each rate was modified by complex body weight-dependent functions. The obvious disadvantage of this approach is the difficulty one encounters in measuring all of the various metabolic rates constants needed for the model.The authors demonstrated that for PCBs and mercury in yellow perch (Perca flavesens) the model can be approximated by:

$$dP/dt = AW^{0.70} - kPW^{-0.58}$$

where A is a constant which combines all the coefficients in the uptake parts of the model, W the body weight, and P the pollutant body burden. Since this is essentially the equation for a simple compartment model, one might expect an equilibrium state to be reached. However the exponents operating on the changes in body weight prevent an equilibrium from actually being achieved. That is, if the relative value of the exponent term on body weight is correct, then the uptake factors for methyl mercury and PCBs have increasing power over the depuration factors as the weight of the aquatic animal increases with time. Thus, if a compound is

persistent, has a high bioconcentration potential, and the animal gains appreciable weight in time, there is more opportunity to capitalize on these seemingly small differences and steady state cannot be reached.

The absolute value of these exponents must vary between species and between various life-stages for each species(Hamelink and Spacie,1977). Kinetic rates and the relative importance of the body weight change as the fish grows, matures, spawns, and finally reaches senescence . Seasonal factors(such as temperature) alter food consumption, habitat selection, etc. Each of these factors contributes to the bioenergetics of the fish and its subsequent bioaccumulation of various contaminants. Thus, to be precise would require a combined biomass-bioenergetics model which incorporates various subroutines for individual fish stocks that might be further subdivided on the basis of sex and year class.As Hamelink and Spacie (1977) concluded this level of refinement would be a monumental task which, with the exception of being informative, would achieve very little in answering more important questions pertinent to the environmental behaviour of various pollutants.

By applying simpler structure-activity concepts developed by Hansch or Veith (Veith et al.(1975), one can achieve a quicker insight into the problem. The environmental behaviour of the pollutants appears to depend more on their chemical-physical properties than on the biological-ecological features of the receiving body. As Hamelink and Spacie(1977) remarked , direct application of a partition coefficient regression appears to underestimate bioaccumulation of compounds having P values

greater than log 6. Compounds in this class often display bioconcentration factors greater than log 5 in whole fish from natural environments. In this case the time required to reach a steady state is extremely long and growth and fat deposition have to be considered in the analysis. Consequently the models developed for these super-pollutants should incorporate the bioenergetics of the fish.

2.3.2.2 Influence of pH on the Bioconcentration of Pollutants

It is well-known that pH does affect the toxicity of ionizable substances such as phenol derivatives. It is surprising how little attention has been paid in so many studies to the influence of pH.

QSARs(Quantitative Structure-Activity Relationships) are becoming increasingly helpful in aquatic toxicity research. Several authors established the toxicity of phenol derivatives and calculated QSARs. Meister(1977) used the mathematical Free-Wilson method and others(Hansch and Fujita(1964), Zitko(1976), Durkin(1978), Kopperman et al.(1974) have used the Hansch approach to calculate QSARs. However, no attention has been paid to the influence of the pH upon the QSARs. Explanations to account for this influence have been published by Lloyd and Herbert (1960) and Tabata(1962). Variations of the two approaches have been presented in the literature in past years. Most researchers share the opinion that the Tabata method

is the most useful(Konemann and Musch, 1981; Saarikovski and Viluksela, 1981).

Tabata studied the influence of pH on the toxicity of ammonia to aquatic organisms. He developed the concept that both ionized and unionized forms of a compound contribute to its toxicity, but the unionized form was usually more toxic than the ionized one (due to the differences in uptake).Konemann and Musch (1981) have used Tabata´s model to develop a formula describing the influence of pH on the toxicity of chlorophenols to fish. They used 11 chlorophenols in their study on guppies(Poecilia reticulata) and determined toxicities at 3 pH values. Using their formula they determined toxicity at another pH. QSARs were established for the LC50´s at all the pH values considered, with log Poct(octanol:water partition coefficient) and pKa as variables(Table 2.4 summarizes their results).

As can be seen the toxicity of the chlorophenols increases with decreasing pH. Maximum toxicity is reached when LC50=1/Tm (Tm is 1/LC50 of the pure molecular form). The pH of 3 represents this situation for all the chlorophenols. Experimental determination of the LC50 at this pH are not possible so Tm can be calculated from the graphical representation as the intercept when plotting 1/LC50 against Ka/([H+] + Ka).

QSARs have been obtained at various pHs using Po/w as a sole parameter or including pKa values as well. The QSARs with pKa were better than the corresponding equations without pKa. According to the authors there are at least three possible causes of the influence of the pKa on the toxicity. First, is

TABLE 2.4

CHLOROPHENOL TOXICITY TO GUPPIES AT DIFFERENT pHs
(from Konemann and Musch, 1981)

LC_{50}'s AND OTHER DATA FOR QSAR CALCULATIONS

Substance	log P_{oct} [a]	pK_a [b]	log LC_{50} at pH			log $1/T_m$
			7.8	7.3	6.1	
Phenol	1.55	9.92	2.52	2.50	2.59	2.55
2-Chlorophenol	2.27	8.52	2.02	1.94	1.74	1.77
3-Chlorophenol	2.27	8.97	1.79	1.70	1.70	1.69
2,4-Dichlorophenol	2.93	7.90	1.56	1.41	1.30	1.30
3,5-Dichlorphenol	2.93	8.25	1.46	1.22	1.20	1.18
2,3,5-Trichlorophenol	3.69	6.43	1.38	0.90	0.65	0.52
2,3,6-Trichlorophenol	3.69	5.80	1.83	1.41	0.68	0.23
3,4,5-Trichlorophenol	3.69	7.55	1.08	0.76	0.76	0.73
2,3,4,5-Tetrachlorophenol	4.42	5.64	1.00	0.52	0.28	−0.19
2,3,5,6-Tetrachlorophenol	4.42	5.03	1.23	0.77	0.23	−0.81
Pentachlorophenol	5.19	4.74	0.46	0.15	−0.32	−1.60

[a] Calculated after Rekker (ref. 13), for the undissociated form.
[b] From Drahonovsky [14].
[c] In μmol/l.

Poct is the octanol:water partition coefficient
Tm is the 1/LC50 of the pure molecular form

the effect of pH on the accumulation: the molecular form of an acid can pass through membranes (the gills or epithelial tissue) in both directions faster than the ionic form. Thus , the uptake and elimination rate constants,with respect to the total concentration of a partly dissociated acid, depend on both pKa and pH and therefore bioaccumulation also depends on these factors. At a given total concentration of the acid, the total uptake rate increases with decreasing external pH, because the relative concentration of the molecular form outside the fish increases, as the relative concentration inside the fish can be assumed to remain constant. When the internal pH is higher than the external pH, ionization can play a role inside the fish, and this will reduce the elimination and therefore increase the accumulation. Second, the toxic action of the phenolic compounds can be caused by either the molecular or the ionic form or both of them. At a given total concentration in fish the concentration of the more active form depends on pKa. Third, the interraction with the receptor of the active form of the chemical can depend on its electron configuration, which also governs the pKa. In this way pKa and this interraction can be correlated. In contrast to the first one, the last 2 pKa influences will not depend directly on the external pH.

Saarikovski and Viluksela (1981) have studied the influence of pH on the toxicity of substituted phenols to the guppy(Poecilia reticulata). The 96-hr LC50 values were determined by a semistatic method in the pH range of 5 to 8. The pH did not affect appreciably the toxicity of 4-chlorophenol, but the toxicity of more acidic phenols decreased as the pH

increased. The changes in toxicity were substantially smaller than they would be ,if only the nonionized form were toxic. The results could be explained by assuming that the phenate ion also contributes to the toxicity, but its molar toxicity decreases with rising pH.The authors main assumption was that the absorption of phenols from water is the main factor influencing the toxicity, which is principally, if not solely, affected by pH changes.

The change in the pH of the surrounding water is not likely to affect the distribution,intrinsic activity,or metabolism of phenols in the fish since the pH of the guppy´s blood is kept within very narrow limits ; because phenols are excreted from fishes mostly as neutral conjugates(Kobayashi,1978), their elimination should also be rather independent of the water pH.

Saarikovski and Viluksela fitted the following equation to their results:

$$LC50_{(pH1)} / LC50_{(pH2)} = (1 + 4^{pH1-pKa}) / (1 + 4^{pH2-pKa})$$

The above equation predicted the changes in toxicity better than the so-called pH-partition hypothesis, which states that acidic and basic drugs penetrate biological membranes only as uncharged molecules(Shore et al. 1957; Hogben et al.1959; Jollow and Brodie 1972). The explanation given by Saarikovski and Viluksela was that both the molecular and the ionic form contribute to the toxicity, conforming to the equation(see Table 2.5 for abbreviation description):

$$1/LC50 = T_{HA} * [HA]/C + T_{A-} * [A-]/C$$

With this kind of model, Levy and Gucinski(1964) arrived at the result that the absorption rate of ionized secobarbital(pKa

TABLE 2.5

TOXICITY OF THE NONIONIZED AND IONIZED FORMS OF 2,4,6-TRICHLOROPHENOL AND PENTACHLOROPHENOL AT VARIOUS pHs (reproduced after Saarikovski and Viluksela, 1981)

2,4,6-Trichlorophenol

pH	[HA]/C × 100	[A⁻]/C × 100	Total toxicity	T_{HA} × [HA]/C	Δ	T_{A^-}	k
	100	0		344			
5	94	6	323	323			
6	61	39	222	210	NS		
7	14	86	86	48	38	44	2.2
8	1.6	98.4	25	5.5	19.5	20	

Pentachlorophenol

pH	[HA]/C × 100	[A⁻]/C × 100	Total toxicity	T_{HA} × [HA]/C	Δ	T_{A^-}	k
	100	0		15800			
5	33	67	6250	5214			
6	4.8	95.2	2275	758	1517	1593	3.0
7	0.5	99.5	602	79	523	526	1.9
8	0.05	99.95	292	8	284	284	

This table was calculated on the basis of the equation:

$$1/LC50 = T_{HA} * [HA]/C + T_{A^-} * [A^-]/C$$

T_{HA} = toxicity of the molecular form
T_{A^-} = toxicity of the phenate ion
[HA] = concentration of the molecular form
$[A^-]$ = concentration of the ionic form
C = total chlorophenol concentration
D = difference between total toxicity and toxicity of molecular form
k = decrease in toxicity of phenate ion as pH increases

7.92) in gold fish is 9.6 times lower than that of the molecular form. Similarly, Broderius et al.(1977) obtained a ratio of 2.3 for the toxicities of HCN and CN- and a ratio of 15 for those of H_2S and HS-.

Table 2.5 reproduced after Saarikovski and Viluksela(1981) shows calculated values for the toxicities of phenate ions for PCP and 2,4,6-TCP. The toxicity of the phenate ion was calculated by substracting the toxicity due to the phenol form from the measured overall toxicity and dividing with the proportional concentration of the phenate ion.

The toxicity of the phenate ion was not constant, but decreased with rising pH. This means that the changes in the toxicity is caused, in part, by changes in the degree of ionization and, in part, by changes in the absorption rate of the phenate ions. With very acidic phenols, the total toxicity should be caused solely by the phenate ions and follow the changes in ion toxicity. On theoretical grounds, the change in the toxicity of the phenate ion should be anticipated. An increase in pH results in a negative surface potential for the outer membranes of the epithelial cells, which should decrease their permeability to organic as well as inorganic anions(Wright and Diamond 1968; Deuticke 1977). The increase in pH may also shift the potential gradient across the gill membrane to a negative direction(McWilliams and Potts 1978), which impedes the diffusion of phenate ions from water to fish without affecting the absorbtion of the uncharged molecules.

In a study published in 1982, Saarikovski and Viluksela presented more results on their studies upon the relation

between the physiochemical properties of phenols and their toxicity and accumulation in fish. This time they determined 96-hr LC50 values of 21 substituted phenols for the guppy (Poecilia reticulata)in the pH range 6-8.They determined regression equations of toxicity on log Po/w and dpKa(difference between the pKa of a substituted phenol and pKa of phenol) at various pH levels in a manner similar to Konemann and Musch(1981). They concluded that lipophilicity is the primary parameter determining toxicity, and that the correlation between toxicity and dpKa is at least partly a consequence of intercorrelation of the parameters. They went even further in establishing an empirical equation to approximate the toxicity of a phenol to guppy at some given pH in the range 6-8:

$$\log(1/LC50_{pH}) = \log(1/LC50_{HA}) - \log(4^{pH-pKa} + 1),$$

where $LC50_{HA}$ is the LC50 value of the nonionized phenol form, pH the pH of the test solution,and pKa the logaritmic ionization constant of the phenol.

When this formula is used to correct the toxicity values for ionization,and new values employed in the regression analysis, the resulting equations are, in principle, independent of the pH of water. They can, for example, be derived from toxicity values measured at different pH levels. Basically they relate the toxicity which phenols would have, if they were nonionized in water, to their physiochemical properties. With proper substitutions they can also predict toxicity at any pH within the range where the empirical equation is valid. The same authors correlated the bioconcentration factor to the Po/w. The log BF value showed a good correlation to the Po/w value only

when corrected for ionization using the following equation:

logBF(corrected) = 1.02logPo/w - 1.82

The excellent correlations between toxicity and log Po/w are very puzzling since the Po/w values used in deriving these equations are for the nonionized form of the phenols. The less acidic phenols are in this form , even at pH 8, but the most acidic phenols appear at and above pH 6 for the most part as phenate ions. These ions do dissolve in octanol as ion pairs, but the apparent partition coefficient of the ion is at least three orders of magnitude smaller than that of the nonionized form (Hansch and Leo, 1979). For this reason a distribution coefficient should be used to determine BF.

Scherrer and Howard(1977) assumed that weak acids appear in the organic phase only in the neutral acid form (ignoring ion pairs) and calculated the distribution coefficients at each pH from the partition coefficients by making use of the Henderson-Hasselbach equation. When values of the Po/w in the regression equations relating toxicity to Po/w were replaced with the new values of the distribution coefficients no improvments to the regressions were observed(Saarikovski and Viluksela,1982).

Fujita(1966) has used another approach, in which log Po/w values are used as such, but the measured LC50s are corrected for ionization in the same way as Sherrer and Howard corrected the log Po/w values. If this approach is used on the regression equations established by Saarikovki and Viluksela(1982) they show weaker correlations than the uncorrected equations or the corrected regression equations using the empirical formula established by the same authors.

The reason for these unexpected results may be found in the multitude of interractions between the parameters considered and the very complex toxic action of the ionizable chemicals. The corrections based on the Henderson- Hasselbach equation fail to improve the correlation because they presume that toxicity is proportional to either neutral or ionized form, when it has been established that both forms contribute to the toxicity,(Saarikovski and Viluksela, 1981).It is also quite possible that the neutral and ionized form have entirely different modes of action. Konemann and Musch(1981) estimated the toxicity of the nonionized phenol form based on the assumption that the toxicities of neutral and ionic form of phenols are additive and independent of water pH. Saarikovski and Viluksela agreed on the additivity aspect but proved that toxicity of the ionic form changes according to the pH.

The importance of lipophilicity and ionization in determining the toxicity of phenols may be rather different in various animal groups. McLeese et al.(1979) measured the toxicity of various phenols to a shrimp(Crangon septemspinosa)in sea water. The results showed a poor correlation with log Po/w and dpKa. When corrected for ionization according to the Henderson-Hasselbach equation, the correlation was quite good. Their results showed that the effect of ionization may be greater in the shrimp than in the guppy.

2.3.2.3 Water Chemistry and its Influence on the Bioconcentration Process

Water characteristics other than pH, such as salinity, hardness, suspended solids load and composition act upon the accumulation of contaminants by the biota. Various species could be affected differently by changes in water quality and a generalization of the observed trends is difficult to do. Muir et al.(1980) showed the importance of the water chemistry on the uptake of organic pollutants by mosquito fish(Gambusia affinis) in river water. Concentrations of all compounds tested were lower in fish in river water than in lab water. Their results suggest that the suspended solids, particularly the organic carbon content of the suspended material, have a great influence on the availability of hydrophobic organic chemicals to fish in natural waters. They also recommended the introduction of a corrective term for the uptake rate constants to account for the effects of suspended solids or suspended organic carbon.

The effects of salinity on the uptake of pollutants plays an important role in estuarine environment. Results of various research work indicate that salinity decreases the uptake of pollutants(Murphy,1970). Whatever the reasons for the difference in the residue ratios, the effect of salinity is extremely important in terms of the interpretation of results from indicator surveys which use samples from areas differing in salinity. The effects of salinity on the uptake of residues were suggested to be caused by differences in osmoregulation by fish at different salinities or by an effect of salinity on the

lipid-water partition of chemicals. Eisler(1972) reported that small variations of temperature, pH, or salinity of test waters could change the LC50 values for organochlorines by at least one order of magnitude.

Water hardness has shown an effect on toxicity tests as well. Holden(1972) and Henderson et al.(1959) showed that DDT and dieldrin are both more toxic in soft water than in hard water at the same exposure concentration.

2.3.2.4 Pollutant Interaction and its Influence on the Bioconcentration Process

Interactions between organochlorines is a proven phenomenon and its effect upon the organochlorines levels in biota have been studied. Organisms which are susceptible to bioconcentration variation due to pollutant interactions must be eliminated from monitoring programs as no logical interpretations of the analytical results may be made to account for the interaction. Macek(1975) tested the acute toxicity of twenty nine two-chemical mixtures to bluegills(Lepomis macrochirus). Eleven of these mixtures exhibited synergism, seventeen exhibited additive toxicities, and one exhibited antagonism. However, Veith et al.(1979) found that the uptake of one chemical was independent of the other chemicals dosed in the exposure solution, providing that the metabolism of the organism subjected to the experiment was not significantly altered by the presence of one of the chemicals.

The possibility of testing more than one chemical in a

single exposure(if the chemicals can be separated for accurate analysis in the presence of each other and they do not interact)is a very attractive proposition. Moreover if an internal standard is adopted, the calculation of a relative BF to the BF of that internal standard would reduce the difficulties in comparing the results of laboratories where different experimental conditions and species are used.

2.3.2.5 Effect of Temperature on the Bioconcentration Factor.

Temperature is another important variable which has a large influence on the bioconcentration factor. It can affect bioconcentration in two ways. First, the water solubility of the pollutants changes as a function of temperature. In general, the solubilities of organochlorines in water increase with increased temperature. Organochlorines exist in water mostly adsorbed to particulate material. An increase in water temperature leads to an alteration of the soluble/particulate ratio by the dissolution of some of the particulate associated compounds. Such a process is probably not restricted to situations where the solubility maxima for a pollutant is exceeded, as temperature changes may be expected to alter the soluble/particulate ratio independent of the concentration in the two phases. It follows that organisms that accumulate at least part of their total body load of pollutant directly from water will contain higher concentrations of organochlorines in waters of higher temperature even if the total organochlorine concentrations at all locations are the same.

Second, there is the case where the concentrations of pollutants are far below the solubility maxima which is actually the most frequent case. The universal explanation accepted is that temperature affects the net uptake of pollutants by altering the metabolic rate of the organisms. Fish are poikiothermous,so rates of metabolism and chemical uptake are linked to the temperature of their environment. Thus, we find that the uptake of chemicals directly from water by fish increases with temperature in proportion to their oxygen consumption(Hamelink and Spacie,1977).

Fig.2.2 reproduced after Veith et al.(1979) shows the variation in BF of Aroclor 1254 with exposure temperatures for fathead minnows, green sunfish, and rainbow trout. It is clear from their results that the temperature has a positive effect on the bioconcentration. The three species used in his experiment show different uptake rates for Aroclor 1254 but all exhibit higher BF with temperature increase. Reinert et al.(1974) ran tests on rainbow trout (Salmo gairdneri) at various temperature levels and proved the increased bioconcentration with the increased temperature(see Fig.2.3).

The temperature effect must be considered when data obtained at different times of the year are used to evaluate the levels of contaminants in the environment. In combination with other seasonal effects such as contaminant use and fish growth and development , temperature contributes to the variable pattern of contaminant levels in biota.

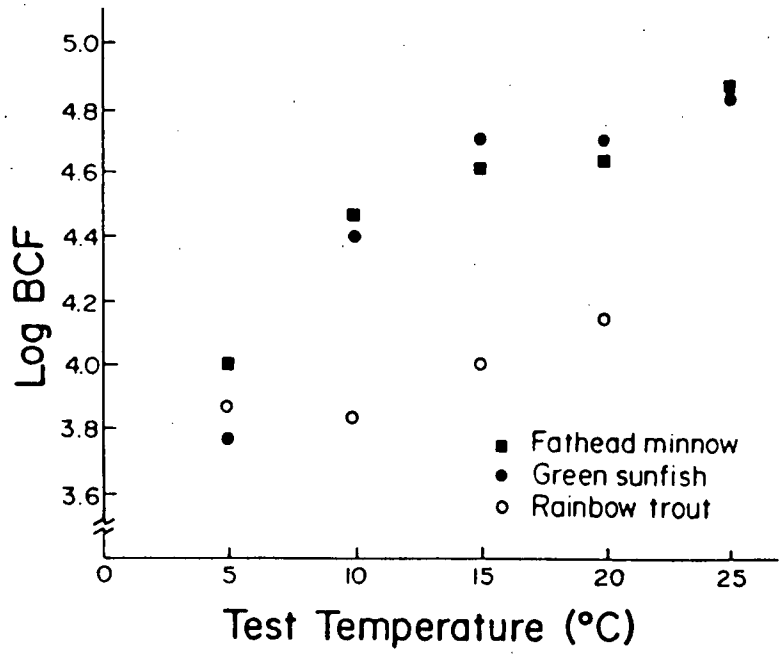

Fig. 2.2. Effect of temperature on the bioconcentration of Aroclor by fish(reproduced from Veith et al., 1979).

2.3.2.6 Effect of Test Species on the Bioconcentration Factor

The use of different test species in determining bioconcentration factors makes the results from different laboratories very difficult to compare. It has been found that different species bioconcentrate the same chemical at different rates, and even within the same species the characteristics of each individual(such as age, size, physiological state) influence the bioconcentration capability. Fig.2.2 (Veith et al., 1979) shows the different values of the bioconcentration factor obtained under identical experimental conditions for the three species used in the tests

2.4 Use of Biological Indicator Organisms to Quantitate Pollutants in Aquatic Environments

Surveys based on the use of biological indicators must be designed very carefully and their results must be interpreted with a practical knowledge of the effects of the interfering parameters.

The term "biological indicator organism" has been used in several different ways in the literature. Some authors have even used this term in ecological studies which make no reference to pollution of any kind. There are mainly two different methods by which biota have been used to assess pollution. This thesis employs a modification of one of these methods. An organism may be used as an indicator of pollution by its presence or absence in a given environment, or as a member of the biota indigenous to

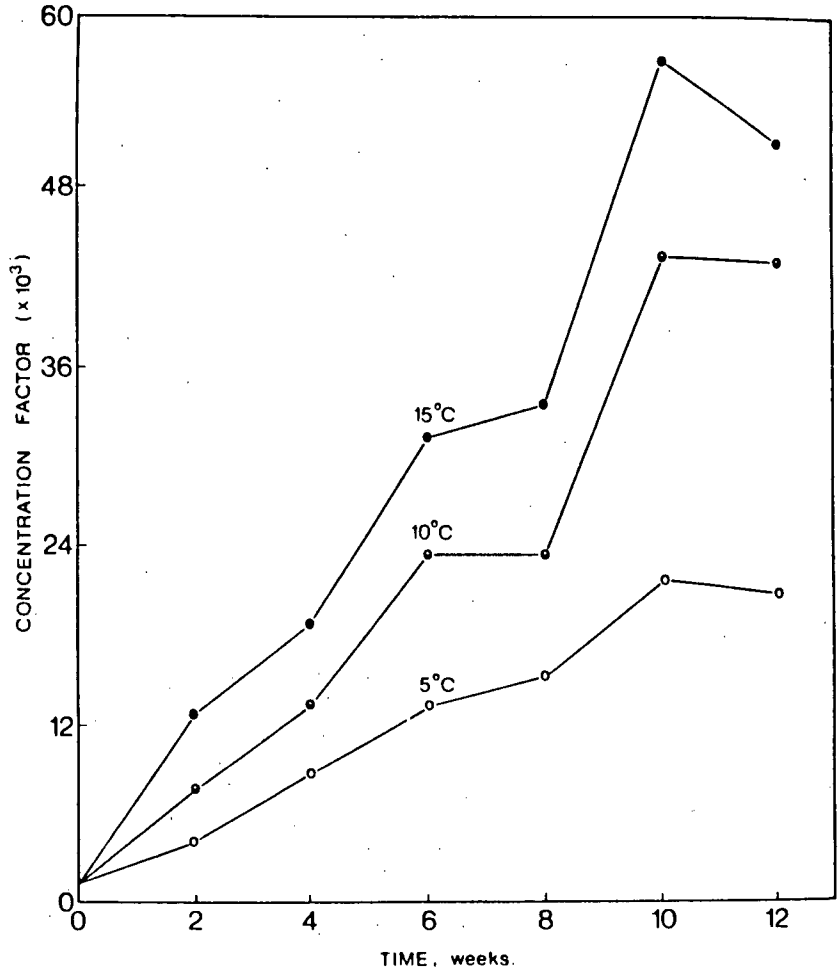

Fig. 2.3. Effect of temperature on the concentration of DDT in rainbow trout. Exposure levels were 176 mg/L at 5 °C, 137 mg/L at 10 °C and 133 mg/L at 15 °C(from Reinert et al., 1974).

the study region, by the accumulation of pollutants from water and/or ingested food. In the second case, the organism acts as an indicator by means of the pollutant content of its tissues, which may be assumed to be an index of the average pollutant availability at the biota collection site. The third mode of pollution assessment with an indicator organism is a variation of the second one in which the accumulation of pollutants in the tissues of the chosen organism is used to quantitate pollution. However, the organism does not belong to the biota of the study region and is purposely implanted at the monitored site.

The necessary attributes for an organism to act as a biological monitor of aquatic pollutants were first suggested by Butler et al.(1971) and amended by Haug et al. (1974) and Phillips (1978). A summary of those attributes is listed below:

- the organism should accumulate the pollutant without being killed by the levels encountered in the environment.

- the organism should be sedentary in order to be representative of the study area.

- the organism should be abundant throughout the study area.

- the organism should be sufficiently long-lived to allow the sampling of more than one year-class, if desired.

- the organism should be of reasonable size,giving adequate tissue for analysis.

- the organism should be easy to sample and hardy enough to survive in the laboratory.

- the organism should tolerate brackish water.

- a simple correlation should exist between the pollutant content of the organism and the average pollutant concentration

in the surrounding water.

- all organisms of a given species used in a survey should exhibit the same correlation between their pollutant content and the average pollutant concentration in the surrounding water, at all locations studied, under all conditions.

When using biota as a pollution indicator a series of factors affecting their pollutant content must be taken into consideration. The way those factors influence the pollutant bioaccumulation must be carefully studied and understood before any interpretation is given to the results of various monitoring surveys using biota.

The major factor that seems to determine the concentrations of organochlorines in biota is the amount of lipid present in the organisms studied. Other factors include species variables such as age, sexual condition , behaviour, and water quality variables such as temperature, pH, alkalinity, oxygen content. Miscellaneous factors such as seasonality , diet and interactions between the pollutants have their influence on the bioconcentration variability.

Differences in the wet weight based concentrations of organochlorines between different teleost species have been ascribed by many authors to differences in the lipid content of these species(e.g. Duffy and O´Connell, 1968; Hannon et al., 1970; Reinert, 1970;Sprague and Duffy, 1971; Carr et al.,1972; Jensen et al. 1972 ; Frank et al.,1974; Linko et al., 1975). Similarly,variation of organochlorine concentrations in the different tissues of a single fish species has been correlated to the lipid content of the tissues(Holden,1962;Reinert, 1969;

Stout et al.,1972; Yoshida et al., 1973; Ernst et al.,1976). Although some data suggest that the variation of organochlorine concentrations in any one species of teleost is markedly less on lipid weight basis than on a wet weight basis this is not always the case (Anderson and Fenderson, 1970). The partial failure of many authors to reach unified conclusions on this matter is due to the interfering effects of other differences(e.g. size or age, metabolic rates,migration, etc.) between individuals in any given teleost population.

Data concerning the effects of lipid on species or tissue differences in organochlorine concentrations amongst organisms other than teleost are sparse. Ernst et al.(1976) reported a correlation between the tissue distributions of DDT,DDE,DDD, and PCBs and the lipid contents of the tissues in the scallop (Chlamys opercularis) and similar data are available for shrimp(Nimmo et al.,1971), sea-lions(Delong et al.,1973) and seals and porpoises(Holden and Marsden, 1967).

Problems such as differences between lipid types in sequestration of organochlorines(Addison and Smith,1974), and the inability of solvent extraction methods to extract certain types of lipid(Vreeland, 1974) are additional concerns if the lipid weight is used as a basis for organochlorine concentrations in indicator organisms. However it is strongly recommended (Phillips,1978) that authors report data concerning the concentrations of organochlorines in biota by both wet weight and lipid weight.

The effect of lipids on the concentration of organochlorines found in aquatic biota are not only important in their own right,

but are also the main cause of the variations seen in such concentrations related to other factors such as age or season. The data available on the effects of age or related factors on the concentration of organochlorines in fish are very contradictory. Zitko(1971) reported higher concentrations of organochlorines in whole herring (Clupea harengus)of average weight 222 g than in those of average weight 59 g. In studies were data were reported by both wet weight and lipid weight the concentrations of organochlorines by wet weight increased in older fish and showed no variations with age if the lipid weight was used to calculate concentrations.(Hannon et al.,1970;Kelso and Frank,1974).

Murphy and Murphy(1971) correlated the uptake of DDT by fish of different sizes to the variation in oxygen uptake rates of these fish. The regression of DDT uptake with weight and of oxygen uptake with weight had almost identical slopes. The conclusion was that the uptake of DDT was proportional to the metabolic rates of fish and it translates into higher organochlorines concentrations in younger fish. With the available data, no universal available conclusion can be reached on the relationship between age and organochlorine content of the biota. It is evident that the age of the organisms sampled is a major interfering parameter in indicator surveys using biota. Sampling for such surveys should aim at taking organisms of similar ages or sizes from all locations studied or at selecting a range of size of fish of each species and subject the results to a detailed statistical analysis.(Anderson and Fenderson(1970). Data on other organisms than teleosts do not clarify the problem

any further.

Seasonality of the concentrations of organochlorines in biota is due to two major factors. The first is the seasonal difference in availability of organochlorines to aquatic organisms which may be correlated to pesticides application times in agriculture, to industrial discharges of organochlorines, to rainfall or run-off or to other factors. The second major factor causing organochlorine seasonality is the seasonal fluctuation of lipid contents in biota. These two factors need not be synchronized with each other and seasonal profiles of organochlorines may exhibit multiple annual peaks in biota due to lack of synchronization. Robinson et al.(1967) suggested that the seasonal fluctuations of DDE and dieldrin in sand eels and cod taken from the Farne Islands(Fig. 2.4) may have been due to migration of fish in and out of the population sampled. However, this hypothesis appears unlikely because of the similarities between the seasonal profiles of the two species. It would seem more likely that the differences reported reflect real differences in the availability of organochlorines throughout the year(Phillips,1978).

Bivalve molluscs have been widely used as indicator organisms and the data reflect the importance of the pollutant source on the seasonal availability of those pollutants. Seasonality of organochlorines has been studied in the sand crab(Burnett,1981) in the amphipod(Gammarus pulex ,Sodergen et al.,1972)in phytoplankton (Jensen et al., 1972), and in zooplankton(Williams and Holden,1973). Their data showed the wide variation of pollutant content in biota as a function of season.

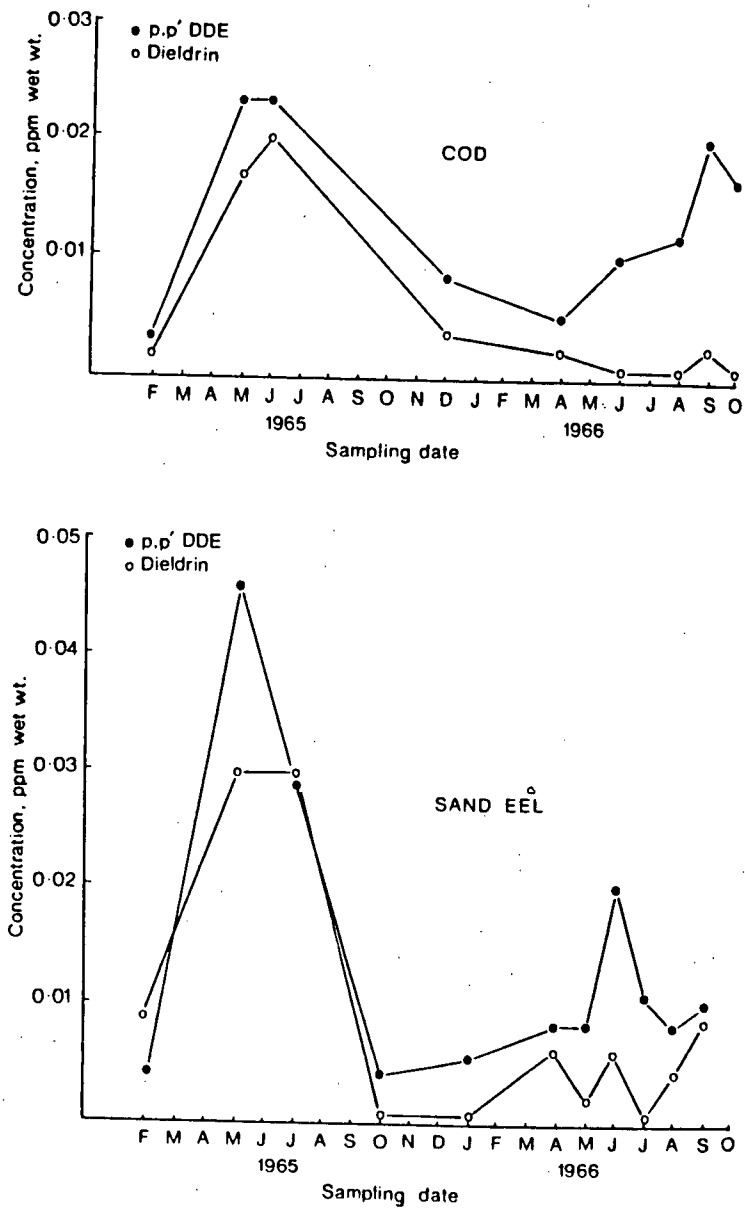

Figure 2.4. Seasonal effects of pesticides levels in fish. The fish, cod(Gadus morrhua) and sand eel(Ammodytes lanceolatus) were caught at different times of the year around the Farne Islands.

The effect of temperature on the uptake of organochlorines by organisms probably also contributes to the seasonality of these compounds in biota(Kellog and Bulkley,1976).Temperature effects are difficult to separate from those of lipid changes and variations in the available ambient concentrations of organochlorines, both of which also contribute to the seasonality. Only controlled laboratory studies can clearly defined the relationship between temperature and bioconcentration.

2.4.1 Pollutional Ecology of the Freshwater Leeches

Sawyer(1974) commented that there is the quantitative, rather than the qualitative, composition of the leech fauna which characterizes the different types of normal or disturbed habitat. Few leech species, if any, are so ecologically restricted that a single environmental factor can determine their distributions. Among the environmental factors that influence the leech distribution, in approximate order of significance, are: availability of food organisms, the nature of substrate, the depth of water, water currents, size and nature of the body of water, hardness, pH, temperature of the water, minimum concentration of dissolved oxygen, siltation and turbidity, and the salinity of the water(Sawyer, 1974).

Species of leeches which feed upon aquatic oligochaetes and dipteran larvae respond indirectly to organic enrichment. The abundance of these leeches in zones subjected to raw sewage discharges(Richardson,1928) reflects their response to the

increased food supply.

Except at extremely low levels, hardness, total alkalinity, and pH have little or no influence on the distribution or relative abundance of leeches. Water temperatures play an important role in the reproductive biology of leeches, primarily by determining the onset of the breeding season. In the laboratory, leeches die at temperatures of 33-35C(Sawyer,1974). High summer temperatures(about 30C or higher) limit the distribution of leeches in both naturally and thermally polluted environments. Most leeches can withstand anaerobic conditions for long periods and are not restricted by temporary oxygen depletion. Siltation and turbidity have a profound effect on the ecology of leeches. Water turbidity reduces the natural predation of other organisms on leeches and leads to high densities and even epidemics. Siltation changes the nature of the substrate to such an extent that leeches have difficulty moving and depositing cocoons. Leeches are relative tolerant to bunker oil and exhibit a very high tolerance for pesticides and other pollutants. LC50 values for various species of leeches exposed to pesticides are readily available in the literature. The LC50´s of the major pesticides, such as dinex, chlordane, diazinon, lindane, mirex , and malathion, vary in the laboratory from 0.5 to 10.0 ppm(Sawyer, 1974).

Besides their high resistance to the toxic action of various chemicals , leeches have also been found to have a considerable bioaccumulation potential for synthetic organic chemicals. According to Navqui and de la Cruz(1973), levels of mirex in the leech Erpobdella punctata were among the highest found in

invertebrates and fish collected from areas treated with the chemical(up to 1.76 ppm).Webster(1967) reported residues of DDT in leeches three months after aerial spraying of the pesticide; no residues were found in the tissues of amphipods and copepods from the same area. D´Eliscu(1975) found 8.1 times higher DDT levels in the tissues of leeches than in clams collected from the Lake Tahoe basin. Other results from the work of the same researcher indicate that the bioaccumulation potential of leeches varies from species to species.

Carey et al.(1983) reported extremely high concentrations of chlorophenols in leeches collected from Canagagigue Creek located in Ontario. Concentrations were 20 times higher than was found in fish sampled at the same locations. As a result of this observation, another study by Metcalfe et al.(1984) emphasized the use of leeches as a potential bioindicator of organic chemical pollution.Their data showed that leeches accumulated very high levels of pollutants (in this case chlorophenols) and the levels found in their tissues were one or two orders of magnitude greater than the levels found in thirteen other benthic invertebrates as well as fish and tadpoles. At one of their sampling sites, leeches accumulated chlorophenols to levels ranging from 40,000 to 140,000 times the average water concentration. Metcalfe et al.(1984) were first to suggest that leeches be used as a "early warning " indicator of organic pollution. Their work on leech bioconcentration capability constituted the starting point of the present thesis. Chlorophenols are widely used in British Columbia by the wood industry and a bioindicator of chlorophenol pollution in the

water system of the province would be a major contribution to present monitoring programs which all rely on discrete, grab water samples.

3. SAMPLING AND METHODOLOGY

3.1 Introduction

The experimental work can be divided in three major sections: in situ Fraser water sampling, in situ field leech experiments, and laboratory controlled leech experiments. This chapter will describe each section in detail with respect to sampling technique, analytical procedures and experimental setup and design.

3.2 In Situ Fraser Water Sampling

Two methods of water sampling were used, namely: grab sampling and automatic sampling. The grab sampling was initially performed in order to obtain basic information upon the CP concentration levels in the study area and to determine the general pattern of CP contamination in the surveyed portion of the Fraser River. Automatic sampling was performed only at one location which was selected on the basis of previous grab sample results, site availability and equipment safety.

3.2.1 Study Area and Sampling Sites

The study area consisted of a stretch of Fraser River, the North Arm, between New Westminster and Iona Island. Fig. 3.1

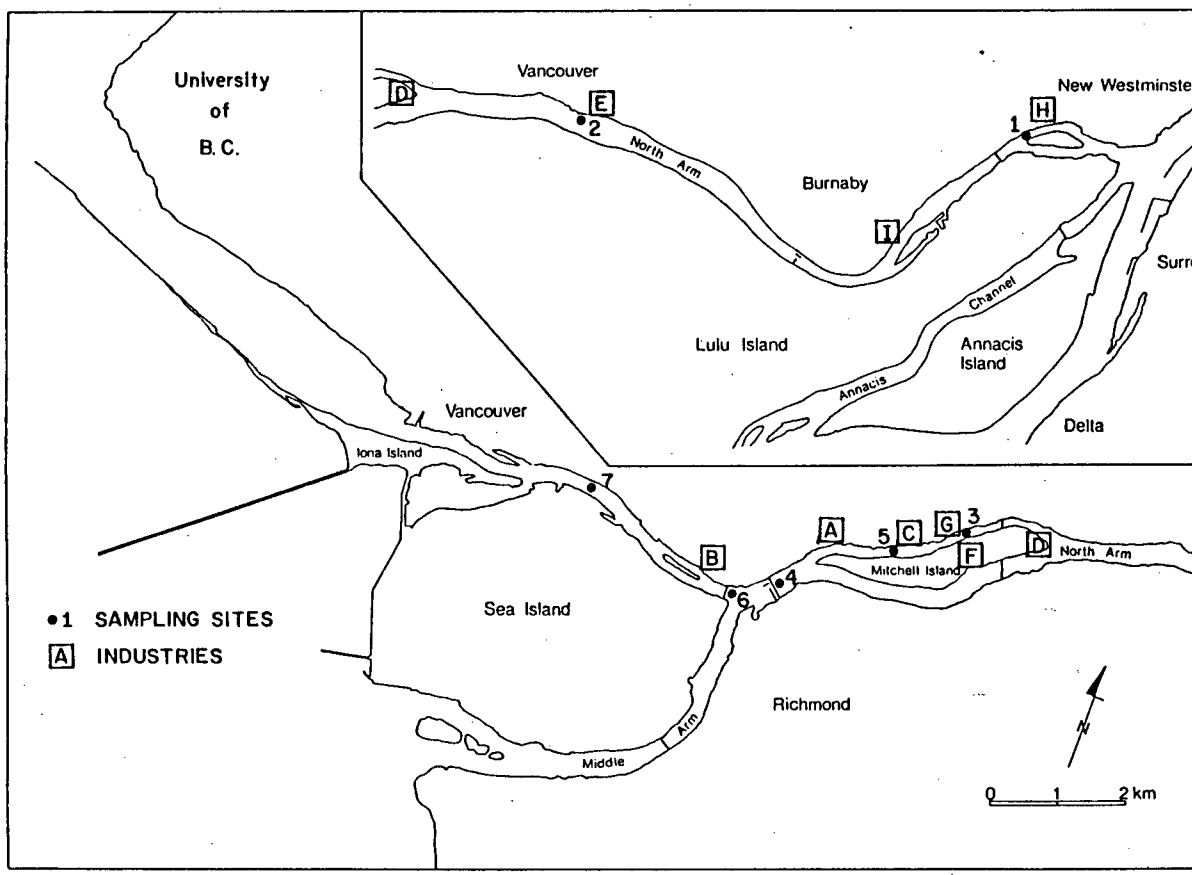

Fig. 3.1. Sampling sites and industries in the study area (sampling stations are identified in Table 4.7 and industries in the text, section 3.2.1).

illustrates the sampling sites and forest industry mill locations. This reach of the Fraser River has a high density of mills using CPs and was considered suitable for a study of chlorophenol pollution:

(A):B.C. Forest Products Ltd., Vancouver;

(B):Canadian Forest Products Ltd., Eburne Sawmills Division;

(C): West Coast Cellufibre Industries Ltd.;

(D): Fraser River Planning Division;

(E): MacMillan Bloedel Ltd., Canadian White Pine Division;

(F):Terminal Sawmills Ltd., Richmond;

(G): Whonnock Industries Ltd., Silvertree Division;

(H): Scott Paper Ltd., New Westminster;

(I): Belkin Packaging Ltd., Burnaby;

Note: the last two mills do not use CPs in their process but their effluents contained CPs(Cain et al. 1980).

Sampling sites locations are indicated in Fig. 3.1 by numbers from 1 to 7 and will be referred to when results are discussed.

3.2.2 Grab Sampling

To assess the general level of contamination in the study area, a series of grab samples were collected between June and October 1984. In June and August samples were collected at access points along the river bank and in September and October samples were collected in midstream using a boat.

The water samples were collected in 4L amber bottles with teflon lined screw caps, and preserved with NaOH(5 pellets/L).

The bottles had been previously cleaned using a three step procedure: step one included washing the bottles with tap water and regular laboratory detergent followed by thorough rinsing with tap water; step two consisted of cleaning the bottles with hexane followed by rinsing with tap water; step three consisted of applying the final rinse to the bottles using distilled water. The samples were transported to the UBC Environmental Engineering laboratory and stored in a cold room at 4 °C. Extraction and further preparation of samples was completed within 7 days as specified in Test Method 604 for phenols (U.S.EPA, 1982). Replicate analyses were performed on many samples to evaluate the reproducibility of the extraction and analytical technique.

Blank samples were prepared and analysed for each sampling trip. A blank consisted of distilled water placed in a regular sample bottle with preservative. This blank was transported to the field and stored in a similar manner to the Fraser River samples. Transportation of blanks to the field was performed in order to subject the blanks to the same outdoor temperature variation and transportation conditions as the field samples.

3.2.3 Automatic Sampling

For automatic sampling, an ISCO automatic sampler which contained 28(500ml) polyethylene bottles was used. All the automatic sampling was done from the loading dock of West Coast Cellufibre Industries Ltd. The sampler was sheltered in a small shack located on the floating dock and was operated by a 12 V battery.The suction tubing was immersed in the water and the

exact point of sampling was 4 feet below the water surface. Every 2 hours a 500 mL sample was taken. After each sample was taken, a purging cycle cleared the suction line to avoid contamination between succesive samples.The sampling process including purging lasted approximate 3 minutes.

The concern of adsorption or leaching problems with plastic bottles was checked by spiking both plastic and amber glass bottles with known amounts of chlorophenols and analysing samples after one week. Very comparable results were obtained and the plastic bottles were considered acceptable for use. NaOH pellets were still used for sample preservation. Standard deviations of the combined samples(contained in plastic bottles and amber bottles) were 3.78% and 12% for TTCP and PCP respectively, values well within the recovery and reproducibility variability. Similar values were obtained for the lower chlorinated phenols.

The individual samples were analysed as such, or a composite sample was made manually. For the composite samples, three consecutive individual samples were combined in equal volumes (400 mL each),in one 1200 mL sample which thus contained combined chlorophenol concentrations over a period of 6 hours. This technique was used in order to reduce the number of samples to be analysed during longer sampling periods(6 or 7 days). In this mode the high frequency of sampling was still maintained(i.e. every 2 hours) without tripling the number of samples to be analysed. With the exception of the first 2-day automatic water sampling experiment, all the other automatic sampling experiments were performed by using 6 hour composite samples.

3.2.4 Water Sample Preparation for Chlorophenols Analysis

The extraction of chlorophenols and further sample preparation for GC analysis followed the methodology outlined by Metcalfe et al.(1984). This technique was originally developed by Chau and Coburn(1974). The technique was further improved by Fox(1978) with two modifications:

1) Alkaline storage at pH 12 to increase solubility, which in turn decreases adsorption to surfaces, followed by acidification to pH 1-2 immediately prior to analysis.

2) Replacement of benzene with toluene as the extraction solvent, because of the suspected role of benzene as a carcinogen. Extraction efficiencies of the two solvents are approximately equal.

The water samples were acidified with 50% H_2SO_4 to pH 1-2 just prior to extraction. The initial volume of the water sample analyzed was different at various experimental times(400 mL, 1000 mL and 1200 mL). The following procedure describes the various reagents quantities used if initial water sample is 1000 mL. Slightly modified quantities were used for the other sample volumes. Appropiate correction factors were used when calculating the concentration factor from the initial sample volume to the final extract volume which remained constant(10 mL) regardless of the starting sample volume.

Acidified water samples were extracted 3 times with chromatographic grade toluene(40, 30, 30 mL). The combined toluene extracts were back extracted 3 times with 0.1 M K_2CO_3 (40, 30, 30mL).

Derivatization using acetic anhydride was used to prepare the extracts for GC analysis. Underivatized phenols do not possess favourable properties for gas chromatographic analysis due to their high polarities and low vapour pressures(Krijgsman and van de Kamp,1977). Derivatization is recommended to form less polar compounds and help overcome the tailing effect and the assymmetric peaks obtained by running pure phenols through the columns. Many derivatization methods have been published. They include formation of ethers, esters, phosphorus esters and silyl derivatives(Krijgsman and van de Kamp,1977). A summary of derivatives with appropiate references is available(NRCC,1982). Derivatization also helps to accomplish sample clean up by eliminating other interfering compounds and gives better separation on the chromatographic column. To the aqueous K_2CO_3 extract, 10 mL of hexane and 1 mL of redistilled acetic anhydride were added. Acetylation was performed by shaking the above reagents for one hour in a 250 mL separatory funnel on an automatic shaker(Model:Wrist Action Shaker; Manufacturer:Callahan). After shaking, the hexane layer which contained the acetylated chlorophenols was removed with a Pasteur pipet and analyzed by high resolution capillary gas chromatography.

The derivatization agent , acetic anhydride, was always freshly redistilled(b.p.=139°C). It is a stable and strong acetylation agent. Stoichiometric calculations showed that 1 mL acetic anhydride used for acetylation was well in excess to the amount of trace chlorophenols found in the sample extracts, so it was assumed that all chlorophenols were totally reacted with the

derivatization agent and were present as acetylated derivatives in the final volume.

3.3 Leech Experiments

As previously mentioned, the leeches used as bioindicator organisms were collected from an unpolluted environment and implanted in bioassay chambers in the study region or used for laboratory controlled experiments. Leeches were collected from the littoral zone of a pristine lake, Kentucky Lake, located some 300 km east of Vancouver, between Princeton and Merritt, B.C. Collection trips were made during the ice free period, usually April to October. The last trip in the fall of 1984 was at the end of September and the first trip in spring of 1985 was mid April which proved to be early for that year which was characterized by a very late spring, with lower than normal temperatures. The lakes in the Merritt area were still frozen at the end of April. Only early in May, were enough leeches collected to permit the continuation of the leech experiments.

For the purpose of identification, leeches were preserved in 85% solution of alcohol. In order to get the leech specimens straight, moderately extended, and undistorted, alcohol was added gradually and used as an initial anesthetic(Moore ,1959). Five individuals of each species were sent to Calgary for identification. Leeches are never identified on the basis of host or habitat. Only the morphology will lead to secure identifications(Moore,1959). With the help of Prof. Davies, Head of the Department of Biology at the University of Calgary the

species of leeches found in Kentucky Lake were identified as follows: Percymorensis marmorata ,Nephelopsis obscura and Glossiphonia complanata.

Only the first two species were used in the experimental work. Our intention was to use only one species in all the experiments in order to keep variables to a minimum. Unfortunately, during May leech collection trips, the slim brown leeches(N.obscura) were in great abundance whereas the large, black leeches(P.marmorata) which had been used the previous fall were in very limited supply and very small in size. For this reason the temperature/bioconcentration factor experiments run in May, 1985, were done with the brown leeches. All other experiments, field or laboratory controlled were done with the black leeches which were first selected because of their apparent abundance(during summer and fall) and large size(0.1g-4.0g).

The leeches were transported to the U.B.C. laboratory in plastic pails, and were stored in a 50 L aerated aquarium containing native lake water. Sand, pebbles, and some wood debris taken from the lake were used to create a more natural environment. A very small percentage of leeches died during the first days of acclimation(5%). Previous storage of leeches in reconstituted fresh water, soft type, as formulated by US EPA (1978) gave us a higher mortality rate(20%), so it was decided to store leeches in water collected from their native environment. Species identified in Kentucky Lake prefer a harder water than the soft type used in the early storage and that explains the difficulty in adaptability experienced by the leeches at first. The majority of leech species are most abundant

in water with total alkalinity above 60 ppm $CaCO_3$. Few species occur in an alkalinity below 18 ppm. Similarly, all leech species are most abundant in water with a pH of 7.0 or higher, and few species occur at pH 6.0 or below(Sawyer, 1974).

Leeches survive well in media of low oxygen content and no bubbling of the water is usually recommended for the maintenance of the adults(Fernandez, 1982); however, the aquarium was aerated to eliminate any foul odours from decomposition processes.

No food was supplied to the leech stock. The species present in the supply lake are reported to feed on oligochaetes,insect larvae and snails(Sawyer, 1974). Since the leeches survive without food for a long period of time (4-6 months), it was elected that no food be provided to them in order to eliminate possible contamination via food source, or possible concentration of the chlorophenols in the ingested food. As a result of feeding, leeches store large amounts of blood or tissue fluid in their gut. Degradation of this food takes days, weeks, or months depending on the species of leech (Fernandez,1982). Bioaccumulation of pollutants via the food chain is considered to play a minor role as compared to the direct uptake from the water matrix and it was not included in the experimental setup.

The aquarium was kept only two thirds full with water to allow the black leeches (P. marmorata), which are amphibious, to get out of the water and crawl on the walls. The other two species are completely aquatic and do not usually leave the water. This characteristic of the black leech made the laboratory controlled experiments a little difficult since the leeches

tended to leave the water and were not exposed to the contaminant in the water for the entire period of exposure. This phenomenon was especially encountered at room temperature(approx. 23°C). At 12 and 4°C the black leeches seemed to remain in the water.

3.3.1 In-situ Field Leech Experiments

Exposure experiments were carried out for periods of six or seven days and 5 leeches were placed in each bioassay chamber. The bioassay chambers were suspended in the water, at 3-4 feet depth, at various locations. The majority of leech field experiments were done at the location where automated water sampling was performed as well. Some leech field experiments were run concurrently with automatic water sampling while others were conducted independently. The cylindrical bioassay cages were made of fine aluminum mesh reinforced on the sides with steel straps. The top of the cylinder was secured with a large hose clamp to facilitate transfer of leeches. The mesh allowed the water to flow freely through the cage. Approximate dimensions of the cylindrical cage were 100 mm in diameter and 300 mm in length. A fishing line was used to tie it securely to a stationary platform or pole. Leeches survived very well the conditions of exposure and none succombed during the field experiments.

Upon completion of the exposure period, leeches were taken to the laboratory where they were immediately weighed and digested for chlorophenol analysis. The leeches were dried with a paper towel and wet weights were determined on an analytical balance. Mann(1953) showed that the amount of water carried over

was sufficiently constant to enable the wet weight to be accurately determined to the nearest 10 mg.

Digestion was performed by using concentrated HCl in a ratio of 1 mL acid to 0.1 g of leech(Metcalfe et al.,1984). The digestion was completed in several hours and usually during the next day the chlorophenols were extracted from the digested leech matrix and the extract prepared for GC analysis.The acid digested leech was extracted with hexane, back extracted in 0.1 M K_2CO_3 and acetylation performed as for the water samples. The final sample volume was 10 mL of hexane which contained the acetylated derivatives of the chlorophenols found in the leech.

Phase separation was often difficult when chlorophenols were being extracted from the digested leech matrix. Centrifugation had to be performed on a few occasions to accomplish separation. Centrifugation had to be perfomed as well for a set of water samples that contained high levels of suspended solids. These samples were collected towards the end of April when the Fraser River was in flood due to the melting snow. Recovery of chlorophenols in the presence of high sediment load was also lower than under normal circumstances and corrections were applied to the results.

3.3.2 Laboratory Leech Experiments

For the controlled exposure of leeches to various concentrations of chlorophenols, one liter of reconstituted fresh water was added to 1400 mL beaker, followed by additions of known concentrations of chlorophenols(non-derivatized standards contained in toluene were used for spiking). The medium hard water formulation recommended by EPA was similar to the Fraser water characteristics and it was used as dilution water in the control experiments(pH 7, alkalinity 50 mg/L $CaCO_3$, hardness 60 mg/L $CaCO_3$). Various concentrations of chlorophenols were used for 24 hours exposure studies and a mixture of 5 chlorophenols at 10 ppb each was used for one week exposure trials. The same mixture of chlorophenols was used for other controlled exposures at lower levels of chlorophenols.

Since experiments were conducted under static conditions, leeches were transferred to a fresh solution of 10 ppb chlorophenols each day throughout the bioassay period; the concentration of chlorophenols in water in which leeches were immersed was checked and it did not change much due to volatilization,photodegradation or bioconcentration of chlorophenols by leeches. At pH 7.0 most chlorophenols are ionized and the process of volatilization applies only to the small fraction of undissociated molecules. Photodegradation was not a problem since most laboratory controlled experiments were run in dark rooms or enclosures. Mass balance was calculated as well, and the amount of chlorophenols accumulated in 24 h by

leeches was insignificant as compared to the total amount contained in the spiked water. Analysis of a spiked one liter dilution water at an initial concentration of 10 ppb was performed (container covered with a glass lid and left on the counter top for one day). After 24 hours, chlorophenols concentrations were 8-9 ppb as compared to an initial concentration of 10 ppb. When all errors due to the extraction, recovery and instrument performance were considered the values indicated that a relative constant concentration of chlorophenols was maintained by daily replacement of the solution.

In the 6 or 7 day experiments, every 24 h, three leeches were digested and extracted for analysis. Parameters considered during leech experiments were contact time, chlorophenol concentrations, temperature, and dilution water pH. Leech size was monitored and it was kept in a very narrow range. A specific experiment having leech size as variable parameter was not carried out.

Constant temperature was obtained by either running the experiments in an enviromental temperature controlled chamber or by using a temperature regulated water bath. pH adjustments were made with additions of 50% sulfuric acid or concentrated caustic(10 N NaOH). For the duration of the pH experiments(24 h), the pH remained within 0.5 units of the initial value. Since only the trend of variation of the bioconcentration factor with pH was monitored this was considered satisfactory.

3.4. Standards Preparation

Stock solutions of all the 19 congeners of the chlorophenols family were made at a concentration of 1mg/mL. Isopropanol was chosen as a solvent because its higher polarity favored the solution of the polar chlorophenols. Ten mg of pure material was weighed and dissolved in pesticide quality 2-propanol(isopropanol) and diluted to 10 mL volume. The chlorophenols were supplied locally by BDH which acted as a distributor for Aldrich Chromatographic Chemicals. Since purity of the compounds was certified at 96% or greater, no correction was made to calculate the concentration of the stock solutions. The stock solutions were kept in amber teflon-sealed screw-cap bottles, and stored at 4 °C in the refrigerator. Stock standard solution were not used for more than 6 months as recommended(U.S.EPA,1982).All the organic solvents used were of chromatographic purity. The stock solutions of each chlorophenol(1 mg/L), were used to prepare various mixtures of chlorophenols at desired concentrations.

In order to determine the retention times of all 19 chlorophenols, 5 standard mixtures of chlorophenols were prepared and analyzed separately for proper compound identification . When in doubt, single compounds were run to confirm a retention time.

Non-derivatized standards were made from stock solutions using toluene as solvent and they were generally used for spiking various samples, to make additions to the controlled bioassay chambers in which leech exposures were performed, and

to make derivatized standards. Derivatized standards were made for every large series of samples and they were used for the calibration files, quality control and monitoring of the detector sensitivity. A concentrated derivatized standard was usually made and diluted to cover the possible range of concentrations encountered in the samples. The most frequently used standard was a derivatized mixture of 5 chlorophenols(2,4-DCP; 2,4,6-TCP; 2,4,5-TCP; 2,3,4,6-TTCP; PCP) at 1 ppm concentration.

For calibration, the external standard technique was used. The most frequently used calibration file was made at three concentration levels, namely: 0.01 ppm; 0.1 ppm; 1 ppm. The ratio of instrument response to amount injected(calibration factor) for the 5 chlorophenols was quite constant over the entire working range which indicated a straight calibration line for the concentrations in question.(if the relative standard deviation of the calibration factor is less than 10%, linearity can be assumed(U.S.EPA, 1982).

Standards were stored in amber vials(10 or 40 mL) with teflon caps and kept in the refrigerator, at 4 °C, when not in use. All the sample vials used in the autosampler were amber with special EC detector caps. The working calibration curve was verified each day that analysis were made and if the response for any compound varied more than 10%, the test was repeated using a fresh calibration standard, or a new calibration factor was determined. In order to achieve consistency of the results, a few older samples which had been analysed and quantified on another occasion were run together with the new set of samples and requantified according to the new calibration curve. In case that

quantitation of those samples varied too much between the two runs(20% variability) correction measures were taken and sometimes entire old runs were rerun together with the new run.

3.5 Analytical Technique for Chlorophenols Separation, Quantification and Identification

The acetylated extracts were analysed for chlorophenols by capillary gas chromatography using the electron capture detector.The temperature program developed allowed a complete separation of the 19 congeners of the chlorophenols family(with the exception of 2,4 and 2,5-DCP which eluted together and could only be separated at very low concentrations).

The method of determination of chlorophenols by capillary gas chromatography developed by Krijgsman and Van de Kamp(1977) served as a starting point in setting up the methodology. Appendix I(Fig.I.1) shows the chromatogram and the relative retention times of the chlorophenol acetates presented by Krijgsman and Van de Kamp. As stressed by these researchers several aspects make this method very attractive for the determination of chlorophenols in environmental samples: selectivity of the extraction; high separation power of the capillary column; and specificity and sensitivity of the electron-capture detector.

Analysis of the derivatized chlorophenols were performed on a Hewlett Packard 5880 A ,gas chromatograph, equipped with an electron capture detector and an autosampler. A DB5 capillary column (30 m x 0.332 mm I.D.) provided good isomer

separation.The DB5 liquid phase of the fused silica capillary column is crosslinked and bonded. It is of non-polar composition: 95% Dimethyl-(5%)diphenylpolysiloxane.

Helium was used as carrier gas at 2mL/min and nitrogen as make-up gas at 20mL/min. Splitless injection was necessary because of the trace levels involved. The inlet was purged after 0.5 min in order to diminish the solvent peaks.

Detector temperature was set at 310 °C and injection port temperature was set at 250 °C. Oven temperature was programmed in three levels as a result of repeated trials to separate the 19 constituents of the chlorophenols family. The temperature program developed was: -level one, from initial oven temperature of 75 °C to 148 °C (15 °C/min); -level two, from 148 °C to 180 °C (3 °C/min); -level three, from 180 °C to 225 °C (10 C/min). A post temperature of 250 °C was used for 5 minutes. A total time of 30 minutes was required to separate all the chlorophenols.

Standard mixtures with CP concentrations as low as 0.01 ppm gave sharp, symmetrical and completely separated peaks of all the chlorophenols.

3.5.1. Identification and Confirmation of the Chlorophenols Presence in the Water and Leech Matrix

Confirmation and identification of chlorophenols present in various samples was obtained by GC/MS (Hewlett-Packard 5985 B), with a mass scanning range of 1-1000 amu. The MS was interfaced to a 5840 gas chromatograph equipped with a DB5 capillary column. A simpler temperature programme was used since only 5 congeners

used in laboratory experiments were run through the GC/MS(2,4-DCP; 2,4,5-TCP or 2-CP; 2,4,6-TCP, 2,3,4,6-TTCP, and PCP).

Standard mixtures at a higher concentration level (100 ppm) were used for chlorophenols identification. This was due to the fact that the full scanning mode of the GC/MS has a sensitivity 100 times lower than the electron-capture detector used for the chromatographic analyses.Since the spectral library (31,500 spectra to date) did not contain the acetylated chlorophenols,the spectra of the chlorophenols standards were filed. A set of electron impact spectra are presented in Appendix II.

The use of positive ion chemical ionization was explored as an alternative method for chlorophenols identification. This method gives a much higher molecular intensity. Appendix II contains the total ion chromatogram and a complete set of positive ion chemical ionization spectra of the chlorophenols monitored during the study .

Leech extracts were also analysed on the GC/MS system and the chlorophenols presence confirmed. A tentative identification of other major peaks present in the leech matrix was performed(see Appendix II for all the pertinent spectra).

Negative chemical ionization is a method of combined chromatographic and mass spectra analysis in the range of the ECD sensitivity. This technique was not explored but single ion monitoring was conducted, which is known to enhance sensitivity up to 1000 times. Appendix II contains spectra obtained through this method. Identification and quantitation of the leech control(blank) samples were done and they confirmed the levels found with the regular GC .

3.5.2 Reproducibility and Technique Evaluation.

To determine reproducibility of the sample preparation for the GC analysis, the extraction step and the derivatization step were assessed separately by calculating recovery of chlorophenols from water and leech matrix and by calculating the derivatization reproducibility. Reproducibility of the results as a function of injection technique, instrument parameters and sample vials condition was evaluated by calculating the injection accuracy. Finally, the method detection limit (MDL) was calculated through a method defined by Glaser et al.(1981).

3.5.2.1 Recovery from Water

At least four 1 L water samples were spiked with 1 mL of 1 ppm mixture of 5 chlorophenols.(2,4-DCP; 2,4,6-TCP; 2,4,5-TCP; 2,3,4,6-TTCP; PCP). The respective spiked water samples were extracted for chlorophenols using toluene and back extracted using the 0.1M solution of K_2CO_3. The extracts were derivatized and analysed for chlorophenols. Results were compared with the derivatized standard of 0.1 ppm concentration.(1 mL of 1 ppm chlorophenols added to 1 L of water and contained at the end of derivatization in 10 mL of hexane leads to a theoretically concentration of 0.1 ppm chlorophenols in the derivatized extract).

3.5.2.2 Recovery from Leeches

At least four leeches were spiked with 1 mL of 1 ppm mixture of 5 chlorophenols. Spiking was done in the vials which contained the acid digested leech. Normal extraction steps were followed and the derivatized chlorophenols contained in 10 ml of hexane were compared with a 0.1 ppm standard of derivatized chlorophenols.

3.5.2.3 Derivatization Reproducibility

Derivatization reproducibility was determined at 2 levels of concentration, namely 0.1 ppm and 1 ppm of the acetylated derivatives. For the 0.1 ppm derivatized mixture of the 5 chlorophenols, one mL of 1 ppm non-derivatized standard mixture of the 5 chlorophenols was added to 100 mL of 0.1 M K_2CO_3 and acetylation performed as usual. The standard deviation was calculated on the basis of 5 derivatized samples. Same procedure was followed for the 1 ppm level. Various concentrations of derivatized standards were obtained in a similar manner. It should be mentioned that an absolute derivatized standard, what is called a check standard which can be usually supplied by the chromatographic material supplier was not available. Hence it was necessary to evaluate the derivatization reproducibility as part of the process of standardization.

3.5.2.4 Injection Accuracy

Twelve injections of a 0.1 ppm standard of the mixture of 5 derivatized chlorophenols were made and the mean and the standard deviations of the results were used to calculate the accuracy of a single injection reported within the 95% confidence interval. The injection variability evaluation contained some of the variability attributable to instrumental parameters and different sample vials.

Proper preparation of the GC prior to running a set of samples was found to be very important. The instrument required at least 4 hours of equilibration before any of the readings could be used. Those 4 hours did not include the column preparation or detector stabilization. Various standards of chlorophenols were run during the 4 hour period and an assessment of the calibration curve was made on the basis of previous runs. New calibration files were used for each day of operation. As a quality control measure after every 10 samples, a standard of known concentration was run in order to confirm the accuracy of the calibration file.

3.5.2.5 Method Detection Limit

Detection limit is one of the most important performance characteristics of an analytical procedure. The detection limit of the method used in this study was assessed according to the method described by Glaser et al., 1981. They defined MDL as an error distribution implying that 99% of the trials measuring the

analyte concentration at the MDL must be significantly different from zero analyte concentration. The procedure of determining the MDL contains the following steps:

-MDL is first estimated.

-samples containing the analyte at the estimated MDL are analysed.

- on the basis of the standard deviation of the seven replicates measurements the MDL is computed as:

$$MDL = t_{(N-1,1-\alpha=.99)} * S$$

where S is the standard deviation of replicate determinations at a fixed concentration and $t_{(N-1,1-\alpha=.99)}$ is the student´s t value for a one-tailed test at the 99% confidence level with N-1 degrees of freedom.

If initial estimates of the MDL are not proximate to the true MDL, the calculated MDL will be much in error. This fact can be easily tested knowing that the estimated MDL is equal to the calculated MDL, if the 95% confidence interval of the calculated MDL contains the MDL value. Detection limit is usually provided by the instrument manufacturer. However this method assesses the MDL to an analytical method and to a sample matrix.

4. RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents results under three main sections: analytical technique evaluation, laboratory experiments, and field experiments. A series of chromatograms and spectra pertinent to the identification and confirmation of chlorophenols is presented in Appendix II.

Laboratory leech experiments are discussed before the leech field experiments but controlled laboratory experimental conditions were established from preliminary field results.

Special laboratory experiments are presented separately at the end of the chapter. These experiments were designed to take place concurrently with field experiments and their parameters were set in such a way that the field experimental results would be easier to interpret. These special experiments are part of the recommended methodology if leech bioconcentration capability is to be used for monitoring purposes.

A general discussion of the experimental data is presented at the end of each section and it constitutes the basis of the final conclusions presented in the following chapter.

4.2 Analytical Technique. Reproducibility and Technique Evaluation.

All the data gathered to evaluate the analytical technique used to quantify the chlorophenols are presented in Table 4.1.

As expected, lower detection limits were obtained for the higher chlorinated compounds of the chlorophenol family, with values as low as 2 ppt for 2,4,5-TCP, 2,3,4,6-TTCP and PCP. Krijgsman and Van de Kamp(1977) report the limit of detection for the pentachlorophenol acetate at 1 pg(equivalent to 1 ppt).

In our study we assumed that the derivatization reaction was 100% complete and reproducibility of the derivatization referred solely to the technique used. Derivatization reproducibility calculated at two levels of concentration, namely 0.1 ppm and 1 ppm, was better at the higher concentration level, with the exception of PCP were a higher deviation was found for the 1 ppm standard.

The yields of PCP acetate from the acetylation of PCP on different stationary phases were determined by Chau and Coburn (1974). They reported values higher than 93% for three different types of columns. The 6´x 1/4" OD glass column packed with 3.6% OV-101-5.5% OV-210 on 80-100 mesh Chromosorb had characteristics similar to the capillary column used.

Average recoveries from water were all in the 90 % range with standard deviations within ± 10%. The initial evaluation of the recovery from water was done using dilution water. Subsequent recovery values have been determined for the river water samples at the time of each field experiment. Recovery values obtained

TABLE 4.1

REPRODUCIBILITY AND TECHNIQUE EVALUATION(1)

Compound	Detection Limit (ppt)	Recovery		Reproducibility		
		Water(2)	Leeches(3)	Derivatization(4) 0.1 ppm	Derivatization(4) 1 ppm	Injection(5) (accuracy)
2,4-DCP	10	100 ± 9.6	83 ± 12.4	± 10.6	± 5.7	100 ± 8.4
2,4,6-TCP	10	98 ± 8.3	99.7 ± 6	± 9.9	± 5.0	100 ± 4.6
2,4,5-TCP	2	95.6 ± 6.0	92 ± 8.5	± 14.5	± 6.0	100 ± 11.5
2,3,4,6-TTCP	2	96.5 ± 7.3	87.5 ± 0.7	± 10.3	± 9.0	100 ± 4.8
PCP	2	95 ± 6.8	84.7 ± 7.5	± 12.3	±18.8	100 ± 7.7

(1) All values expressed as percent unless indicated.
(2) Water samples were spiked with 1 ppm mixture of the 5 CPs; mean and st. dev. calculated for n=4.
(3) Leeches were spiked with 1 ppm mixture of the 5 CPs; mean and st. dev. calculated for n=4.
(4) Derivatization reproducibility was determined at 2 concentrations. A mixture of the 5 CPs at 0.1 ppm and at 1 ppm was derivatized . The standard deviation was calculated on the basis of 5 derivatized samples (n=5).
(5) This is the accuracy for one injection reported with 95% confidence. The accuracy is calculated on the basis of 12 injections (n=12).

for the river water were usually in the same range as those obtained for the dilution water. Chau and Coburn(1974) reported similar recoveries of PCP from distilled and tap waters(88% average). Lake Ontario water spiked by them resulted in recoveries from 84 to 93 %. Recovery of chlorophenols from the extraction-acetylation step was 80-100% in the work of Krijgsman and Van de Kamp and 90% or better in the work of Metcalfe et al.(1984), which makes our results very comparable.

Recovery values lower than the above mentioned range were obtained for the river water collected during the experiment between April 18 and April 25,1985. The river water contained a higher sediment load at that time of the year and the recovery values were 52% for TTCP and 44% for PCP(suspended solids load in the Fraser River water varies from 18 to 118 ppm, with the highest values during freshet (Drinnan and Clark, 1980). For that particular run, corrections were applied to the concentrations of chlorophenols in the water.

Average recoveries from leeches were also very good with over 80% recovery and a maximum of 12 % deviation from the mean for the DCP. The fact that the derivatized extracts did not need to be further concentrated enhanced the recovery both from the water and the leech matrix.

Reproducibility of results as a function of injection technique, instrument parameters and sample vial condition was very good. The degree of accuracy obtained by using one injection per sample was in the same range as other statistical characteristics of the analytical technique therefore quantitative determination of chlorophenols was done on the basis

of one injection per sample.

4.2.1 Chromatograms of Acetylated Chlorophenols: Standard Mixtures, Water and Leech Samples.

The working range of concentration for the standard solutions was found to be 0.01 to 1 ppm. This range seemed to contain the field chlorophenols concentrations as well as the chlorophenols levels bioconcentrated in the leech during the exposure experiments. Sharp, symmetrically and completely separated peaks of all the chlorophenols were obtained over all this range. Fig. 4.1 presents a chromatogram of a standard mixture of all the congeners in the chlorophenol family , at 0.01 ppm concentration. Corresponding to their elution times the peaks are identified in the figure footnotes.

As the sensitivity of the detector responds to the number of chlorine atoms in the molecule, one can see that the higher peaks correspond to the tri, tetra and penta chlorophenols and the very short peaks correspond to the mono-chlorinated chlorophenols.

Chromatograms at a lower concentration level, such as 0.001 ppm, contained only peaks of the higher chlorinated chlorophenols. In the case of 2,4 and 2,5-DCP which elute together and were not separated in the chromatogram at 0.01 ppm, a complete separation was obtained at lower concentration of the standard mixture. Since at 0.001 ppm and lower, the monochlorinated chlorophenols do not appear on the chromatogram, the chromatogram obtained at 0.01 ppm was chosen for illustrative purposes.

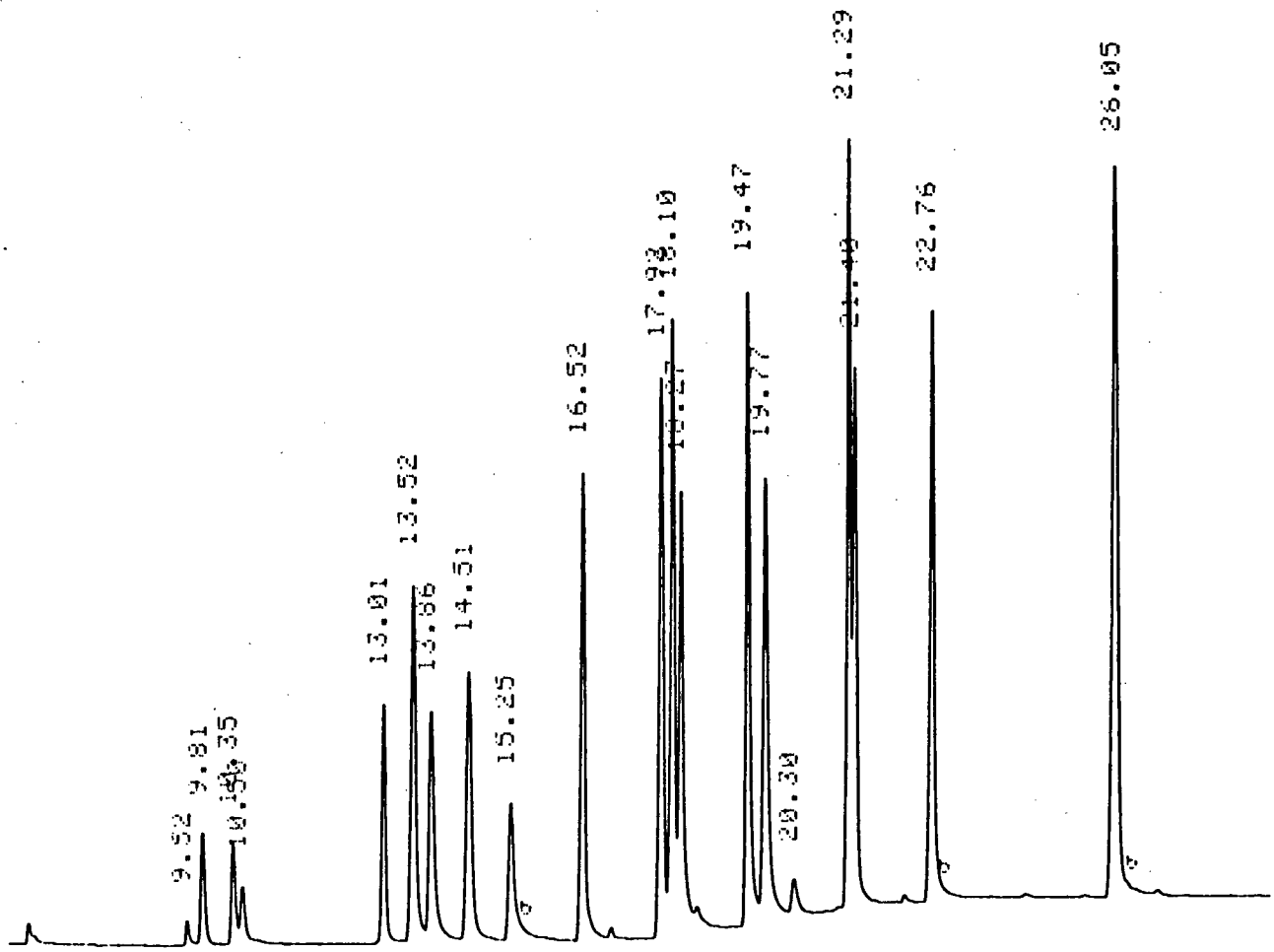

Fig. 4.1. Chromatogram of acetylated chlorophenols. All the 19 chlorophenol congeners were separated in this run with the exception of 2,4 and 2,5-DCP which eluted together. Concentration of the standard mixture was 0.01 ppm of each congener. Peaks identification according to retention time:

9.81 min : 2-CP	10.35 min : 3-CP
10.50 min: 4-CP	13.01 min : 2,3-DCP
13.52 min: 2,4-DCP	13.52 min : 2,5-DCP
13.86 min: 2,3,5-TCP	14.51 min : 2,6-DCP
15.25 min: 2,3,4-TCP	16.52 min : 2,4,6-TCP
17.92 min: 3,5-DCP	18.10 min : 2,3,6-TCP
18.27 min: 2,4,5-TCP	19.47 min : 3,4-DCP
19.77 min: 3,4,5-TCP	21.29 min : 2,3,5,6-TTCP
21.40 min: 2,3,4,6-TTCP	22.76 min : 2,3,5,6-TTCP
26.05 min: PCP	

If one compares the illustrative chromatogram(Fig. 4.1) with the chromatogram from Appendix I (Fig.I.1) obtained by Krijgsman and Van de Kamp(1977) one can notice the high level of resolution achieved in both. One difference is that their chromatogram contains only 13 congeners and they are at various concentrations in order to keep the peak height uniform. They do report the relative retention times of all the 19 congeners, however no chromatogram accompanied their results to verify complete separation of all the compounds.

For the laboratory controlled experiments a mixture of only 5 chlorophenols was used. Their chromatogram is represented in Fig.4.2. The level of concentration used in this run was 1 ppm and the peaks are in order of elution: 2,4-DCP(14.40 min) 2,4,6-TCP(17.46 min) 2,4,5-TCP(19.10 min) 2,3,4,6-TTCP(22.21 min) and PCP(27.25 min). One can see that the size of the peaks corresponding to this concentration is much larger than for the 0.01 ppm standards(see Fig. 4.1). Most sample concentrations were found to be in the neighbourhood of 0.1 ppm, the intermediate concentration(see Fig. 4.3).

A typical chromatogram of a Fraser River water sample spiked with a mixture of chlorophenols was very similar to a standard solution chromatogram(see Fig. 4.4). The extraction and acetylation steps manage to clean up the water samples of most interfering and unwanted compounds. One can also see that the retention times of the chlorophenols are very reproducible even when run on different days.

A typical water blank chromatogram is represented in Fig. 4.5. Four peaks of interest were identified: 2,4,6-TCP;

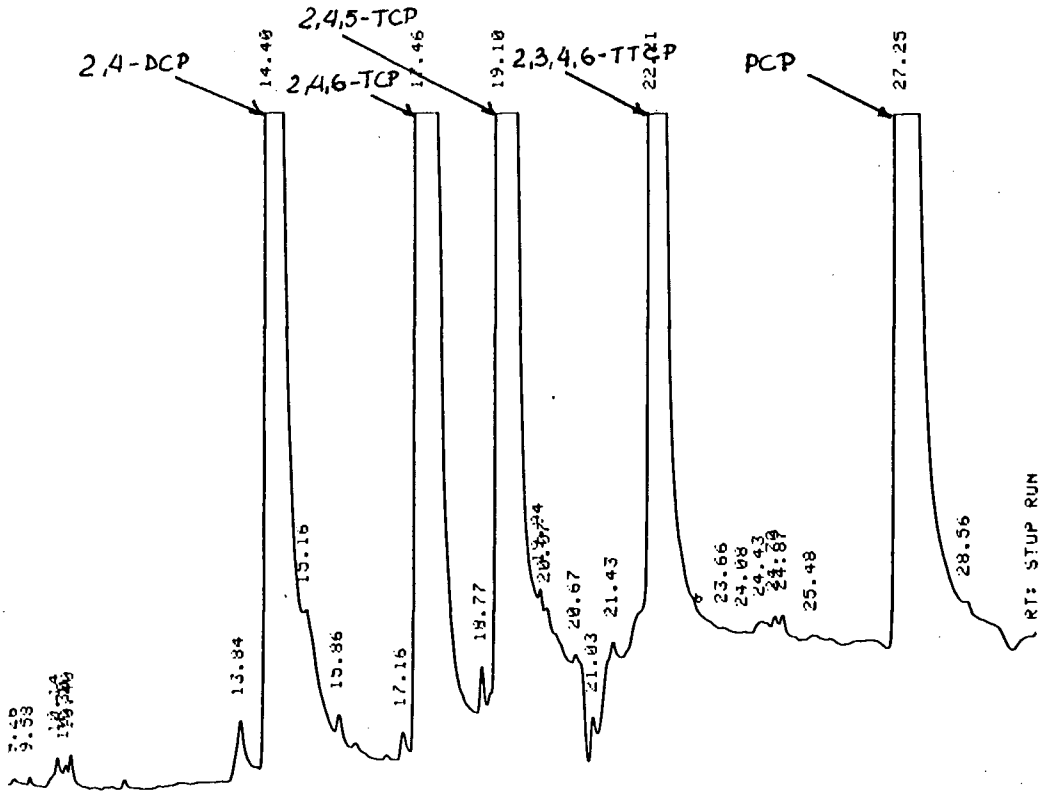

Fig.4.2. Chromatogram of a 1 ppm standard mixture of 5 chlorophenols.

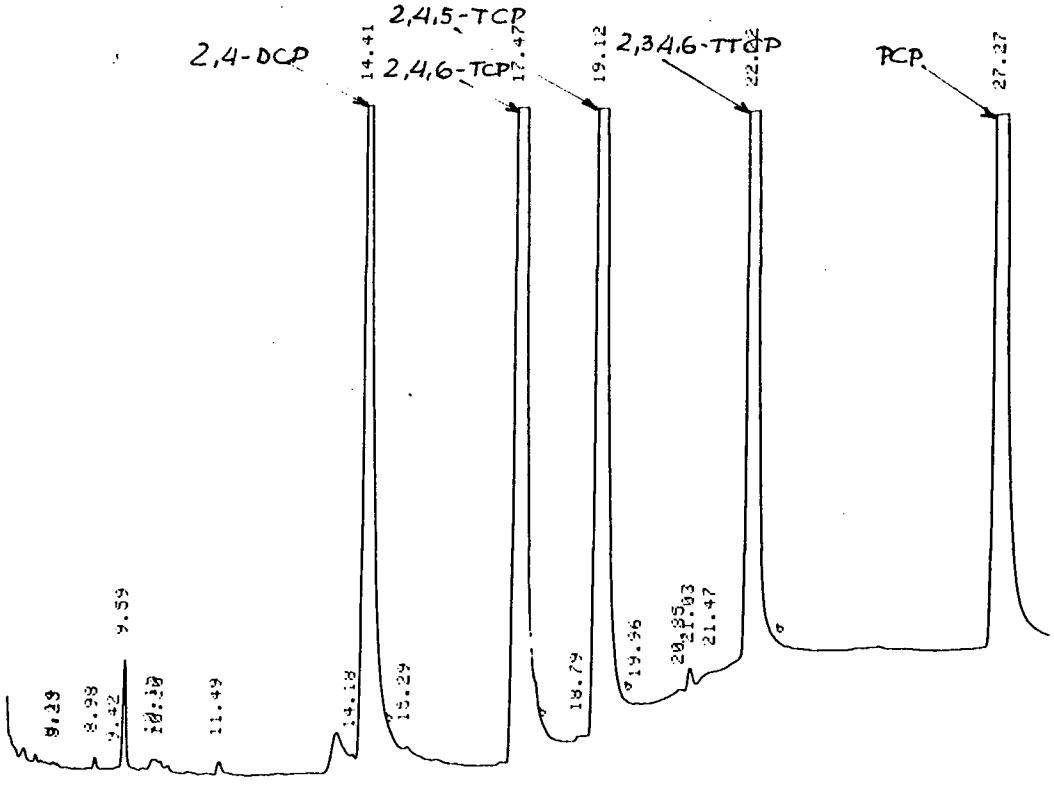

Fig.4.3. Chromatogram of a 0.1 ppm standard mixture of 5 chlorophenols.

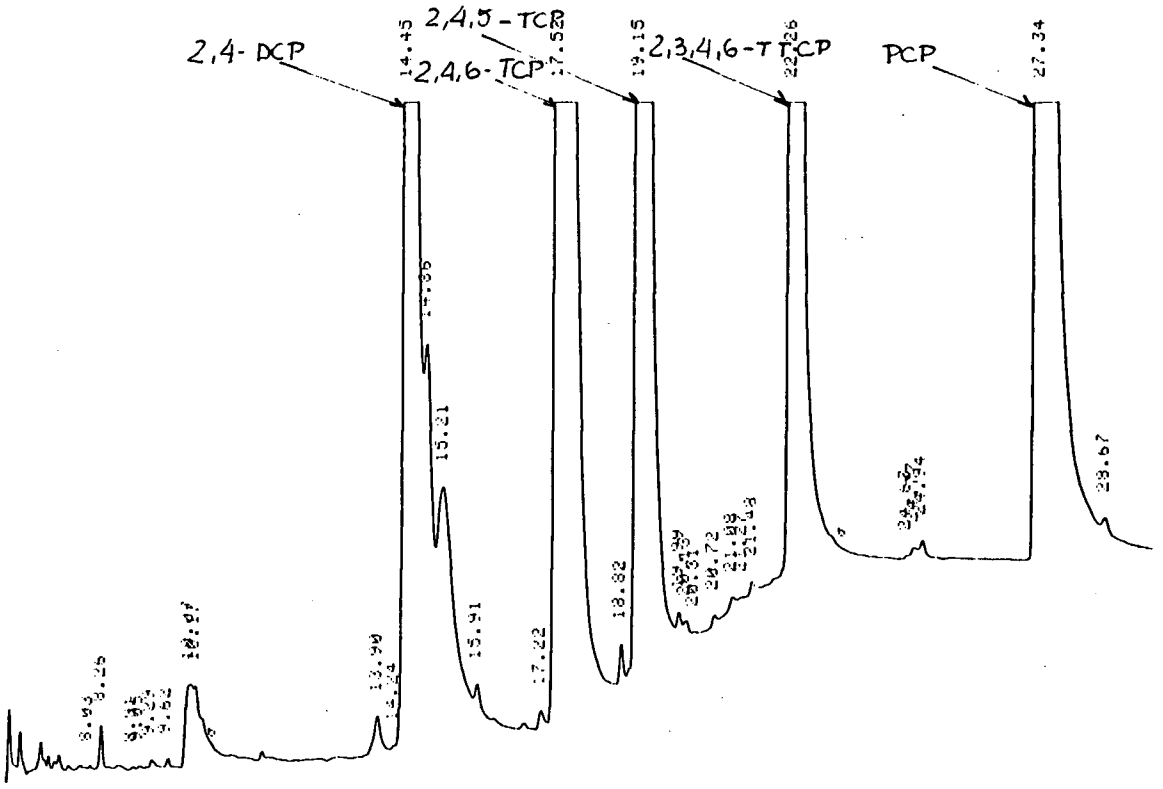

Fig.4.4. Chromatogram of a Fraser River water sample spiked with a mixture of 5 chlorophenols(same chlorophenols as in the standards from Fig.4.2 and 4.3. Spiking level: 10 ppb.

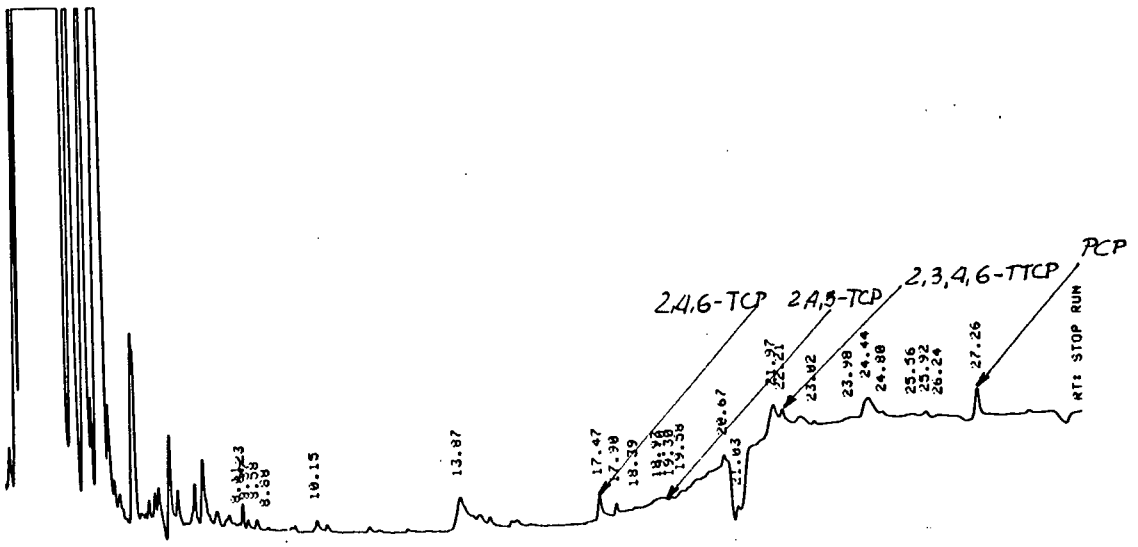

Fig.4.5. Chromatogram of a water blank.

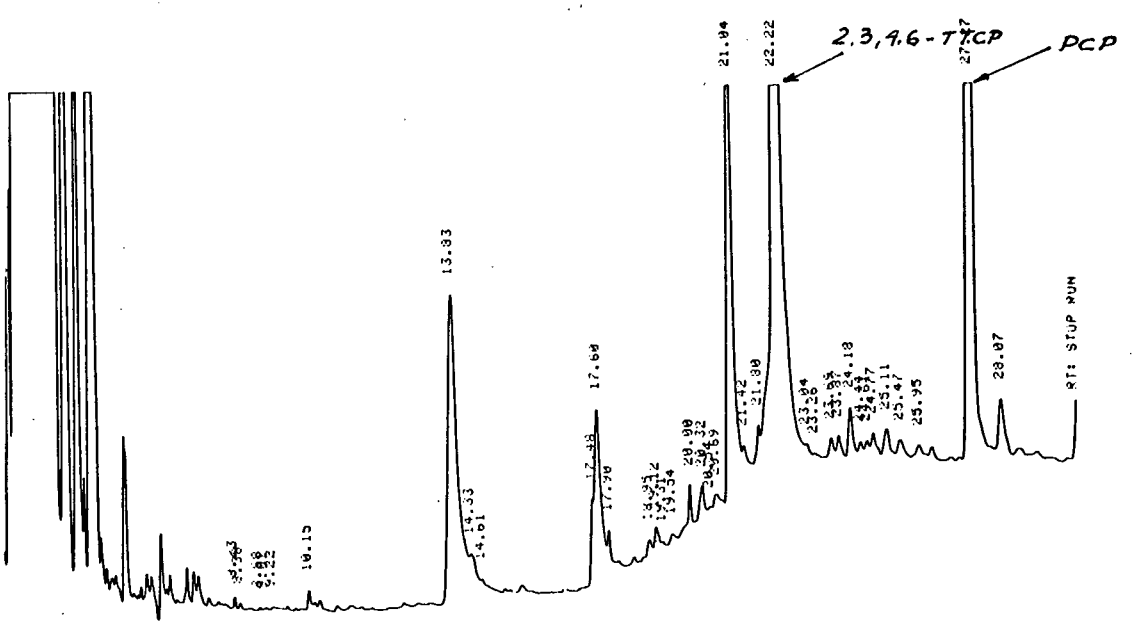

Fig.4.6. Chromatogram of a Fraser River water sample. Date of sampling: September 13, 1985.

2,4,5-TCP; 2,3,4,6-TTCP and PCP. Only the TTCP and PCP were quantified at 8 ppt and 6 ppt respectively. The other identified peaks were present in concentrations below the detection limit.

Generally, the chlorophenol values for water blanks were in the neighbourhood of 10 ppt. These values were considered as background concentration and substracted from the values of chlorophenols found in the river water samples.

A chromatogram of acetylated CPs extracted from a Fraser River water sample is represented in Fig. 4.6. Peaks of interest are TTCP(at retention time: 22.22 min) and PCP (at retention time: 27.27 min). Quantification of those peaks gave 2.268 ppb TTCP and 0.345 ppb PCP. This water sample was concentrated 120 times by the extraction technique prior to the GC analysis.

A chromatogram of acetylated CPs extracted from a leech is presented in Fig. 4.7. This particular leech had been exposed to Fraser River water for 6 days in the interval September 12-18/1985. The leech extract was diluted 10 times prior to the GC analysis. The leech chromatogram is much more complex than the chromatogram of a water sample taken during the same time interval(Fig. 4.6).

A typical chromatogram of the acetylated CPs extracted from a control(blank) leech is presented in Fig. 4.8. If compared with a water blank chromatogram (see Fig.4.5), the control leech chromatogram contains very many peaks. In the chromatogram from Fig.4.8, four peaks of interest were identified and their quantification gave the following values: 47 ppb for 2,4-DCP; 24 ppb for 2,4,5-TCP; 36 ppb for 2,3,4,6-TTCP and 76 ppb for PCP.

The levels of chlorophenols found in the control leeches

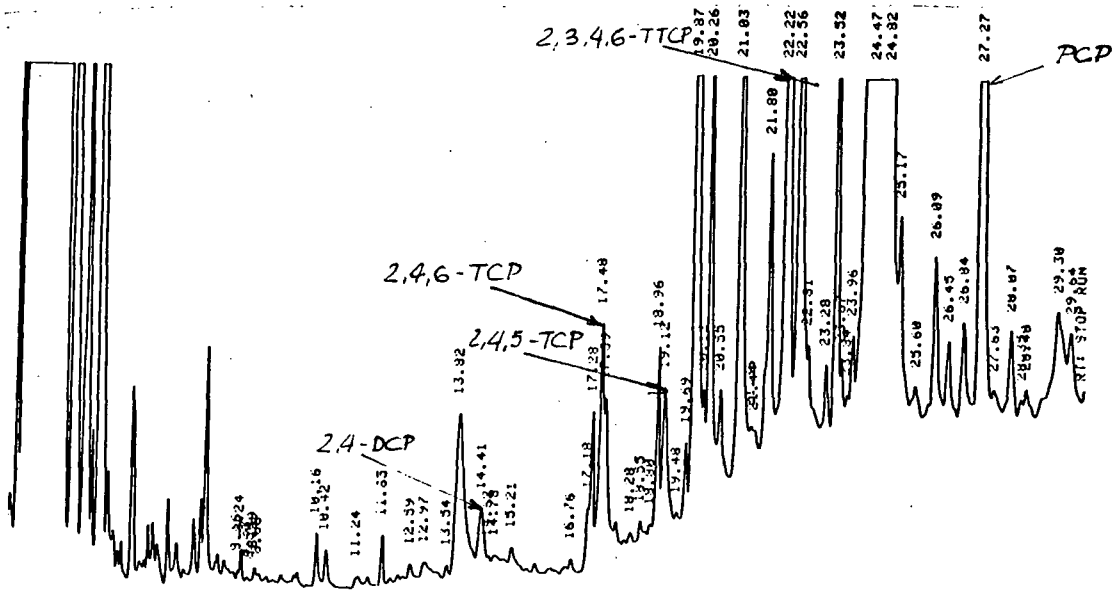

Fig.4.7. Chromatogram of a leech exposed for 6 days to the Fraser River water.

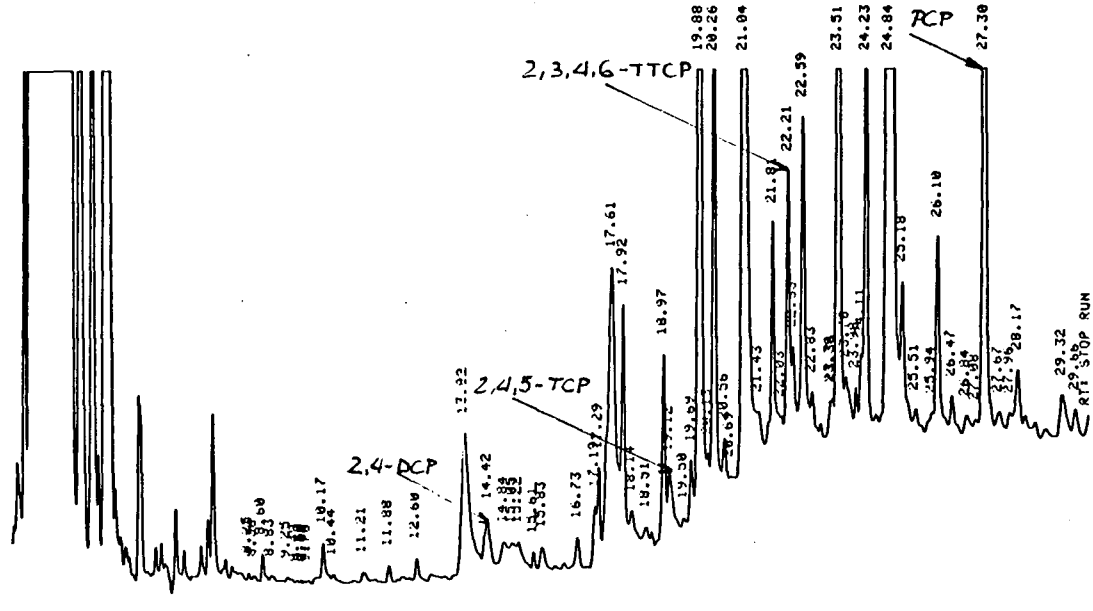

Fig.4.8 Chromatogram of a leech blank.

were always high, and a viable explanation for these high background levels is still to be found. The peaks were confirmed by GC/MS analysis to be chlorophenols and not some other compounds with similar retention times.

Control leeches were collected together with all the other leeches from Kentucky Lake and analysed at arrival in the Vancouver laboratory. The lake is located in a remote area and should be contaminant free, but as other researchers have reported, low background concentration of CPs were present in pristine environments(Pierce et al., 1977). They reported a 0.5 ppb of PCP in the water column of their control pond. If a similar or even lower concentration was present in Kentucky Lake, given the high bioconcentration capability of leeches, the high level in the control leech could be explained. Unfortunately, the Kentucky Lake water was never tested for chlorophenols. The consistency of CPs in the control leeches made it necessary to accept this contamination as an experimental condition(average and standard deviation of the CPs found in six control leeches were 16 ± 13 ppb for 2,4,5-TCP, 25 ± 8 ppb for TTCP and 91 ± 36 ppb for PCP).

The only step taken to improve the results reliability was to select the size of the control leeches. Size selection was done by keeping the control leech size in the same size range with leeches used in the experiment for which the control leech was to be considered. The size range and influence of size on the bioconcentration capability will be discussed further in a separate section.

4.2.2 Identification and Confirmation of Chlorophenol Acetates Through Mass Spectrometry

All the illustrative spectra pertinent to this section are contained in Appendix II, to which references are made throughout the text.

As outlined in the previous chapter, three methods of mass scanning were used for identification and confirmation of chlorophenols in various sample matrices: electron impact, positive ion chemical ionization and single ion monitoring.

4.2.2.1 Electron Impact GC-MS Spectra

Standard mixtures used for this method were scanned and spectra of the acetylated derivatives of the chlorophenols present in the standards used were filed in the spectral library of the Applied Science GC-MS laboratory.

Fig. II.1 presents a chromatogram of a 100 ppm standard mixture containing: 2-CP(mol wt=170); 2,4-DCP(mol wt=204); 2,4,6-TCP(mol wt=238); 2,3,4,6-TTCP(mol wt=272); PCP(mol wt=306). Fig. II.2 to II.6 present the EI spectra of each acetylated chlorophenol in this standard.

A leech extract was scanned by this method and a tentative identification of unknown peaks was made. Fig.II.7 presents the chromatogram of a leech extract which was exposed to a standard mixtures of chlorophenols for 7 days(2-CP; 2,4-DCP; 2,4,6,-TCP; 2,3,4,6-TTCP and PCP). Three other peaks were identified as dibromophenolacetate, tribromophenolacetate and underivatized

pentachlorophenol. No explanation was found for the presence of the brominated chlorophenols in the leech matrix. Underivatized pentachlorophenol indicated that this particular phenol was not exhaustively derivatized during the preparation step, however, the peak was very small in comparison with the derivatized chlorophenol peak.

4.2.2.2 Positive Ion Chemical Ionization Spectra

The same standard mixture was used for this scanning method. Fig. II.8 presents a typical total ion chromatogram of the standard mixture and Fig. II.9 to II.13 present the spectra of each individual acetylated chlorophenol obtained through this method.

The ion source pressure used was 0.2 x 10-4, using methane as ionizing gas, and temperature programming had a constant rate of 8 C/min from 75 C to 280 C.

4.2.2.3 Single Ion Monitoring Spectra

Parameters used for this method are contained in Table II.1 from Appendix II. The method was applied to various samples such as leech extracts, Fraser River water samples and standard mixtures. A semiquantitative analysis of the samples was also tried. Forty-five picograms appeared to be the detection limit of the method.

Fig. II.14a to II.14e present the chromatogram and the spectra from a leech extract, and Fig. II.15a to II.15e present

the chromatogram and the spectra pertinent to a Fraser River water sample.

A control(blank) leech extract was analysed through this method of increased sensitivity and the presence of chlorophenols at high levels was confirmed. This confirmation was necessary for the assessment of the background concentration in all the leech experiments.

4.3 Laboratory Leech Experiments

A series of laboratory experiments was set up at the beginning of the experimental work in order to determine the range of concentrations to be used to make the exposure solutions. These laboratory data, together with the levels of chlorophenol found in the Fraser River during the preliminary grab sampling trips, constituted the basis to establish the parameters for the laboratory controlled experiments.

4.3.1 Preliminary Leech Exposures

The preliminary exposures covered three areas of importance to help set the experimental parameters, namely, 24 hour exposure, depuration and pH influence on bioconcentration activity.

4.3.1.1. 24 Hour Bioconcentration Ratios

The concentration of chlorophenol found in leeches after 24 h exposure was divided by the solution concentration in order to obtain the bioconcentration ratios. The term of bioconcentration factor was avoided since the equilibrium state was not reached in such short time and it would have been improper to use the term of bioconcentration factor if its definition was respected(see section 2.3.1).

The results of the 24 h exposure tests at various chlorophenol concentrations are summarized in Table 4.2.

TABLE 4.2

CONCENTRATION RATIOS IN LEECHES AFTER 24 HOUR EXPOSURE

Compound	Exposure solution concentration		
	10 ppb	20 ppb	30 ppb
2,4-DCP	40	40	54
2,4,6-TCP	58	50	79
2,4,5-TCP	60	72	88
2,3,4,6-TTCP	60	72	100
PCP	60	88	135

Note:solutions contained 10 ppb, 20 ppb and 30 ppb of each of the CPs listed in the composition ; average leech weight = 1.35 g, standard deviation = 0.37 g ; experimental temperature = 22 C. pH = 6.5.

Only one leech was exposed at each concentration of the solutions used for the exposure tests and the results have no statistical value. However, one can see that the general trend is to exhibit higher bioconcentration ratios as the concentration of the exposure solution is increased and the trend is more evident for the higher chlorinated chlorophenols(i.e. TTCP and PCP).

For the same concentration of the exposure solution the lowest ratio corresponds to the least chlorinated chlorophenol. This is expected since it is known that the bioconcentration factors relate positively to the Po/w values, which increases with the number of chlorine atoms in the molecule(Konemann and Musch, 1981).

No attempt was made to determine if these results fit equations developed by various researchers(Saarikovski and Viluksela, 1982) since the values determined in this preliminary experiment were not true equilibrium bioconcentration factors.

Exposure of leeches to a solution of 1 ppm TTCP (1000 ppb) for 24 h gave a bioconcentration ratio of 100. This would indicate that at higher exposure concentration, the bioconcentration activity levels off and does not continue to increase with increased concentration. This behaviour follows the theory developed by Majori and Petroni(1973) who stated that the concentration in biota cannot exceed a certain maximum concentration(concentration of binding centers). They also stated that the rate of uptake is proportional not only to the concentration of the chemical in water but to the concentration of free binding centers.

The same experiment was repeated for 1 ppm PCP , but the PCP concentration proved to be deadly to the leeches and no conclusions were drawn from that experiment, except that the dead leeches seem to loose the bioconcentrated chlorophenol once they die(very low levels of PCP were found in the dead leeches). The same observation was made on other occasions when dead leeches were analysed after various exposure experiments when some deaths occurred . It was therefore decided that chlorophenol concentrations in dead leeches were never to be used . The phenomenon of chemical release upon death by leeches was also observed by J.H. Carey from Canada Centre for Inland Waters,Burlington, Ontario (personal communication).

4.3.1.2 Depuration Tests

The depuration experiments were carried out for one week. Leeches exposed in Fraser River for one week were used for the depuration experiment. At the end of the exposure time, three of the initial five leeches were analysed immediately after collection and two underwent depuration. At the end of 7 day exposure in clean water(dilution water, which was changed every day), the concentration of chlorophenol in the leech matrix was at the same level as found in the leeches analysed right after the 7 day exposure in the river (see Table 4.3).

The depuration rate constant was not determined since more data would have been required along with an extended period of depuration. Depuration rate constant determination was beyond the scope of this research. Personal communication with J.H Carey,

TABLE 4.3

CHLOROPHENOLS IN LEECHES EXPOSED IN THE FRASER RIVER ESTUARY

Sampling Site	Location	Date (1984)	Exposure[1] /Depuration	TTCP[2] µg/g	PCP[2] µg/g
2	Kerr St.	Oct. 4-11	7/0	0.476	0.226
		Oct. 4-11 /Oct. 11-18	7/7	0.540	0.311
5	Mitchell Is.	Oct. 4-11	7/0	2.946	0.846
		Oct. 4-11 /Oct. 11-18	7/7	3.733	0.771

(1) Exposure and depuration time in days.
(2) Average values in µg/g wet weight, n=2 for leeches that underwent depuration, n=3 for leeches analyzed immediately after collection.

confirmed a very slow rate of depuration for chlorophenols in the case of leeches(initial concentration of the chlorophenol in the leech was reduced by 50 percent in approximately 6 months). For this research which had the goal of using leeches for relatively short(seven days) exposure experiments as pollution biointegrators, the estimate of depuration for one week was satisfactory.

The determination of the depuration rate constant(K2) would have been necessary if the bioconcentration factor was to be calculated by the relationship:

$$BF = K1/K2$$

Determination of BF was chosen to be made by direct measurements of the partitioning at the steady state, which conforms to the equation:

$$BF = C(organism)/ C(water)$$

4.3.1.3 pH Experiments

The last of the preliminary experiments determined the effects of pH on bioconcentration. As with all preliminary experiments, it served to set the parameters for more detailed experiments. Only the general trend of the bioconcentration process was monitored. The experiment was carried out to validate the theory behind the pH influence upon bioconcentration under our experimental conditions. The results are presented in Table 4.4

TABLE 4.4

CONCENTRATION RATIOS AS A FUNCTION OF pH FOR 24 HOUR EXPOSURES

Compound	pH		
	9.2	7.5	5.0
2,4-DCP	42	88	73
2,4,6-TCP	-	57	90
2,4,5-TCP	13	51	115
2,3,4,6-TTCP	13	73	115
PCP	29	71	125

Note: experimental temperature= 4C; solution at 10 ppb of each CPs; the multipliers are calculated from the concentrations found in leeches at the end of 24 hours, expressed in ug/g wet weight, divided by the solution concentration taken as constant over the exposure period; average leech weight = 0.079 g, standard deviation = 0.0061 g.

Except for 2,4-DCP, the trend shows an increase in bioconcentration with the decrease in pH. The theory developed by Konemann and Musch (1981) on the pH influence upon the toxicity of CPs states that the nonionic form is preferentially concentrated in the tissues. Since lipophilicity is a characteristic of the nonionic form , partitioning into the biota is favored under low pH conditions. The nonionic molecules seem to penetrate the epithelial tissues easier than the charged molecules.(see Chapter 2 for more details).

All mathematical relationships between BF and Po/w, developed by various researchers, fit the general form:

$$\log BF = a \log Po/w + b$$

where a and b are constant.

pH influences the solubility in water of the chlorophenols as it is known that the nonionic form is far less soluble than the ionic form (NRCC,1981). Increased solubility in water will

adversely affect the value of Po/w and indirectly change the value of BF since the two parameters are directly proportional to each other.

Saarikovski and Viluksela(1982) developed a relationship for the chlorophenol series linking toxicity directly to dpKa.

$$\log (1/LC50) = 0.36\ dpKa + 0.69$$

where dpKa = pKa(phenol) - pKa(substituted phenol)

Since the 1/LC50 values can be substituted with BF values(Kobayashi et al., 1978) a direct proportional relationship is obtained between BF and dpKa of various chlorophenols. dpKa's are higher for TTCP and PCP(4.59 and 5.34 respectively) than for the other chlorophenols(Saarikovski and Viluksela,1982) and that explains the higher bioconcentration ratios obtained in their case.

A decrease in pH affects the bioconcentration of the higher chlorinated phenols more, since they have lower values of pKa. In the case of 2,4-DCP which has a pKa value of 7.90, our results were a little confusing because bioconcentration was higher at pH 7.5 than at pH 5.0. In this pH range, 2,4-DCP is mostly undissociated, and one would expect somewhat comparable concentration ratios at the two pHs. What can be concluded from the results is that this phenol is not influenced in the same manner by pH as the other chlorophenols. The unusual behaviour of 2,4-DCP was consistent with other experiments that will be presented.

The initial pH of the dilution water was 7.5. The pH gradually decreased to 6.5 after a day of exposure which was probably attributable to the low buffering capacity(alk. = 50

mg/L $CaCO_3$). For longer experiments the pH varied between 6.0-6.5. Additional buffering capacity was avoided to prevent any stress on the leeches. However, since the median values of pH in the Fraser River Estuary varies from 7.0 to 8.0 (Drinnan and Clark, 1980) these experimental conditions were considered satisfactory to allow comparison to field conditions.

4.3.1.4 Summary of Results

The 24 h exposure experiment gave values for the bioconcentration ratios from 40 to 135. At the same concentration level, the higher chlorinated phenols exhibited higher ratios than the lower chlorinated phenols(88 for PCP as compared with 50 for 2,4,6-TCP, at a solution concentration of 20 ppb).

Higher concentration of the exposure solution seemed to increase the ratios. This phenomenon was observed however for moderate increases in concentration(a few times). At a drastic increase in concentration(50 fold) the bioconcentration ratios leveled off(in the case of TTCP the ratios were 72 at 20 ppb, 100 at 30 ppb and 100 at 1000 ppb).

The depuration rate was very slow for the system leech/ chlorophenol. It was assumed to be negligible for the duration of all the experiments which never exceeded one week.

Dead leeches released the accumulated chlorophenols and their CPs levels were never included in any experimental results.

The change in pH affected the bioconcentration as the theory predicted. Lower pHs lead to higher bioconcentration ratios and the effect of pH was more pronounced for the higher

chlorinated phenols with lower pKa values than for the other chlorophenols. pH should not have a direct influence on the bioconcentration of CPs during the field experiments since the Fraser River water pH is more or less constant. Any localized variation of pH is rapidly neutralized by the great dilution capacity of the river. The pH influence must be considered when laboratory experiments are set up. The pH should be kept constant and the field and laboratory pH should be similar if the results are to be compared.

4.3.2 Bioconcentration Factors Determination

As mentioned before, the bioconcentration factor should be calculated by determination of partitioning at steady-state. This method is by far the most accurate of the three methods used (one other method uses relationships between BF and Po/w and one uses the reaction rates constants; see Esser and Moser, 1980).

The major shortcoming of the direct measurement method is that it sometimes takes a long time to establish the steady state conditions.

4.3.2.1 Bioconcentration of Chlorophenols in a 7-Day Laboratory Experiment

The first laboratory experiment in which the bioconcentration factors of various chlorophenols were calculated was carried out for 7 days. A mixture of 5 chlorophenols was used at a concentration of 10 ppb each. Experimental temperature was

22 °C and the large black leeches(P.marmorata) were used. Average leech weight was 0.332 g with a standard deviation of ±0.257 g. Most of the graphical results(see Fig.4.9) are averages for n=2 and a few are for n=1, so the statistically significance of this test is very limited.

The purpose of this 7-day exposure experiment was to determine the pattern followed by leeches in the bioconcentration process as they were intended to be used for monitoring purposes and field exposure time never exceeded 7 days. The calculation of the bioconcentration factors was determined from the concentrations found in the leeches at the end of the exposure period. Fig.4.9 shows that steady state conditions were not quite reached after 7 days. Therefore, the values obtained for the bioconcentration factors could be in error and they were used with caution. Bioconcentration factors were 2155 for PCP, 2111 for TTCP, 1450 for 2,4-DCP and 1340 for 2,4,6-TCP ;2,4,5-TCP had an unexpected value high value of 2280.

Lower experimental temperatures used later lead to more accurate bioconcentration factors since the steady state was reached within the experimental time. The fact that at 22 °C steady state was not reached and that it was reached at lower temperatures seemed to indicate that the higher temperature delayed the steady state. This is contradictory to the observation made by Saarikovski and Viluksela (1982) about the accumulation of phenols by guppies. They stated that steady state was reached more rapidly at high temperature.

If one needs an accelerated test to determine bioconcentration factors, calculation using rate constants of

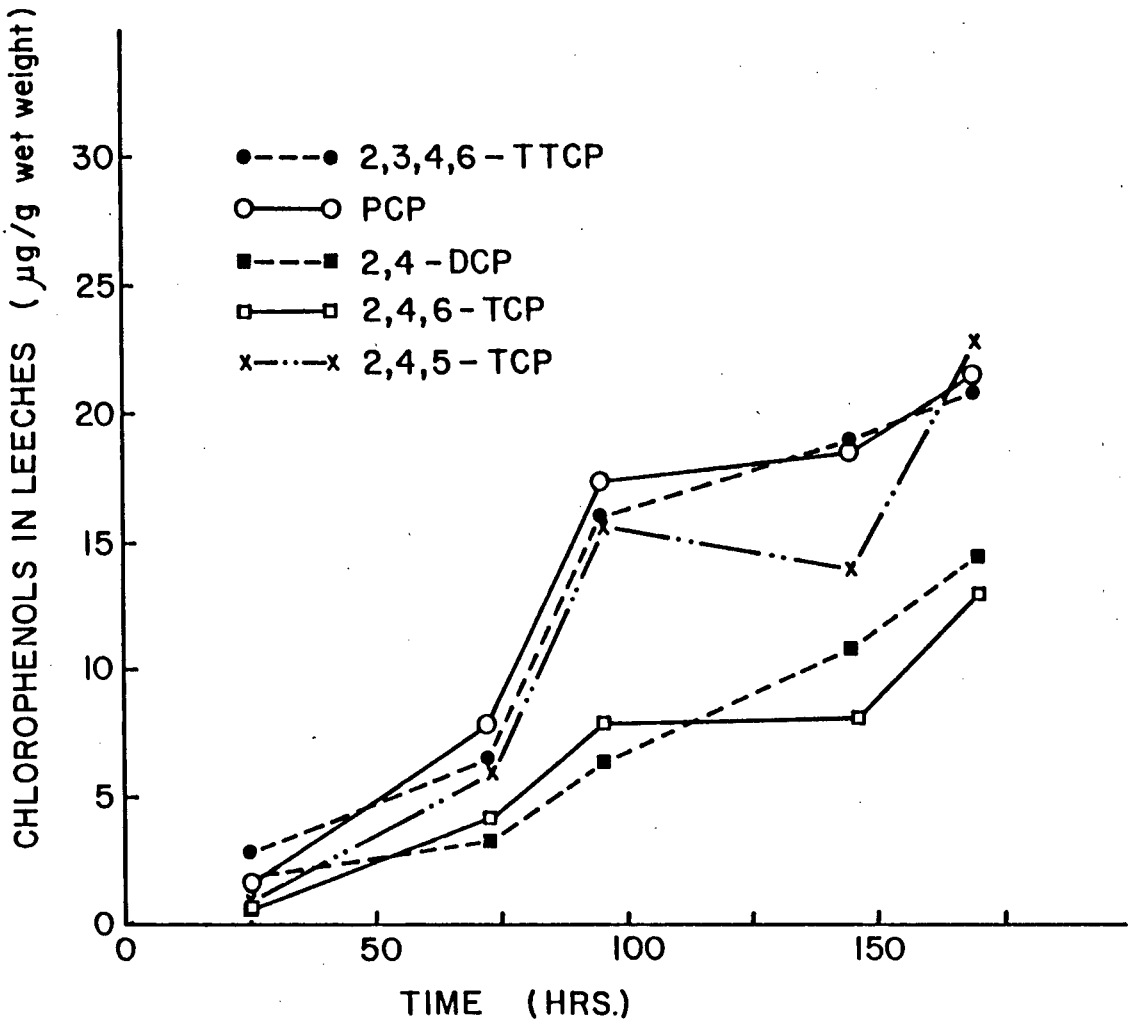

Fig.4.9 Bioconcentration of chlorophenols by leeches in a seven-day laboratory experiment. Experimental temperature was 22 °C. The leech species used was P. marmorata.

uptake and clearance is probably a better method(Branson et al.,1975).

4.3.2.2 Bioconcentration of Chlorophenols at Different Temperatures

The data obtained during these temperature experiments are graphically represented in Fig. 4.10, Fig. 4.11 and Fig. 4.12. The values represented are averages for n = 3. Most standard deviations were in the range of 10 to 15 % of the average values, however a few reached 20-25 %. The standard deviations were not presented on the graphs in order to keep the data presentation less cluttered. Fig. 4.13 shows the variability(standard deviation) that occurred in the case of TTCP. It is obvious that the largest variations were obtained at the highest experimental temperature of 22 °C. A similar pattern of variation of the standard deviation occurred for the other chlorophenols.

The size of leeches used in these experiments was kept as uniform as possible. Table 4.5 shows the size distribution used in the three experiments.

TABLE 4.5

SIZE OF LEECHES USED IN TEMPERATURE EXPERIMENTS

temperature	4 °C	12 °C	22 °C
average size(g)	0.826	0.669	0.845
S D (g)	±0.214	±0.160	±0.274

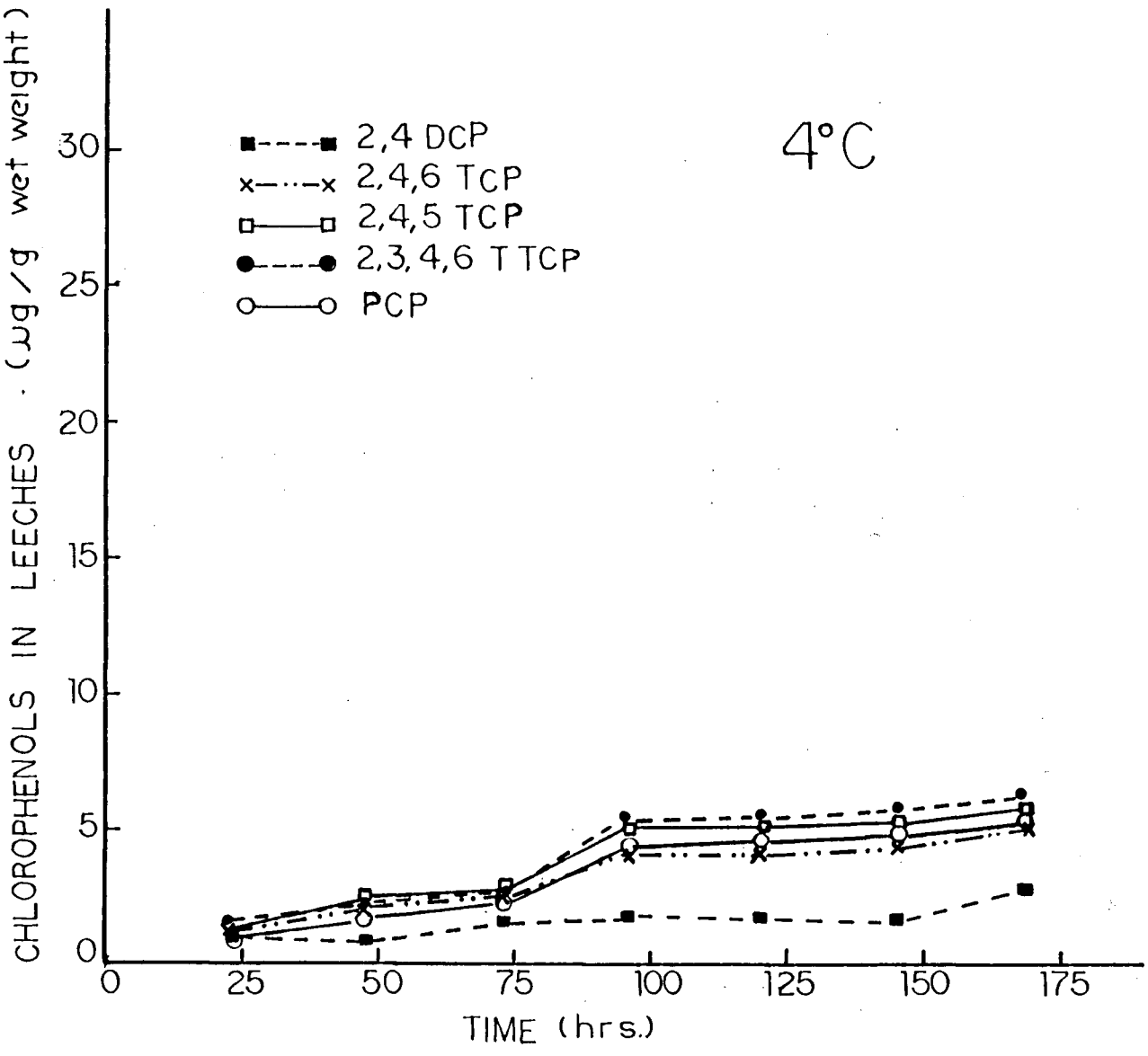

Fig.4.10 Bioconcentration of chlorophenols by leeches in a laboratory experiment at 4 °C. Concentration of the exposure solution was 10 ppb of each chlorophenol. The leech species used was N. obscura.

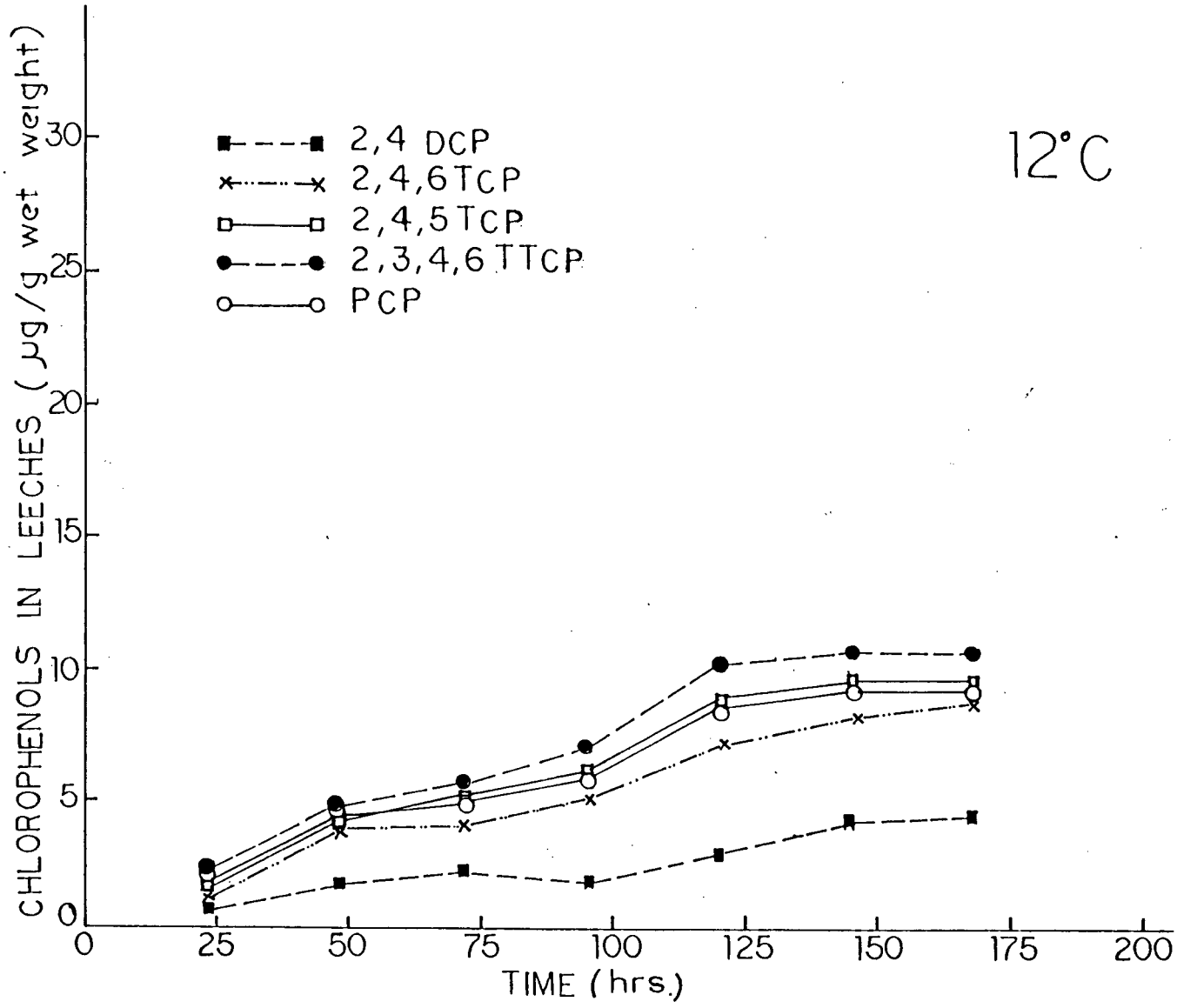

Fig.4.11 Bioconcentration of chlorophenols by leeches in a laboratory experiment at 12 °C. Concentration of the exposure solution was 10 ppb of each chlorophenol. The leech species used was N. obscura.

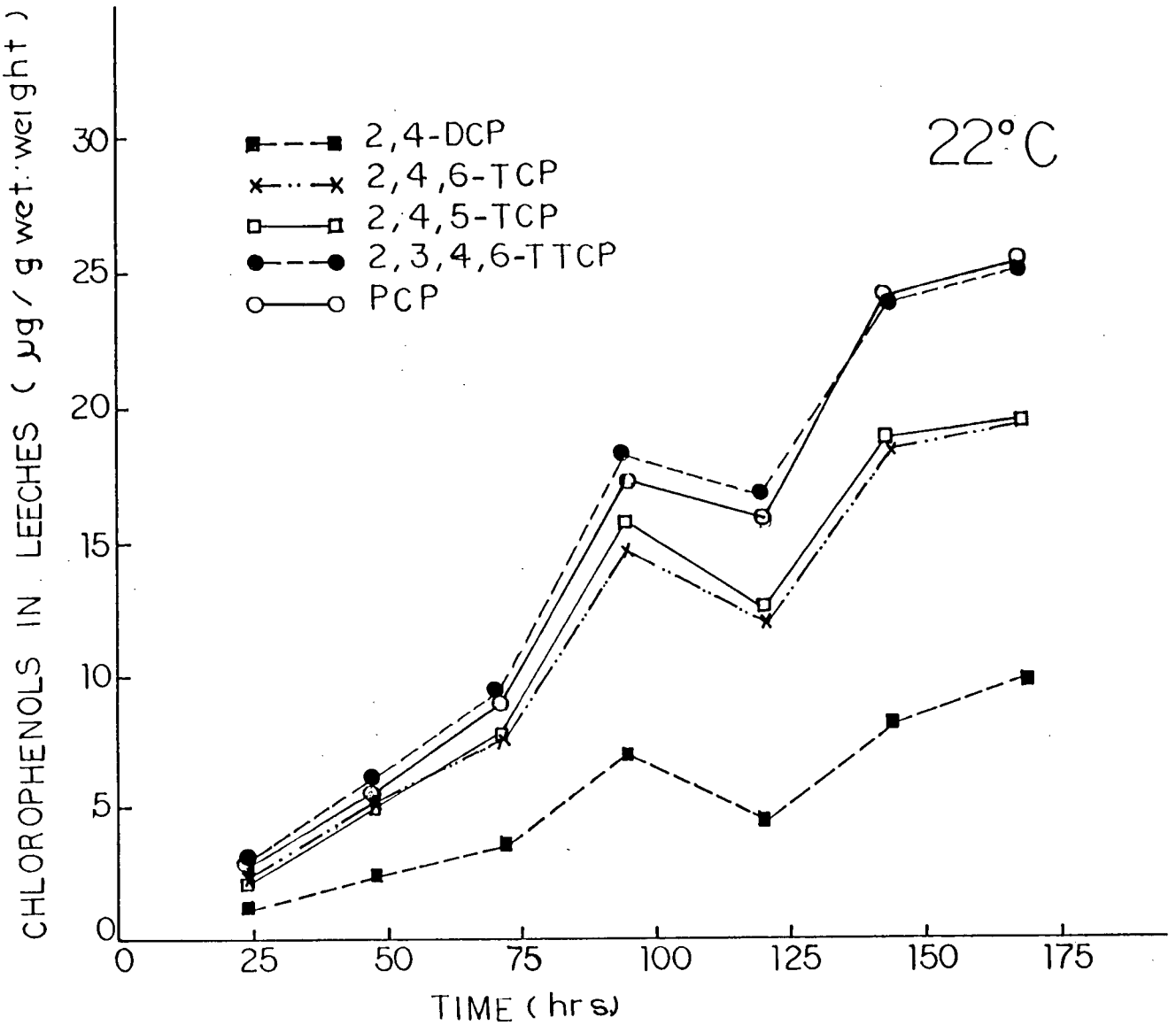

Fig.4.12 Bioconcentration of chlorophenols by leeches in a laboratory experiment at 22 °C. Concentration of the exposure solution was 10 ppb of each chlorophenol. The leech species used was N. obscura.

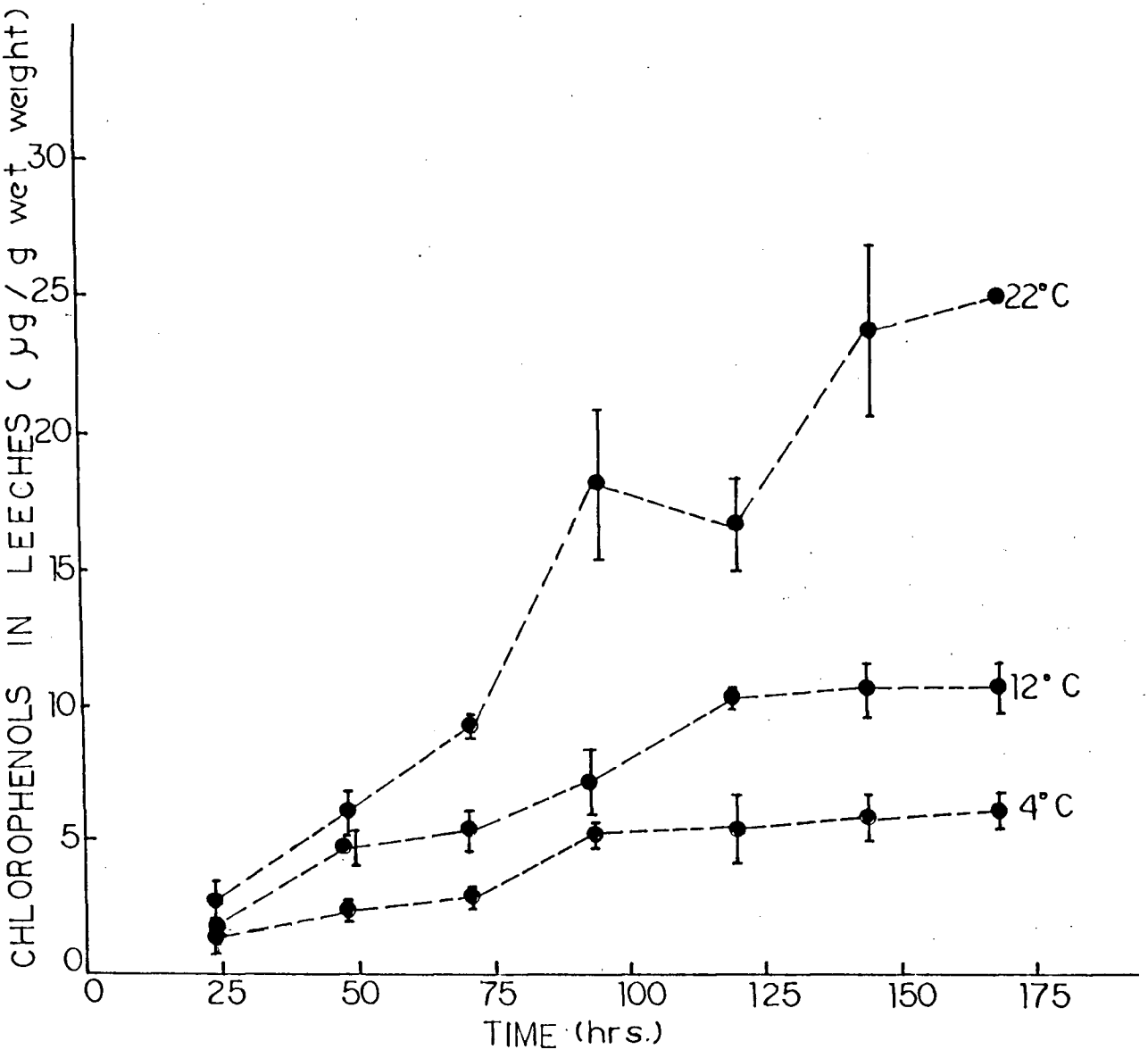

Fig.4.13 Bioconcentration of 2,3,4,6-TTCP by leeches at different temperatures. The data was compiled from the three laboratory experiments shown on Fig.4.10 to 4.12. The standard deviations are shown on this graph.

The temperature related experiments were the only experiments carried out using brown leeches(N. obscura). These leeches were used as substitute for the black leeches which were not available in sufficient quantity at that time of the year(April 1985). Subsequent testing proved that the difference in species did not have a major influence on the bioconcentration capability(see section 4.3.2.3); a uniform size was more important.

Table 4.6 presents the bioconcentration factors obtained at various experimental temperatures.

TABLE 4.6

EFFECT OF TEMPERATURE ON THE BIOCONCENTRATION OF CHLOROPHENOLS BY LEECHES(1)

Compound	Log Po/w	Bioconc. factor		
		Temperature		
		4 °C	12 °C	22 °C
2,4-DCP	3.08	282	424	980
2,4,5-TCP	3.80	593	969	1948
2,4,6-TCP	4.03	524	869	1988
TTCP	4.45	614	1059	2501
PCP	5.15	525	935	2508

At 4 °C(see Fig. 4.10) steady state adsorbtion of chlorophenols was reached relatively fast, namely, after 4 days of exposure. With the exception of 2,4-DCP, the bioconcentration factors were all close to 500(Table 4.6). A lower bioconcentration factor for 2,4-DCP was expected considering that the log Po/w of this compound is 3.08 (Saarikovski and Viluksela, 1982).

Previous equations developed for log BF in relation with log Po/w gave a higher value for BF for increasing Po/w values(Saarikovski and Viluksela, 1982). However, this relationship was not followed in our experiments. The bioconcentration factor was higher for 2,4,5-TCP than for 2,4,6-TCP(if bioconcentration conforms to their log Po/w values the BF should be reversed) and the 2,3,4,6-TTCP bioconcentration factor was higher than the PCP bioconcentration factor(which again should be reversed).

The graph at 4°C shows that the four CPs bioconcentrate practically at the same level, regardless of their Po/w values. It seems that in the case of leeches the values of Po/w have little influence upon the bioconcentration factor.

The experiment carried at 12°C followed the same general pattern as the one at 4°C. Steady state was reached after 5 days and with the exception of 2,4-DCP, the bioconcentration factors for all the other chlorophenols were about 1000 (Table 4.6).

The same discussion applies for this temperature and same discrepancies were noticed between the experimental values of bioconcentration factors and the values of bioconcentration factors calculated from equations developed by various researchers. As mentioned for the 4 °C experiment, the bioconcentration factors of the chlorophenols were grouped at the same level, regardless of the corresponding Po/w values(with the exception of 2,4-DCP). Bioconcentration factors for 2,4,5-TCP and 2,4,6-TCP , and for TTCP and PCP were again reversed when compared to their Log Po/w.

Temperature was a very influential parameter. At 12 °C, the bioconcentration factors were almost doubled as compared with the

4 °C bioconcentration factors.

A higher temperature resulted in higher BFs. At 22 °C(see Fig. 4.12) the bioconcentration factors were more than twice the values measured at 12 °C(Table 4.6).

Again, the bioconcentration factors did not follow the pattern established by equations relating BF to Po/w. However, the BF values were grouped in relation to relative Po/w values. 2,4,5 and 2,4,6-TCPs exhibited almost the same values for BF, and TTCP and PCP, with the highest Po/w values, had similar BF values.

If the equation log BF = 0.85 log Po/w- 0.70, developed by Veith(1974), using fathead minnows as the experimental organisms is applied to our data, and the calculated BFs are compared to the experimental BFs , one can see that they do not follow the same pattern. The best fit to Veith´s equation however, is obtained by the bioconcentration factors obtained at 22 °C, which is close to the experimental temperature (25 °C) used by Veith(see Table 4.7).

TABLE 4.7

EXPERIMENTAL AND CALCULATED BFs

Compound	Log Po/w	Log BF	BF	Exp. BF (22 °C)
2,4-DCP	3.08	1.918	83	980
2,4,5-TCP	3.80	2.530	339	1948
2,4,6-TCP	4.03	2.725	531	1988
TTCP	4.45	3.080	1208	2501
PCP	5.15	3.680	4758	2508

If other equations were considered, they gave different absolute values for the bioconcentration factors, but in all the

cases, the BF values increased with higher Po/w(Esser and Moser, 1980). We concluded from our data that for leeches, the bioconcentration factors did not follow the pattern developed by various researchers for other experimental organisms.

To summarize, the higher experimental temperature(22°C) had a few noticeable effects on the bioconcentration factor:

- the steady state took longer(at least 7 days) to be established when compared with 4 and 5 days for 4 °C and 12 °C respectively.

- the bioconcentration factors at 22 °C were doubled if compared with the bioconcentration factors obtained at 12 °C and quadrupled if compared with BFs at 4°C.

- a simple linear correlation between the logaritmic values of BF and Po/w as described by various equations was not followed by the experimentally determined BFs. Bioconcentration factors of various CPs differentiated only slightly on the basis of their Po/w values.

Fig.4.13 presents the TTCP concentrations found in leeches at the three levels of temperature, 4, 12, 22°C. The graph shows the influence that temperature had upon the bioconcentration factors. If BFs are plotted against temperature the effect of temperature upon bioconcentration becomes more obvious.

Regression equations were calculated for the temperature-BF relationship and they could be used when experimental data have to be normalized at a certain temperature. This is especially useful when data from different laboratories are to be compared.The main limitation to the application of the equations for interpretation of field experiments is that they can be used

to normalize only equilibrium bioconcentrations.

Regression equations were calculated for BF and log BF versus temperature(T). A very good correlation was obtained between BF and T with R^2 values between 0.9934 and 0.9648 but a better correlation was obtained for the log BF and T , with R values higher than 0.9910. The regression equations are listed as follows:

-for 2,4-DCP:

BF = 62 + 39.5 T R^2 = 0.9648

LOG BF = 2.305 + 0.039 T R^2 = 0.9910

-for 2,4,5-TCP:

BF = 274 + 65.5 T R^2 = 0.9934

LOG BF = 2.650 + 0.029 T R^2 = 0.9990

-for 2,4,6-TCP:

BF = 81 + 82.6 T R^2 = 0.9731

LOG BF = 2.614 + 0.027 T R^2 = 0.9990

-for TTCP:

BF = 43 + 106.4 T R^2 = 0.9731

LOG BF = 2.639 + 0.034 T R^2 = 0.9980

-for PCP:

BF = -97 + 112 T R^2 = 0.9732

LOG BF = 2.549 + 0.038 T R^2 = 0.9960

TTCP and PCP were bioaccumulated at the same level by leeches. Knowing that at the same exposure concentration and under the same experimental conditions PCP is more toxic to leeches than TTCP, one can attribute the lower LC50 of PCP to its intrinsic toxicity. This is contrary to Kobayashi et al. study(1978) who attributed the higher toxicity of chlorinated phenols

in goldfish to their relative accumulation in fish.

The experiment presented in Fig. 4.12 was carried out under very similar experimental conditions as the experiment presented in Fig.4.9(the length of both experiments was 7 days, experimental temperature was 22 C in both, and the experimental exposure solution was made up of the same chlorophenols at 10 ppb each). The two experiments lead to similar results. In both cases the equilibrium was not quite reached after 7 days and the bioconcentration factors did not seem to depend too much on the Po/w. The main difference between the experimental conditions was that they used different species of leeches, namely the black leech(P. marmorata) and the brown leech(N.obscura). This difference might be enough to account for the differences between the experimental bioconcentration factors obtained in the two experiments.

The brown leech exhibited a somewhat higher bioconcentration capability than the black leech since the bioconcentration factors were 20 % higher for the brown leech(in the case of TTCP and PCP). The data in the experiment that used the black leech could also be slightly biased by the fact that they represent single experimental points. Therefore , the black leech experiment has a greater experimental error than the experiment using brown leeches where data are obtained by averaging 3 points. The influence of the leech species upon the bioconcentration factor was studied in more detail in separate experiments presented in the following section.

4.3.2.3 Species Influence on the Bioconcentration

Since previous experiments indicated that the two leech species bioconcentrated at slightly different levels, a specific test to determine speciation differences was performed. The experiment was carried out at 4 °C. The exposure solution was made of the same CPs used throughout all the experimental work, at a concentration of 10 ppb each. A total of 9 leeches of each species(P. marmorata and N.obscura) were subjected to the exposure solution for 6 days. Three leeches of each species were analysed for chlorophenols after 24, 96 and 168 hours.

At the end of the six days(168 hours) the concentration of TTCP found in the black leech was 3.52 ug/g wet weight(average of 3 leeches) and 4.92 ug/g wet weight in the brown leeches(average of 3 leeches).In the case of PCP, the concentration in the black leeches was 3.16 ug/g wet weight (average of 3 leeches) and 4.33 ug/g in the brown leeches(average of 3 leeches).

The results showed once again that the brown leech had a higher bioconcentration capability than the black leech. According to the experimental data, bioconcentration was 40% higher in the case of TTCP and 37% higher in the case of PCP.

Since at 22 °C the difference in bioconcentration between the two species was only 20% in the favor of the brown leech, one can conclude that the higher temperature made the absorptive performance of the two leech species more uniform. The size of leeches used in the two experiments were quite different and this could have contributed to the variation in the bioconcentration factors; the bioconcentration capability of the brown leech could

have been even higher than the reported value if they had similar weight/ surface area ratio to the black leech. Average weight for the black leech was 0.239 g and for the brown leech was 0.683 g(the black leech is usually larger than the brown leech , but at the time of that experiment the black leeches were very small).

4.3.2.4 Weight of Leech Specimens and Its Influence on the Bioconcentration

The size of leech was originally not selected as a variable parameter in any of the experiments. Only observations made during experimental work used to study other variables led to a few conclusions on the effects of the size of the leeches on bioconcentration.

Theoretically, bioconcentration is a surface process achieved by respiration. The organisms pass water containing contaminants through the epithelial tissue and retain and bioconcentrate those contaminants in their body. If they have a lipophilic structure, the contaminants will be preferentially deposited in the fat tissues of the biota.

The concentration of chlorophenol in leeches was expressed as ug/g wet weight and not g/unit of surface area. That means that smaller the leech, the higher the ratio of surface area to weight. Therefore, a small leech is expected to exhibit a higher bioconcentration factor. Also, as bioconcentration was correlated to organism bioenergetics(Norstrom et al., 1976), a higher bioconcentration factor was expected from the younger organisms since they have a higher metabolic rate than the older, larger,

ones.

These considerations made it necessary to try and keep the size range of leeches as narrow as possible. In selecting leeches, a maximum standard deviation of 40% was set as the desired criterion. In some cases, due to the limited supply of leeches, it was necessary to use leeches from a wider weight range.

A few observations were made in respect with the bioconcentration calculated for various size ranges used in the same experiment:

-when a very small weight was used along with larger weights, the small leeches had occasionally very high concentrations of chlorophenol. A viable explanation for this could be based either on the higher surface area per unit weight for smaller leeches or on extraction and preparation techniques: a very minor error or contamination could have been artificially magnified when the amount found was calculated on the basis of a smaller weight.

- the extracts of control(blank) leeches of larger specimens were more complex(the chromatograms exhibited more peaks) than the extracts of control leech of smaller specimens. This observation made us use large control leeches for large experimental leeches and small control leeches for small experimental leeches.

4.3.2.5 Summary of Results

Experimental temperature was the most important parameter when determining bioconcentration factors. No bioconcentration factor should be considered unless the experimental temperature at which it was determined is given.

Temperature affected the length of time required to reach the steady state and the magnitude of the bioconcentration factor. Steady state was reached in 4 days at 4°C, in 5 days at 12°C and in 7+ days at 22°C. TTCP and PCP had bioconcentration factors of approximately 500 at 4°C, of about 1000 at 12°C and of about 2500 at 22°C. Regression equations were established and the R^2 values obtained were all higher than 0.96 which showed a good fit of the equations to the data. The equations could be used to normalize bioconcentration factors to a preset temperature.Their use is limited to equilibrium bioconcentrations and to a concentration of the exposure solution in the range of 10 ppb. It has been previously shown (see section 4.3.1.1) that bioconcentration capability is influenced by the exposure solution concentration.

The assumption that steady state condition was reached in the 4 and 12°C experiments was based on the fact that the CP concentrations found in leeches were not significantly different after 4 and 5 days respectively. However, a solid confirmation of the steady state condition can be only given by a longer exposure experiment, of 30 to 45 days. Log Po/w values of the chlorophenols used in the exposure experiments were not well correlated to the level of bioconcentration in leeches. The general equation: Log BF = a Log Po/w + b , was not followed in

the case of leeches exposed to chlorophenol. Higher experimental temperatures seemed to enhance the influence of the Po/w upon the bioconcentration factor .

The two species used in the experiments exhibited different bioconcentration factors which were approximately 40% higher, at 4 C, for the brown leech than for the black leech. Higher experimental temperature(22 C) seemed to lower the difference in bioconcentration between the two species. The brown leech exhibited a bioconcentration factor only 20 % higher than the black leech when the experimental temperature was 22 C.

Size of leech should be seriously considered for the selection of specimens for various experiments. A narrow size range should be kept for more reproducible results. Empirically, it was decided to work with a maximum of 40% standard variation and to keep the leech weight in the same order of magnitude.

4.4 Field Experiments

Field experiments were conducted in the North Arm of the Fraser River to determine the levels of chlorophenol pollution. Water samples were collected and analyzed for chlorophenols and exposure trials were made with leeches to evaluate their potential as bioassay organisms for chlorophenol bioconcentration. Water sampling was carried out independently, to evaluate the temporal and spatial distribution of chlorophenols in the river, and in synchrony with leech bioassays to validate the ability of the leeches to integrate chlorophenol pollution over time.

4.4.1 Water Sampling

Water sampling was performed in two ways: grab sampling and

automatic sampling. The sampling sites are located in Fig. 3.1. They were numbered from 1 to 7 and are identified by geographical landmarks(Table 4.8).

4.4.1.1. Grab Sampling

The main objective of the grab sampling was to investigate the general distribution of chlorophenol pollution in the area of study and help select an area for the detailed sampling and leech studies. Table 4.8 presents the data from the grab sampling trips.

The only two chlorophenols detected in the grab samples were TTCP and PCP. This is consistent with the main source of chlorophenol pollution to the North Arm of the Fraser River which is the forest industry. Other reported sources are the sewage treatment plants but our sampling sites were not in the vicinity of sewage outfalls.

The grab samples, which were taken either from the shore or from a boat, represented reasonable well-mixed water samples. The values ranged from nd(not detected; detection limit for TTCP and PCP is 0.002 ppb) to high values recorded at sampling site 5, Mitchell Island location, namely, 11 ppb TTCP and 2.25 ppb PCP. On the basis of these high values and the known busy and active forest industry in this area, the Mitchell Island area was chosen for a permanent site for the detailed sampling and leech exposure program.

The interpretation of the grab sampling data lead to the following conclusions:

- A low background level of 2,3,4,6-TTCP,at concentrations

TABLE 4.8

GRAB SAMPLING RESULTS[1]

Sampling Site No.	Location	Date (1984)	2,3,4,6-TTCP (ppb)	PCP (ppb)
1	Scott Paper	Sept. 11	nd	nd
		Oct. 4	0.480	nd
		Oct. 11	nd	nd
2	Kerr St.	June 28	0.470	0.040
		June 28	0.580	0.003
		Sept. 11	nd	nd
		Oct. 4	nd	0.120
		Oct. 11	nd	nd
3	Fraser St.	Aug. 1	0.060	0.010
4	Oak St.	Aug. 1	0.440	0.110
5	Mitchell Is.	Sept. 10	0.614	nd
		Oct. 4	11.00	2.25
		Oct. 11	0.180	nd
6	Arthur Laing Bridge	Oct. 4	1.560	0.140
		Oct. 11	nd	nd
7	Wood Is.	Sept. 10	0.132	nd

[1] See Fig.3.1 for location of stations. nd = not detected. Detection limit for TTCP and PCP is 0.002 ppb. Detection limit for the other chlorophenols is 0.010 ppb but they were not observed at this level.

between 0.1 and 0.4 ppb was almost always present in the study area. PCP was present at a lower concentration level(0.03 to 0.1 ppb).

- Stations in the upper reaches of the North Arm, such as Scott Paper and Mac Millan Bloedel, exhibited lower levels of CPs(more values close to the detection limit and blank values). This could be due to a series of factors such as: cleaner or ceased operations, a lower frequency of grab samples which missed any sporadic discharges that occurred, and the fact that water from the lower stations could be subjected to multiple dosing attributable to a greater tidal influence in this area(Joy, 1975).

- Higher chlorophenol levels were found but they were sporadic. The grab sampling technique could have missed other high levels of CPs and the need for a more integrated sampling technique became obvious.

The high level of TTCP found at station 5 provided a guideline for selection of the concentration to use in the laboratory experiments: 10 ppb concentration was used to make up the exposure solutions. This level of chlorophenol was not acutely toxic to leeches and since it was the highest level that could be found in the river, this level led us to believe that the leeches would survive the field exposures.

During the subsequent automatic sampling, concentrations of TTCP at the same level were recorded.

4.4.1.2 Automatic Sampling

The automatic sampling used a 2 hour sampling frequency. Depending on the total length of the sampling period the water samples were analysed as discrete or composite samples.

In January 1985, the automatic sampler was placed on the loading dock of the West Coast Cellufibre Industries Ltd.(near station # 5). All the automatic sampling was done at this location. One has to bear in mind that the data regarding the chlorophenol levels at this location are representative for that particular location and not for the level of chlorophenol in the Fraser River Estuary. This particular sampling station was selected with the expectation that the water would contain some high concentrations of chlorophenol. The presence of contaminant in water was needed in order to be able to demonstrate the capability of leeches to bioconcentrate it. The presence of chlorophenol in that location was probably due to either surface runoff containing the contaminant, improper disposal of the rinse waters from the clean-up operations, or to leaching from the treated lumber.

The data of a two-day sampling experiment are represented in Fig.4.14. All the graphed points are analytical results for discrete samples taken every two hours. This two-day experiment demonstrated how ineffective single grab samples were in documenting chlorophenol contamination. Two peaks of high concentrations of TTCP and PCP occurred over a period of 10 hours and, as the graph shows, a grab sampling method could have missed them both if sampling was done at the wrong time. The mill

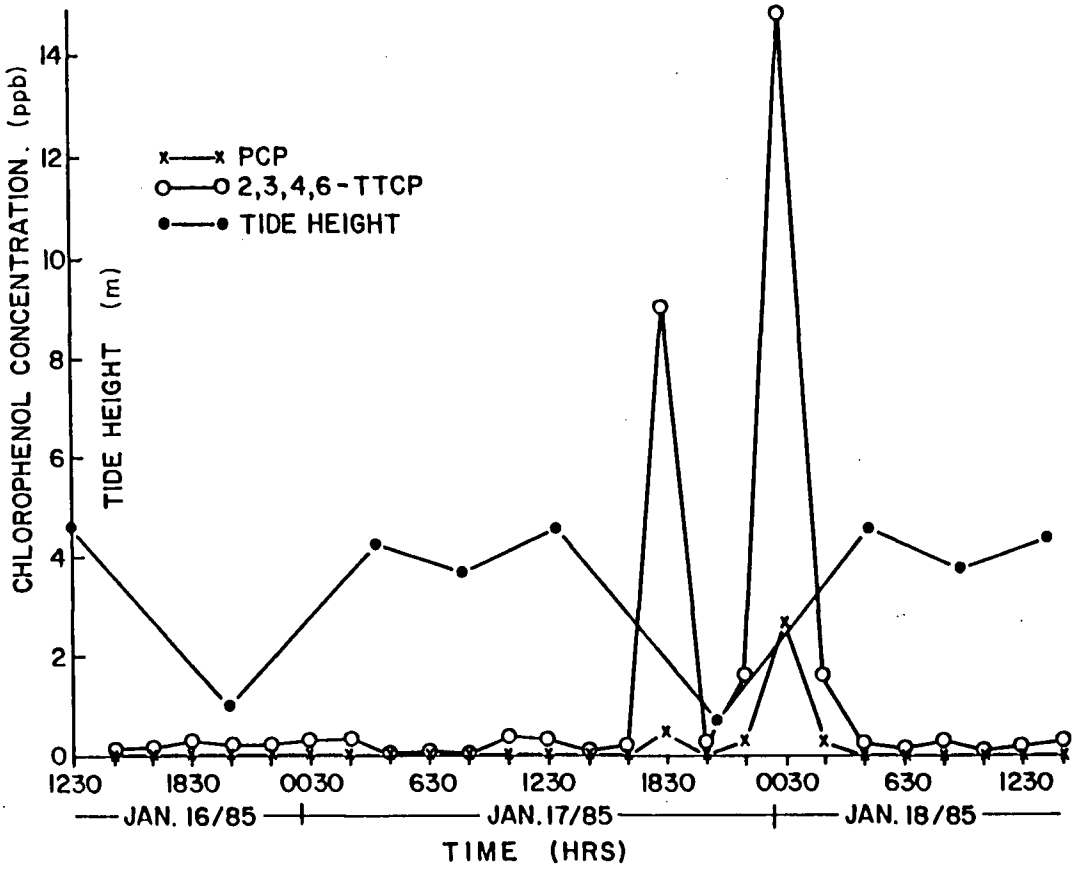

Fig.4.14 Chlorophenol concentration at Mitchell Island over a two-day period. Average concentrations were 1.250 ppb TTCP and 0.167 ppb PCP. Water temperature was 3-4 °C. Daily average water flow for the sampling period was 1330 to 1410 m3/s(Water Survey of Canada, 1985 data).

adjacent to the site of sampling, was in operation 24 h/day, 5 days a week and the high plumes of chlorophenol could have been due to a spill or midnight clean-up operation. If the tidal effect was considered, the two peaks measured at that particular location could have been the result of just one discharge of contaminant which took place at some other location and which appeared as two separate peaks due to the ebb and flood of the tide.

The Fraser River water temperature and the average daily flow range for the sampling periods are given on all the figures pertinent to water sampling. The river flow is measured at the Port Mann pumping station since no recording is done at the North Arm(Water Survey of Canada, 1985 data). The North Arm flow is estimated(personal communication) at 10 -14 % of the flow at Port Mann. The river flows are to be used as relative values to compare the flows in the study area at various times of the year. In January, the Fraser River flow was at its lowest point and dilution was minimal. Any spike of contaminant was present in the river at its maximum concentration. Low river flows were also recorded in February and March. This is the most critical time for the Fraser River to receive any toxic discharge in terms of dilution capacity. Plumes of contaminants are also easier to detect by sampling the river at short intervals. At periods of high flow, the contaminant spikes are diluted more rapidly and their presence is detected more as a plateau determined by the average concentration. During periods of high flow, the turbidity of water increases and this can affect the availability of the contaminants to the biota as the suspended particles compete for

the contaminants. Also, recovery of those contaminants from the water matrix is more difficult due to the high load of suspended solids and special recovery tests must accompany the sample analysis.

Tidal height was represented on all the graphs(Canadian Tide and Current Tables, 1985). Again, only the relative values of the tide height must be considered since the tidal height that is represented is taken at Point Atkinson. The reason for including the tidal height in the graphical representation of the water sampling results was to show the estuarine characteristics of the study area. The tidal cycle influences the dilution factor and the direction of movement of the contaminant plume.

The results were presented differently when longer sampling periods were involved. Since composite samples were used in order to keep the number of analyses reasonable and to maintain the same sampling frequency(every two hours),the results were represented as histograms covering a six hour period. To facilitate compound identification on the graph, PCP histograms were represented as triangular instead of rectangular areas. The composite samples were obtained by manually mixing three consecutive samples taken at two hour intervals. The concentration of the composite samples represented the average chlorophenol concentration for the six hours. High concentrations were measured, but their levels were lower than found during the two day experiment since high concentrations were diluted by mixing with more dilute samples collected during this period.

The automatic sampling experiment carried out between March 31 and April 6, 1985(see Fig. 4.15), gave an average

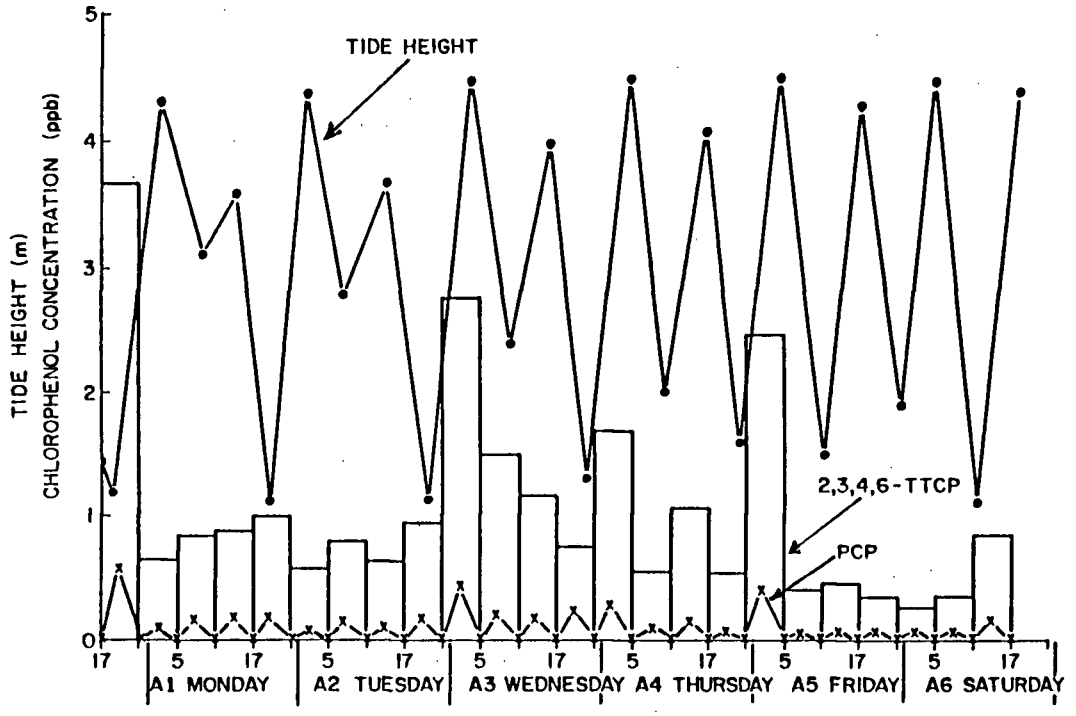

Fig.4.15 Chlorophenol concentrations at Mitchell Island over a six-day period. Average concentrations were 1.058 ppb TTCP and 0.175 ppb PCP. Water temperature was 4 °C. Between April 1 and April 6, 1985, the daily average water flow was 1790 to 2020 m3/s(Water Survey of Canada, 1985 data).

concentration of chlorophenol over the entire sampling period of 1.058 ppb TTCP and 0.175 ppb PCP. Concentrations as high as 3.678 ppb TTCP were reported. This high value for a composite sample could mask an even higher peak which was averaged by mixing with two other less contaminated samples.

What was characteristic of this experimental period was the repeated occurrence of high levels of chlorophenol between the same hours of each day: 11 p.m. and 5 a.m. on April 3, 4, and 5.This could not be related to the tidal oscillation since similar tidal variation was experienced during the daylight period. The regular frequency of the high levels of chlorophenol could more realistically be related to a midnight clean up operation by the lumber treating industries adjacent to the sampling site. Again, because of the current reversal over the tidal period it was quite impossible to pinpoint the exact location where the discharge took place. The purpose of the water sampling was not to identify the guilty party, but to establish the level and pattern of variation of chlorophenol contamination in water.

The comparison of the average daily flows at the beginning of April with the average daily flows during the January experiment showed that a greater dilution occurred in April. This could be one of the possible explanations for the lower average concentration of chlorophenol in early April than in January(1.058 ppb of TTCP in April as compared with 1.250 ppm of TTCP in January).

The seven day experiment between April 18 and April 25, 1985(see Fig.4.16) showed an even lower average concentration of

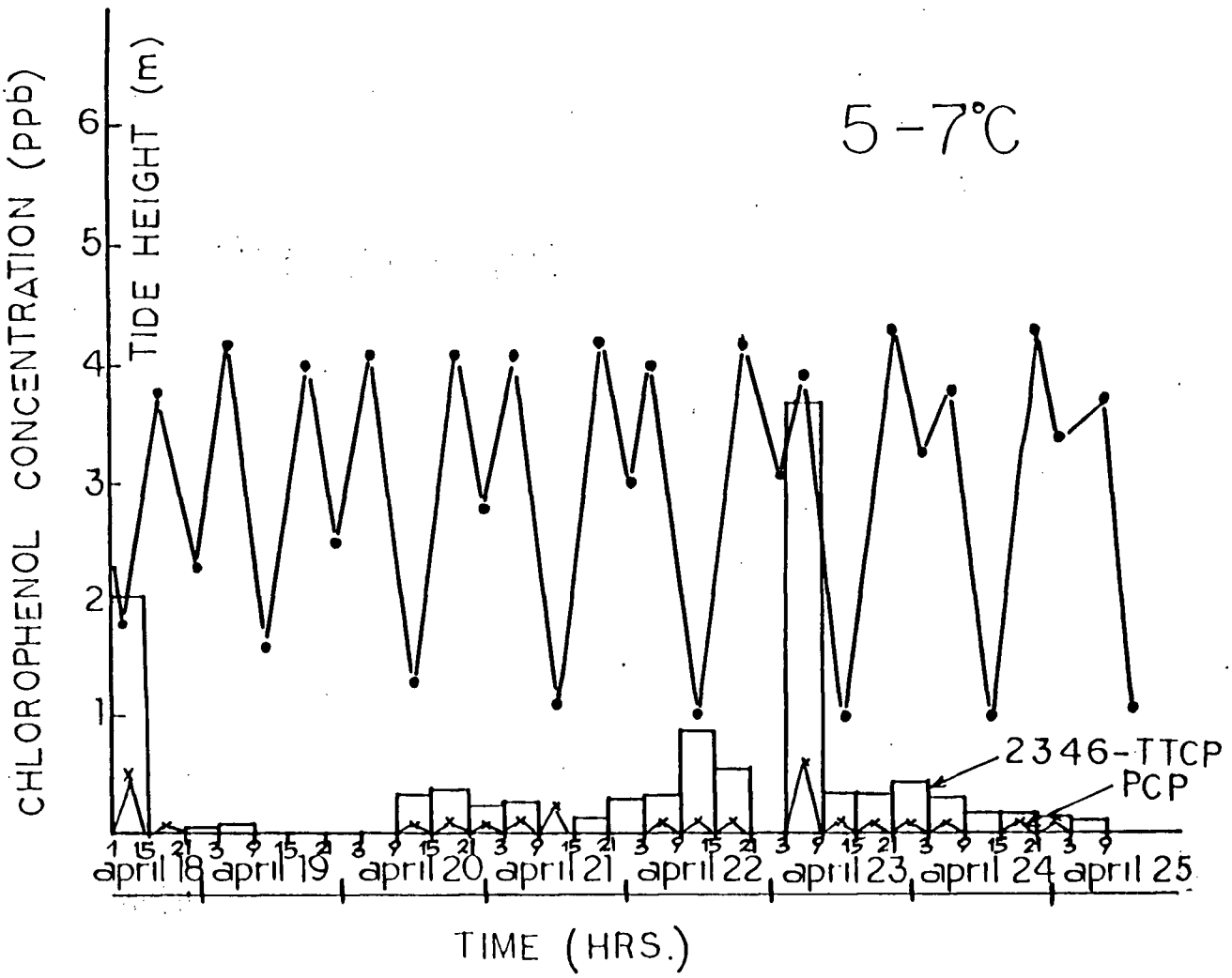

Fig.4.16 Chlorophenol concentrations at Mitchell Island over a seven-day period. Average concentrations were 0.430ppb TTCP and 0.080 ppb PCP. Water temperature was 5-7 °C. Between April 18 and 24, 1985, the daily average water flow was 3490 to 3840 m3/s(Water Survey of Canada, 1985 data).

chlorophenol in water: 0.430 ppb for TTCP and 0.080 ppb for PCP. This could be again due to the higher dilution capacity of the river at that time of the year. Cleaner operations and less lumber processed in this time could also be a viable explanation for the decreased level of pollutant.

Due to the high content of suspended solids in the river water at the time of increasing flow, recovery values had to be applied to the analytical results(52% recovery for TTCP and 44 % recovery for PCP).

The last of the series of automatic water sampling experiments was carried out in September 1985(see Fig.4.17). The average concentration of chlorophenol at this time of the year was found to be 1.680 ppb for TTCP and 0.428 ppb for PCP. The river flow was decreasing in early fall but the change in flow was not as dramatic as occurred during the spring. Average daily flows for the duration of the September experiment were a great deal lower than during the last April experiment. A general characteristic of the chlorophenol level in the September experiment was a more uniform concentration over the sampling period. Two higher values were found, but in general the levels of chlorophenol exhibited little variation from the mean concentration.

4.4.1.3 Summary of Results

The automatic sampling experiments showed how ineffective grab samples could be and how easily high spikes of contaminants can be missed if the sampling time did not coincide with their occurrence. The CPs levels showed a large variation with time. Peaks as high as 14 ppb TTCP were reduced to the background level

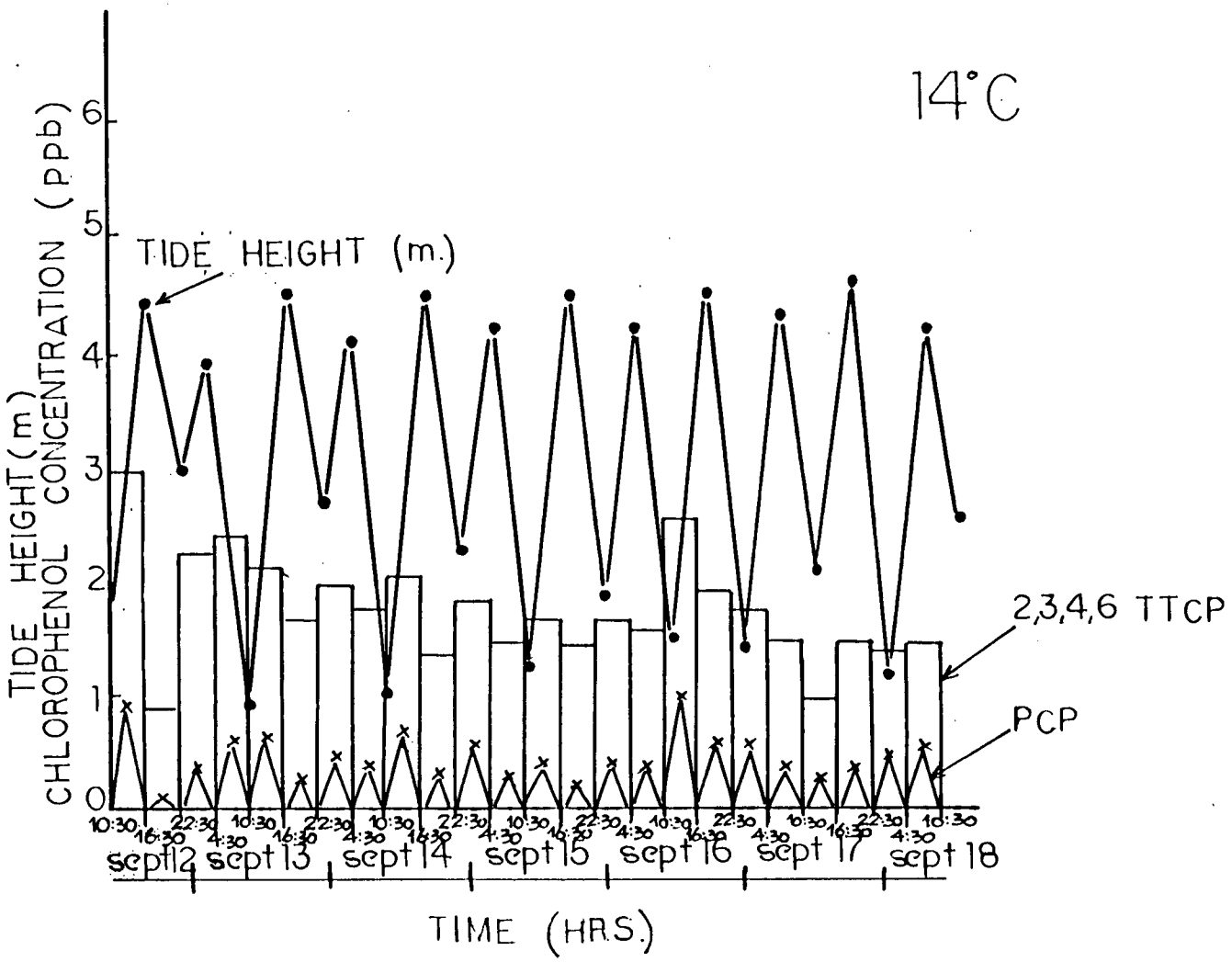

Fig.4.17 Chlorophenol concentrations at Mitchell Island over a six-day period. Average concentrations were 1.680 ppb TTCP and 0.428 ppb PCP. Water temperature was 14°C. Between September 12 and 18, 1985, the daily average water flow was 1760 to 2740 m3/s(Water Survey of Canada, 1985 data).

in only two hours.

The river flow seemed to play an important role for the contaminant levels found in the water at various times of the year. The water quality such as reflected by the suspended solids content affected chlorophenol recovery from the water matrix. At times of high loads of suspended solids, the recovery values had to be included in calculations.

High levels of chlorophenol, found during the automatic water sampling experiments, were very comparable to high levels reported by previous studies conducted in areas impacted by the forest industry operations(EPS, 1979). The background levels for the area of study estimated at 0.1 ppb to 0.4 ppb for TTCP and 0.03 ppb to 0.1 ppb for PCP were also confirmed by Carey (unpublished data) who sampled the same area in 1984.

4.4.2 Field Leech Experiments

Table 4.3 contains the results of the first leech exposure experiment in which leeches were exposed for seven days to the chlorophenol levels in the Fraser River water. The experiment used 5 leeches at each of the two locations. After 7 days of exposure, three leeches were analysed for chlorophenol and two underwent depuration. The leeches exposed at the Mitchell Island location exhibited a high chlorophenol concentration(2.946 ug/g TTCP and 0.846 ug/g PCP). This indicated that higher levels of chlorophenol should be encountered in the water collected at that location.This was confirmed by the high concentration of chlorophenol(11 ppb TTCP and 2.22 ppb PCP) detected at that

location on October 4.

Considering that no chlorophenols were reported in the grab samples collected on October 4 and 11 at the Kerr station, the level of chlorophenol found in leeches after 7 days exposure at that site, indicated that chlorophenols were present in water at some periods during this 7 days exposure.

All the other leech exposure experiments were carried out at one location:the loading dock of the West Coast Cellufibre Ind., where the automatic sampler was located.

Two of the leech exposure experiments were accompanied by water sampling. For these experiments, the pattern of chlorophenol variation was available and the average concentration of chlorophenol during the exposure could be calculated(April 18-25, 1985, and September 12-18, 1985). The leeches exposed for 7 days, between April 18 and 25, 1985, contained a chlorophenol concentration of 124 ppb for TTCP and 55 ppb for PCP. The average chlorophenol concentration in the water was 0.430 ppb TTCP and 0.080 ppb PCP(see footnotes Fig.4.16).

The leeches exposed for 6 days between September 12 and 18, 1985, contained a chlorophenol concentration of 273 ppb TTCP and 196 ppb PCP. The average chlorophenol concentration in the water was 1.680 ppb TTCP and 0.428 ppb PCP(see footnotes Fig 4.17) .

Comparing the chlorophenol levels found in the leeches at the end of the two exposure experiments it is clear that leeches exposed to a higher average chlorophenol concentration at a higher temperature accumulated more contaminant in their tissues. The fact that one experiment was carried out for 6 days and the other for 7 days should not influence the final bioconcentration

level very much since it was shown before that a steady state condition is reached in 5 days at 12 °C temperature and 4 days at 4 °C. During the two leech experiments presented above, the water temperature was in the neighbourhood of these values(see footnotes of Fig. 4.16 and 4.17).

The chlorophenol concentration during the experiment from September 1985 was quite constant and it could be possible that a steady state was reached. For the experiment from April 1985 the steady state was not achieved because of the high variation of the chlorophenol concentration. There is considerable variation in chlorophenol levels in the Fraser River Estuary, thus the leeches are subjected to a wide dynamic range that they must integrate over an exposure period. Only estimates of the average chlorophenol concentration in water can be made on the basis of the leech chlorophenol content at the end of the exposure experiments.

Metcalfe et al.(1984) reported chlorophenol levels in leeches at 40,000 to 140,000 times the average chlorophenol concentration in water. This does not mean that the bioconcentration factor of those leeches is in this high range of values. The leeches sampled must have been exposed to a high concentration of chlorophenol for some time prior to their collection. Since the depuration rate of chlorophenol from the leech matrix is a very slow process, the leeches still contained the accumulated contaminants in their tissues. They exhibited an artificially high bioconcentration factor because of the low chlorophenol concentration in water at the time of sampling.

Two more leech field experiments were carried out between May

31- June 7, 1985, and September 27- October 3, 1985. They were not accompanied by automatic water sampling and the interpretation of the CPs levels in the water from levels in leeches was done solely by using the laboratory data.

The leeches exposed between May 31-June 7, 1985, had TTCP at 235 ppb and PCP at 57 ppb . The leeches exposed between September 27-October 3,1985 had TTCP at 28 ppb and PCP at 29 ppb. Table 4.9 contains a summarized presentation of the field leech experiments carried out at the West Coast Cellufibre location . Pertinent information, such as size of leeches, water temperature and concentration of CPs in the water (if available) are listed for easy reference.

Interpretation of the leech field results will be done in section 4.6.

4.5 Specially Designed Laboratory Exposure Experiments

The bioconcentration capability of leeches and the slow depuration rate of contaminants from their tissues make the use of leeches an attrative proposition for monitoring purposes. They cannot be used as titrants of the chemicals present in the water column since it is virtually impossible to account for all the variables in a natural system. However they seem to be reliable indicators of past and present pollution and estimates of the pollution level can be made on the basis of their pollutant content.

Implanted for various lengths of time in the region of study , they will integrate the concentration of contaminant and should

TABLE 4.9

FIELD LEECH EXPERIMENTS

Exp. No.	Duration	Date (1985)	Water Temp	Leech Weight	CPs in Leech TTCP	CPs in Leech PCP	CPs in Water TTCP	CPs in Water PCP	Observations
1	7 days	April 18-25	5-7 C	0.4 g	124 ppb	55 ppb	0.430 ppb	0.080 ppb	Low recovery values
2	7 days	May 31-June 7	8 C	0.6-1.0 g	235 ppb	57 ppb	-----	-----	
3	6 days	Sept 12-18	14 C	1.2-1.3 g	273 ppb	196 ppb	1.680 ppb	0.428 ppb	
4	6 days	Sept 27-Oct 3	12 C	1.0-1.9 g	28 ppb	29 ppb	-----	-----	Values close to the leech blank

signal unusual high levels of contaminant by unusual high contaminant concentration in their tissues.

For a proper interpretation of the field results one has to consider the major parameters that influence bioconcentration. It has been shown that some important parameters that regulate bioconcentration are temperature, suspended solids, hardness, pH of water and leech size. These parameters can be relatively easily monitored and their influence upon variability of the results reduced to the minimum. River pH varies little during the year and can be easily matched in a laboratory experiment. Size of leeches can be controlled by carefully selecting the specimens. Temperature of the river water does change during the year but it stays relatively constant for short periods such as 6-7 days, and again can be reproduced in a laboratory experiment.

Temperature, pH, alkalinity and hardness are water characteristics easy to reproduce. Other characteristics such as suspended solids are harder to match because not only the load of suspended solids influences the bioconcentration process (Muir et al., 1980) but the composition of those solids as well (i.e. a higher organic load will have more impact on the availability of chlorophenol to the aquatic biota).

A laboratory controlled experiment in which all these parameters are matched with the river water characteristics has to be run if an accurate estimation of the contaminant level in the water is to be made on the basis of the contaminant level found in the leeches. For a field test to be successful one of its main characteristics is for it to be simple. If a laboratory controlled experiment is to be run concomitant with the leech

field test, it has to be kept uncomplicated as well.

Special laboratory experiments were designed with all the above considerations in mind and their main purpose was to help in estimating the field results.

For practical reasons, the concentration of the exposure solution must be chosen somewhere in the range considered to be safe for a natural environment such as the Fraser River Estuary.

At present, there are no such regulatory limits for chlorophenol. Fox(1980) suggested 0.4 ppb PCP as the safe concentration for the protection of aquatic life. Concentrations in the range of 1 ppb have been chosen for the laboratory experiments. On the basis of the results obtained in these experiments, the level of CPs found in leeches was used to estimate the average chlorophenol concentration in water during the exposure.

4.6 Interpretation of the Field Results on the Basis of the Laboratory Experiments

Laboratory experiments were set up to reproduce at best the field conditions: water characteristics(temperature, pH, hardness, alkalinity) and exposure time. Since no regulatory values are imposed for CPs, arbitrary concentrations of 0.5 ppb, 1 ppb, and 2 ppb were used.

The results of the specially designed laboratory experiments are shown in Table 4.10. They represent averages of three leeches . Only the levels of TTCP and PCP are presented since these are the only two CPs found in the river. Experiments 1 and 2 were

TABLE 4.10

SPECIAL LABORATORY CONTROLLED LEECH EXPERIMENTS

Experiment No.	Duration	Water Temperature	Exposure Solution CPs Concentration[a]	CPs in Leech[b] TTCP	PCP	Leech Weight
1	7 days	4°C	0.5 ppb	84 ppb	27 ppb	1.7-2.8 g
2	7 days	4°C	1.0 ppb	231 ppb	157 ppb	1.0-2.7 g
3	6 days	14°C	1.0 ppb	121 ppb	191 ppb	2.2-2.8 g
4	6 days	14°C	2.0 ppb	238 ppb	383 ppb	2.4-2.9 g

[a] The exposure solution was made of five chlorophenols (2,4-DCP, 2,4,6-TCP, 2,4,5-TCP, 2,3,4,6-TTCP, PCP), each at the indicated concentration. Dilution water was made after EPA formulation for medium hard water (see section 3.3.2).

[b] Only the concentration of TTCP and PCP are given because only they are to be used to estimate the average concentration of CPs in water during the leech field experiments; only TTCP and PCP were found in the Fraser River water.

carried out at 4 C to reproduce the best winter conditions and experiments 3 and 4 were carried out at 14 C to reproduce the summer and early fall conditions of the Fraser River water.

The higher CPs concentration exhibited by leeches in experiment 2 compared with experiment 1 was expected since the CPs concentration in the exposure solution was twicw as high. However, if one compared results of experiment 2 and 3, both carried out at the same CPs concentration of 1 ppb, one could see that the results of experiment 2 were unusually high. The two experiments differed by the experimental temperature, and it was expected that the higher temperature would result in a higher level of CPs in the leech tissue(see section 4.3.2.1).

Experiment 4, carried out at the same temperature as experiment 3, but at a twice concentration in the exposure solution, showed higher bioconcentration levels. They reflected the increased dosage of CPs.

No viable explanation was found for the high results obtained in experiment 2 and a replicate experiment was not carried out to confirm or reject the values. Its data were not used in the interpretation of the field results.

The fact that the experiments were carried out for various lengths of time(6 or 7 days) should not influence the final results. It was shown before that the steady state condition was established in 4 or 5 days for the experimental temperatures used(see section 4.3.2.1) . This means that the bioconcentration factor should not change after 5 days of exposure.

Since TTCP was the main chlorophenol found in the Fraser River water the estimation was done only for this compound.The

excercise consisted in comparing the CPs levels in the field leeches with the CPs levels in the leeches exposed under laboratory conditions to known concentrations of chlorophenol solutions. The field conditions were matched by the laboratory conditions and the chlorophenol concentration was considered to be the only variable. Matching of field and laboratory conditions was not perfect as field leeches were exposed to dynamic changes of chlorophenol levels and other water parameters(suspended solid content, salinity, etc.) over the bioassay period. Temperatures were not normalized to an identical value since equilibrium conditions were not considered to be reached under field conditions. Only the general trend of variation of bioconcentration with temperature was considered when making the estimate.

Field experiment 2(see Table 4.8 for all the field experiments carried out at West Coast Cellufibre location) showed CPs levels in leeches of 235 ppb for TTCP and 57 ppb for PCP. The level of TTCP was similar to the level reached in laboratory experiment 4. The CPs concentration used in that experiment was 2 ppb. The temperature at which these field and laboratory experiments were done differed from each other. With the knowledge that at higher temperatures the bioconcentration is higher, an average value of 2 ppb TTCP in water during field experiment 2 was very conservative estimate.

Field experiment 4 showed very low levels of CPs in leeches. A resonable estimation would conclude that no measurable levels of CPs were present at this location during that interval of time, since the levels found were very close to the control

leech CPs levels.

For the leech experiment carried out in October 1984 at the Kerr St. location(see Table 4.3) when TTCP was found at 476 ppb and PCP at 226 ppb, one can estimate the concentration of TTCP in water as being higher than 2 ppb for the entire period of exposure(field water temperature was similar with the laboratory experiment 4 temperature). It was shown by the grab sample taken at the beginning and end of the experimental period that TTCP was below the detection level. This means that high concentrations of TTCP occurred at various times during the exposure period and they brought the average TTCP concentration at over 2 ppb for the entire length of the exposure.

The leech experiment carried at the same time at the Mitchell Island location(see Table 4.3) showed levels of CPs in leeches even higher: 2946 ppb for TTCP and 846 ppb for PCP. The average TTCP concentration was definitely higher than 2 ppb. The grab sample taken at the beginning of the experiment indicated 11 ppb TTCP and 2.25 ppb PCP. The leeches exposed at this location exhibited the same level of TTCP concentration with leeches exposed for 35 hours to a solution of 10 ppb TTCP(comparison was made with the temperature controlled experiment carried out at 12 °C, a temperature similar to the water temperature during the field exposure). This made us estimate that a high concentration of TTCP was present at that particular location for an extended period of time or that the frequency of high levels of TTCP in water was very high. Another possibility is that a very localized spill at even higher concentration of TTCP occurred at that location for a shorter time. A very high concentration for a

short time would have led to the same bioconcentration level as lower concentration for a longer time.

Field leech experiments 1 and 3 were accompanied by automatic water sampling. For these experiments the average CPs concentration in water was known. If one tries to estimate the concentration of TTCP during field leech experiment 1 using the laboratory experiments, a concentration higher than 0.5 ppb, lower than 2 ppb and about 1 ppb would be estimated. Since the measured average TTCP concentration was 0.43 ppb this made the estimate approximately twice as high as the real value.

If the results of field experiment 3 were used to estimate the average concentration of TTCP on the basis of the laboratory experiments, a concentration of 2 ppb TTCP was estimated for the river water at that location. The measured value was 1.68 ppb TTCP and that made the estimated value within ± 15% of the real value.

Considering that those estimates are made using a living organism as the integrator of the contaminant level, the acquired accuracy is well within expected values. Experiments using biota are considered successful if the order of magnitude can be accurately predicted.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

As shown by the grab sampling program results and by the extreme variations of chlorophenols levels during the high frequency automatic sampling periods, the actual system of monitoring pollution levels is totally inadequate. The use of periodic grab samples to monitor pollutant levels is not representative for the pollutant presence in the area under survey. Since a high frequency sampling program would run into enormous costs, the use of a bioindicator organism is the only viable proposition.

Leeches proved to be a very suitable organism for monitoring of chlorophenol pollution and only more research can prove if they can be used to estimate the time averaged concentration of other contaminants. Preliminary laboratory controlled leech experiments indicated that the leeches have great bioconcentration capabilities for chlorophenols and set the main parameters for further laboratory controlled experiments.

During field experiments, only two chlorophenols were detected in the water samples: 2,3,4,6-TTCP and PCP. The average concentration for TTCP determined during the various sampling periods, carried out between January 1985 and September 1985 at the automatic sampling location, was always around the 1 ppb level. PCP concentrations were about 4 to 5 times lower. This indicated that the main source of chlorophenol pollution in the

study area was the forest industry(West Coast Cellufibre Indutries Ltd) which uses formulations of the two chlorophenols in ratio of 4 to 1, TTCP to PCP.

Temperature proved to be a major parameter influencing the bioconcentration factor of chlorophenols in leeches. Bioconcentration increased with temperature. All chlorophenols exhibited the same bioconcentration factors with the exception of the 2,4 DCP. The relationships established by various researchers between the Po/w and BF were not followed by laboratory exposure experiments with leeches.

For the purpose of using leeches as bioindicators of pollution in a river environment, absolute values of the bioconcentration factors have little importance since equilibrium state is never reached under environmental conditions. What is more relevant for the use of leeches as bioindicators is their ability to concentrate chlorophenols to high levels and show a low depuration rate from their matrix. Leeches are capable of reflecting high concentrations of pollutants that have occurred at various times over a long period of exposure.

pH influence on the bioconcentration of chlorophenols followed the expected trend for ionizable compounds: bioconcentration increased at lower pH. pH is not as important as temperature, since in a river environment pH does not change much. Any drastic changes of pH are usually localized and rapidly neutralized by dilution. pH has to be considered when dilution water characteristics are matched to the characteristics of the body of water monitored by the use of leeches. Other water characteristics, such as suspended solids content and

composition, have an impact on the accumulation of chlorophenols in biota tissues. This variable was not studied, but higher loads of suspended solids were reflected in the results of chlorophenol analysis by low recovery values. When recovery values were too low, corrections had to be made to the results.

Estimation of the average concentration of chlorophenols in the water on the basis of the accumulated chlorophenols by the leeches gave values within the same order of magnitude with the real chlorophenol concentration in water monitored by automatic sampling. The worst estimate was twice as high as the actual monitored chlorophenol concentration. For a biological sample this estimation is very good.

Laboratory controlled experiments, using a series of concentrations of the exposure solution, had to be run in order to compare the bioconcentration of chlorophenols in leeches, at various levels of pollution. They proved to be an effective aid to the interpretation of the field results.

5.1 Recommendations

Recommendations under this section will cover two main aspects: the use of the results of this research in setting up the methodology for a monitoring program using leeches, and future research needed for better understanding the bioconcentration capability of leeches.

A basic test using leeches as bioindicators of chlorophenol pollution in an aquatic environment should consist of two tests: a laboratory controlled exposure experiment and a field leech

exposure test. Both exposure tests should be run for the same duration, using the same number and size range of leeches. The exposure solution used in the laboratory controlled experiment should match closely the characteristics of the water where levels of pollution are surveyed. Temperature and pH should be identical with the field values. The chlorophenol concentration selected for the laboratory controlled exposure should be similar to levels set by regulations or other considerations.

At the end of the exposure time, the levels of the bioconcentrated chlorophenols in the field and laboratory leeches are to be compared. If a significantly higher value was obtained in the field leeches than in the laboratory leeches, this would indicate a pollution problem in the surveyed environment. Since the maximum acceptable levels were exceeded, a more intense monitoring activity for that location must be initiated to pinpoint the source and the extent of the pollution. An estimation of the average chlorophenol concentration in water could also be made. This estimation would define the range of contamination and determine the severity of the pollution problem. If the field leeches exhibited lower levels of chlorophenols than the laboratory leeches this would indicate chlorophenol levels within the limits established by regulations. This kind of monitoring program will effectively reveal the areas of serious contamination and signal occasional spills or malpractices of waste disposal.

As a need for further research on bioconcentration activity of leeches the following areas should be investigated:

- the implementation of a program of growing leeches under

laboratory conditions: this would assure a supply of leeches all year round, a uniformity in size, and more consistent and lower background levels of contamination.

- the study of the bioconcentration process kinetics: determination of the uptake and depuration rates; determination of the bioconcentration factors using the kinetic approach.

- the study of the influence of the load and composition of the suspended solids upon the availability of the chlorophenols to the leeches. Assessment of the impact this delayed load of chlorophenols might have upon other aquatic organisms in the region of study.

In order to have such a monitoring program in place, a more serious involvement of the regulatory agencies is required. Water quality criteria for chlorophenols have to be set for the Fraser River Estuary and an efficient biomonitoring program will benefit both the environment and people who use the resource.

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APPENDIX I

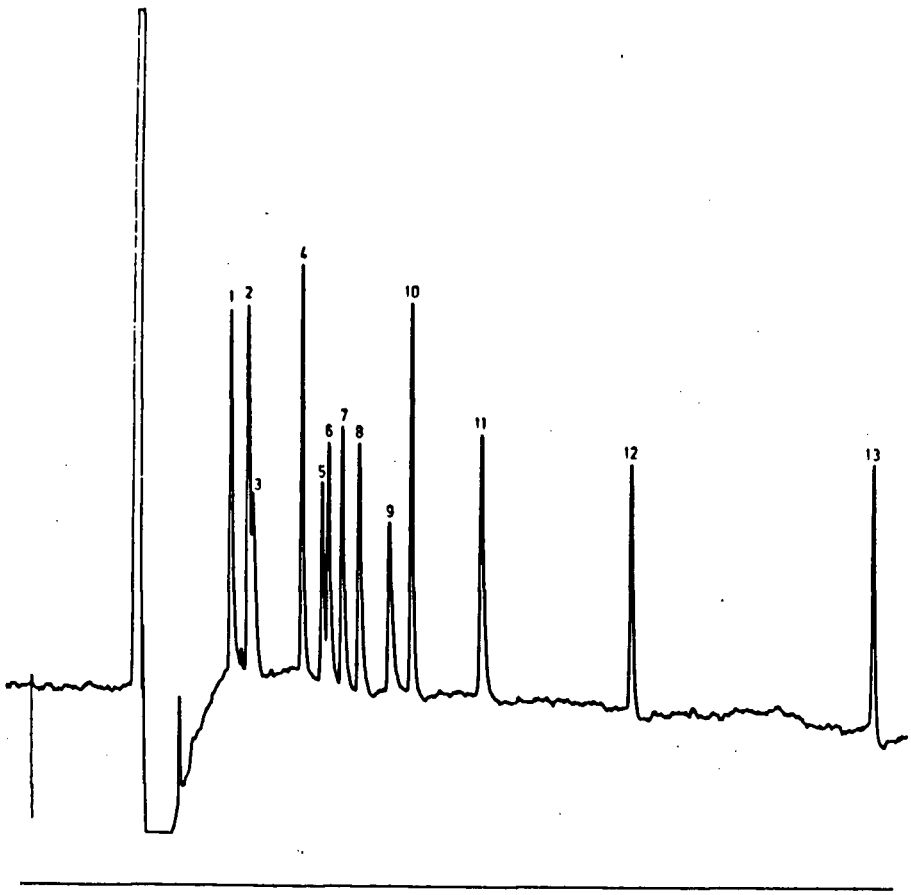

Fig.I.1. Separation of chlorophenol acetates. Linear programming from 95 C to 180 C at 3 C/ min was used. The concentration of the components(ng/uL) is given in parantheses. Peaks: 1 = 2-chlorophenol acetate(2.21); 2 = 3-chlorophenol acetate(2.22); 3 = 4-chlorophenol acetate(2.12); 4 = 2,6-dichlorophenol acetate(0.090); 5 = 2,5-dichlorophenol acetate(0.081); 6 = 2,4-dichlorophenol acetate(0.086); 7 = 3,4-dichlorophenol acetate(0.084); 8 = 2,3-dichlorophenol acetate(0.092); 9 = 3,5-dichlorophenol acetate(0.084); 10 = 2,4,6-trichlorophenol acetate(0.049); 11 = 2,4,5-trichlorophenolacetate(0.059); 12 = 2,3,4,6-tetrachlorophenol acetate(0.049); 13 = pentachlorophenolacetate(0.018), after Krijgsman and Van de Kamp,1977.

APPENDIX II

TABLE II.1

SINGLE ION MONITORING OF THE CHLORINATED PHENOLS

Parameters	Group 1: 2,4-DCP	Group 2: 2,4,5-TCP 2,4,6-TCP	Group 3: 2,3,4,6-TTCP	Group 4: PCP
Start Time(min)	16.5	19.0	23.0	26.0
Run Time(min)	2.5	4.0	3.0	3.0
Mass 1 (chlorine isotope pattern)	162	196	230	264
Mass 2 (chlorine isotope pattern)	164	198	232	266
Mass 3 (molecular ion)	204	238	272	306

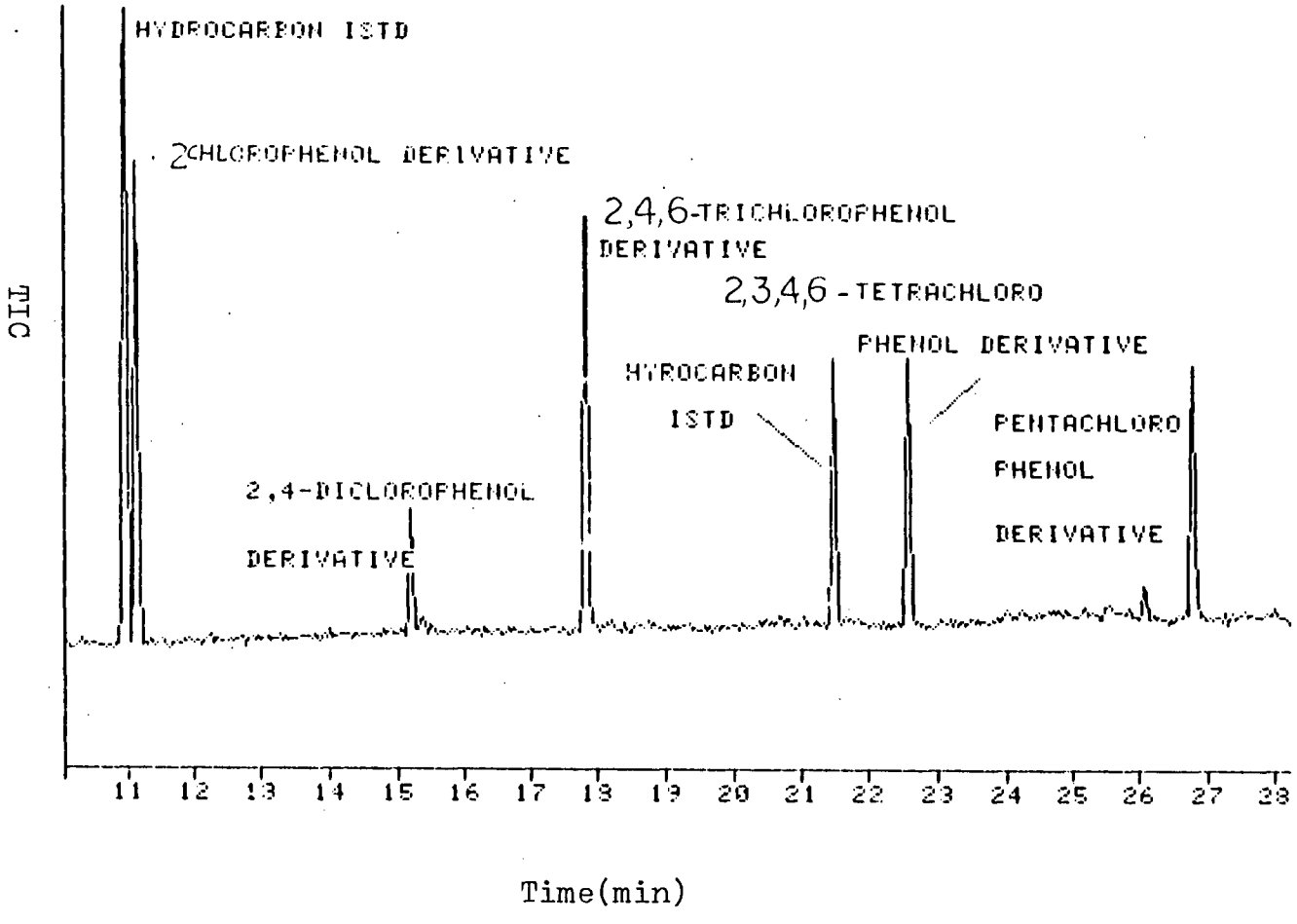

Fig.II.1. Total ion chromatogram of a standard mixture of chlorophenols used for mass scanning by electron impact. Standard components at 100 ppm concentration each.

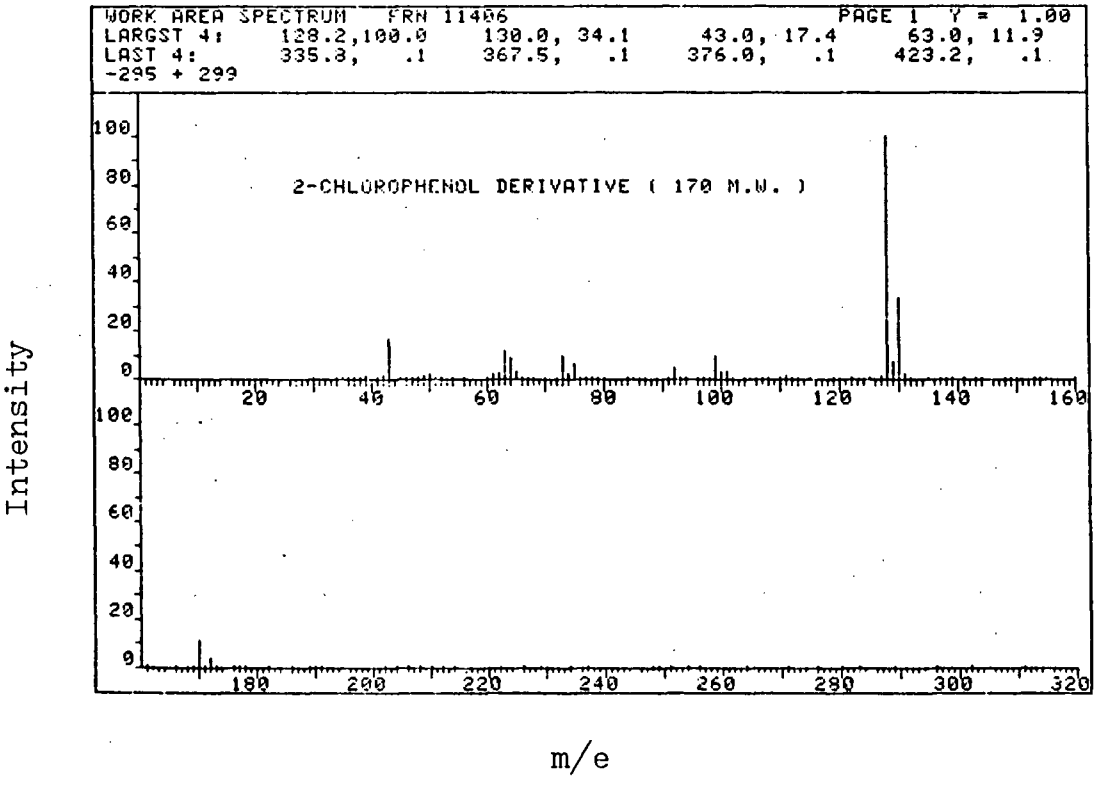

Fig. II.2. Mass spectra of 2-CP acetate, obtained by electron impact. (1) Values for peak intensity are measured relative to the most intense ion which is given a value of 100.

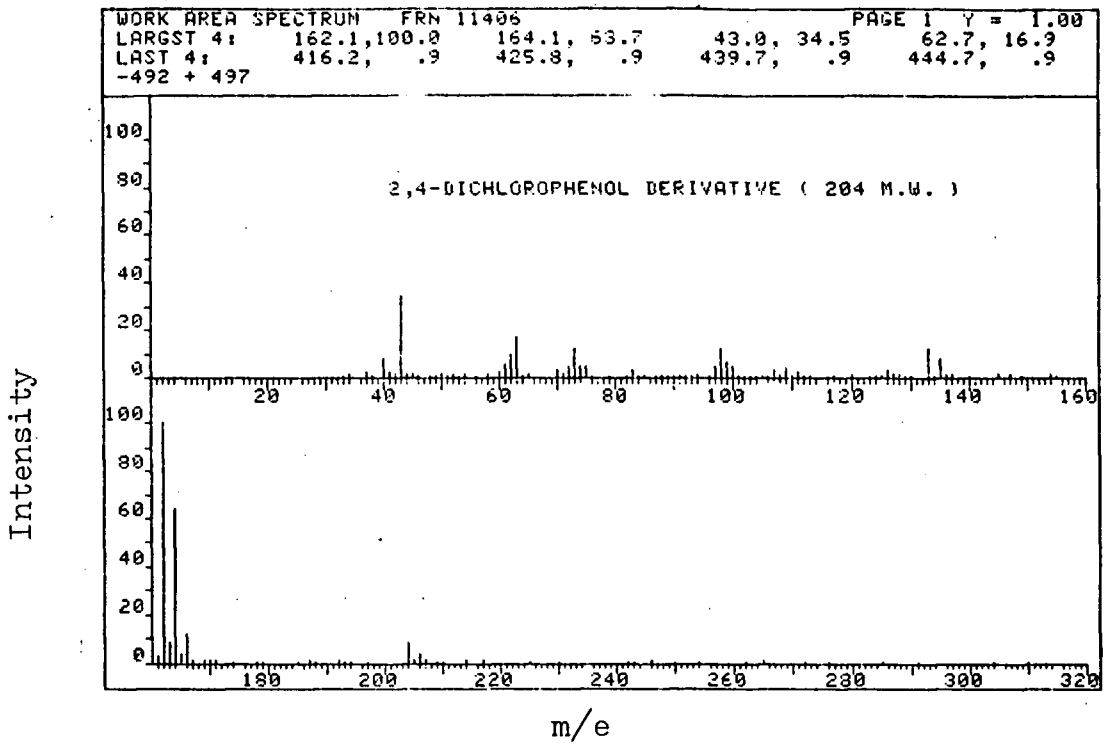

Fig. II.3. Mass spectra of 2,4-DCP acetate, obtained by electron impact.

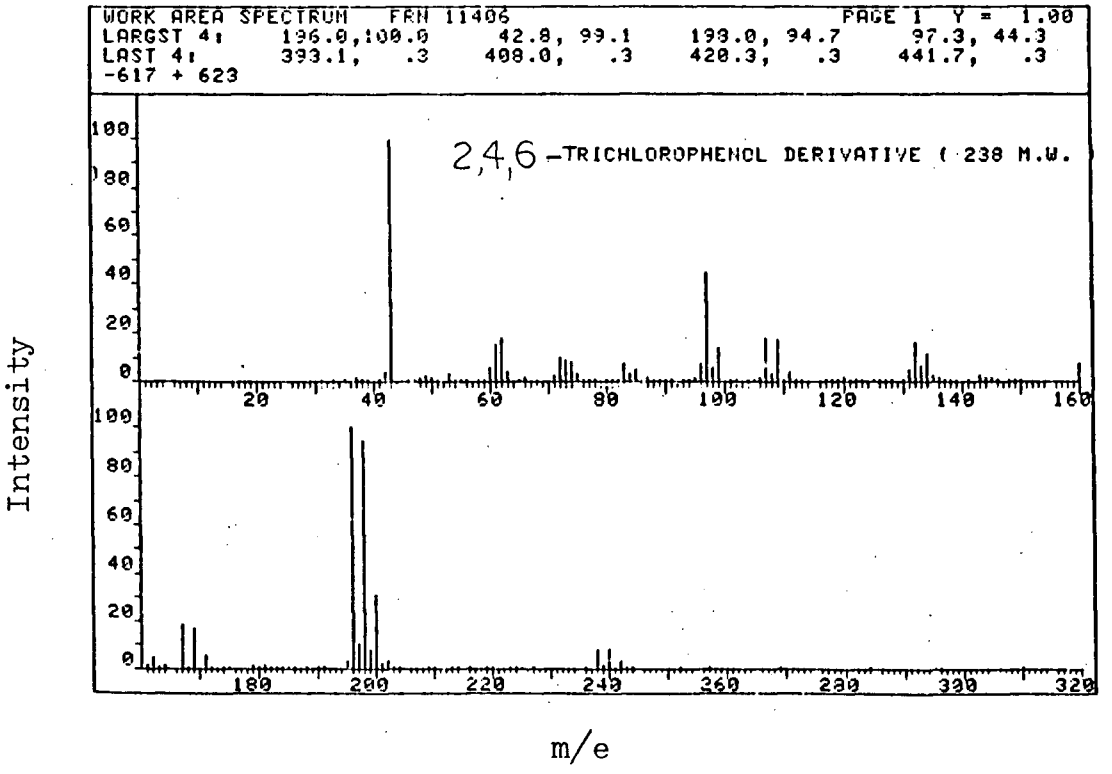

Fig.II.4. Mass spectra of 2,4,6-TCP acetate, obtained by electron impact.

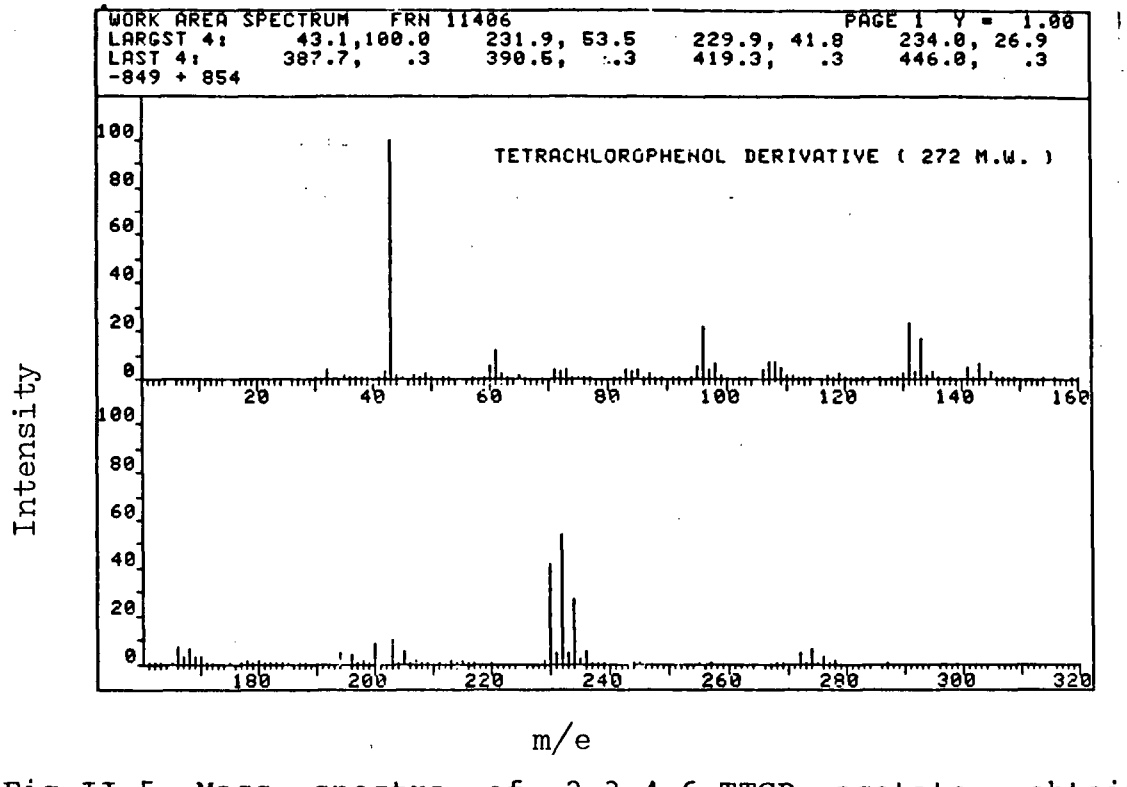

Fig.II.5. Mass spectra of 2,3,4,6-TTCP acetate, obtained by electron impact.

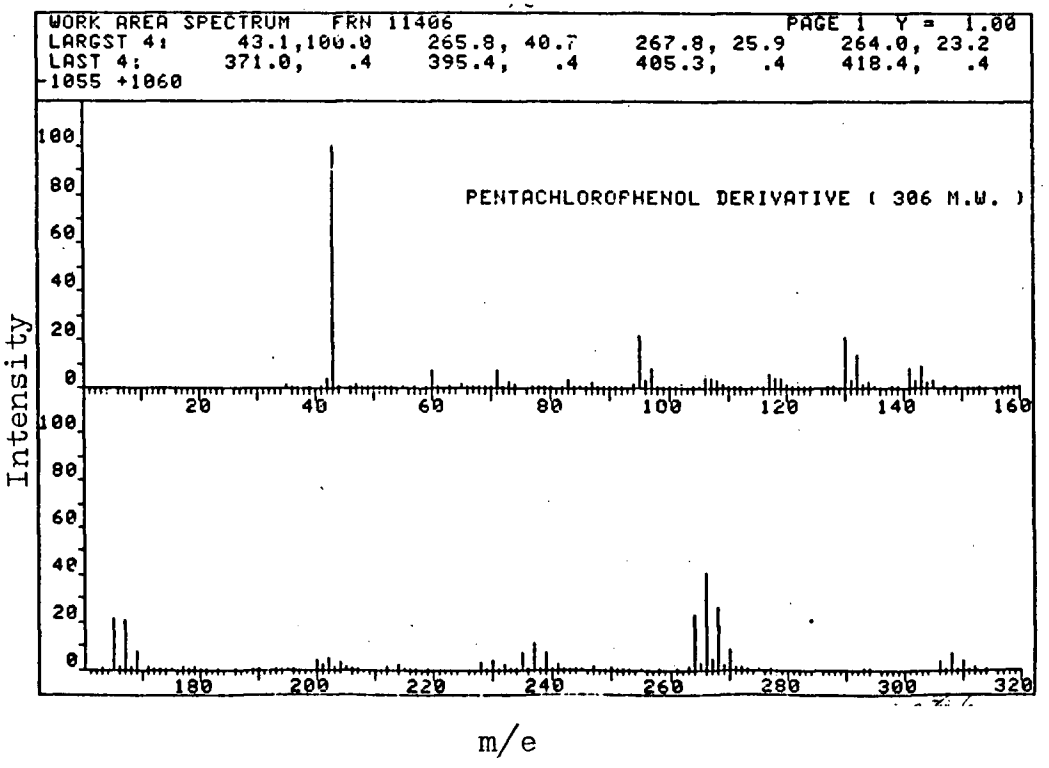

Fig. II.6. Mass spectra of PCP acetate, obtained by electron impact.

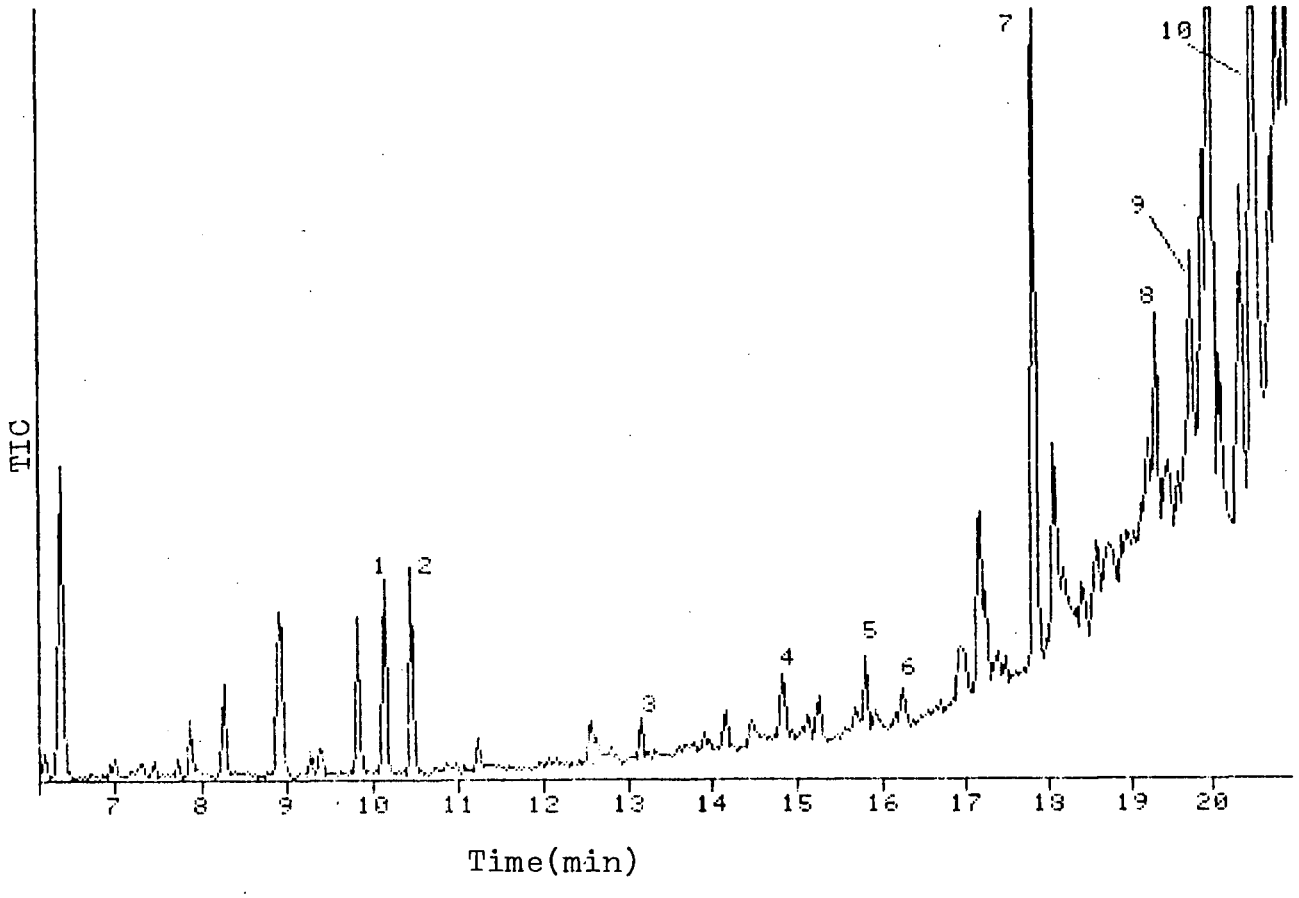

Fig. II.7. Total ion current(TIC) chromatogram of a leech extract. The leech was subjected to 7 day exposure to a standard CP solution. The standard contained 2-CP, 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TTCP and PCP which are identified as peaks 2, 3, 4, 7, and 10, respectively. Peaks 6, 8, and 9 are identified as dibromophenol acetate , tribromophenol acetate, and underivatized PCP, respectively.

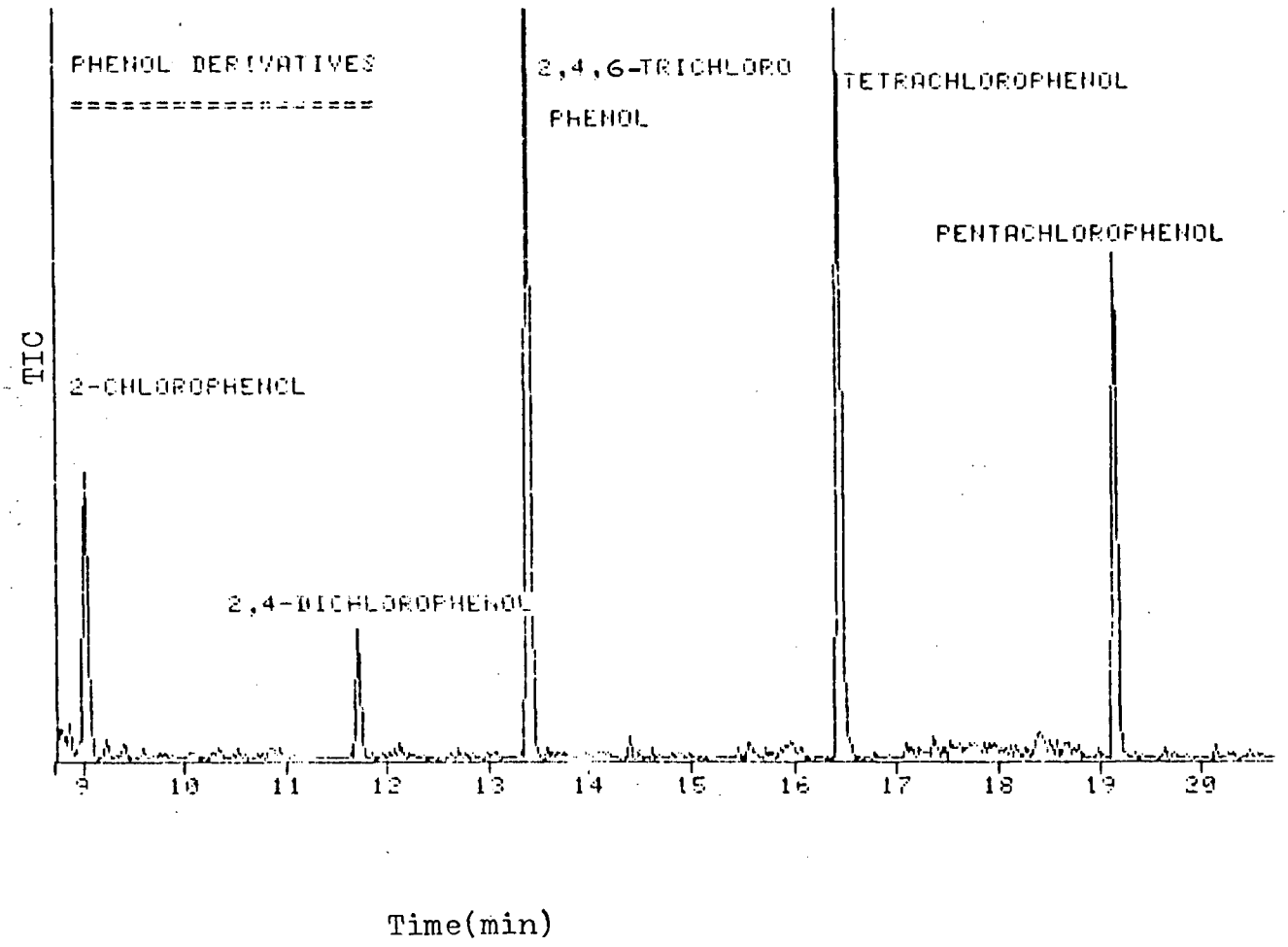

Fig. II.8. Chromatogram of a standard mixture of CPs used for positive ion chemical ionization. Ion source pressure = 0.2 x 10^{-4}. Methane used as ionizing gas.

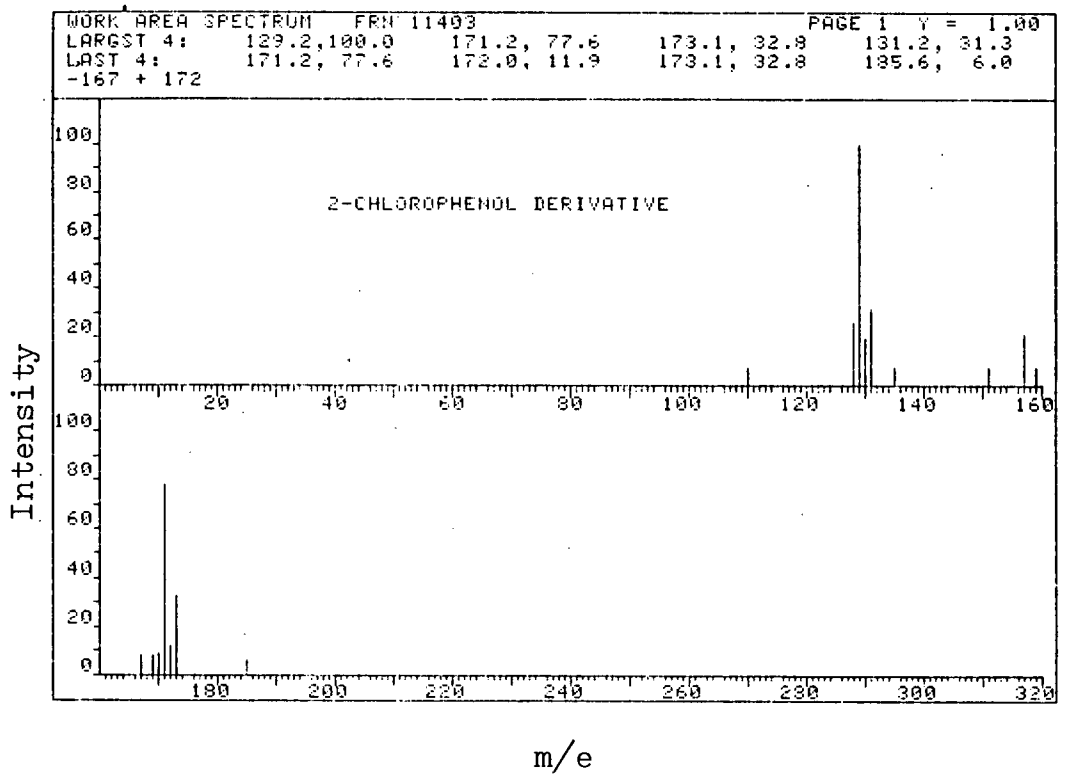

Fig. II.9. Mass spectra of 2-CP acetate, obtained by chemical ionization.

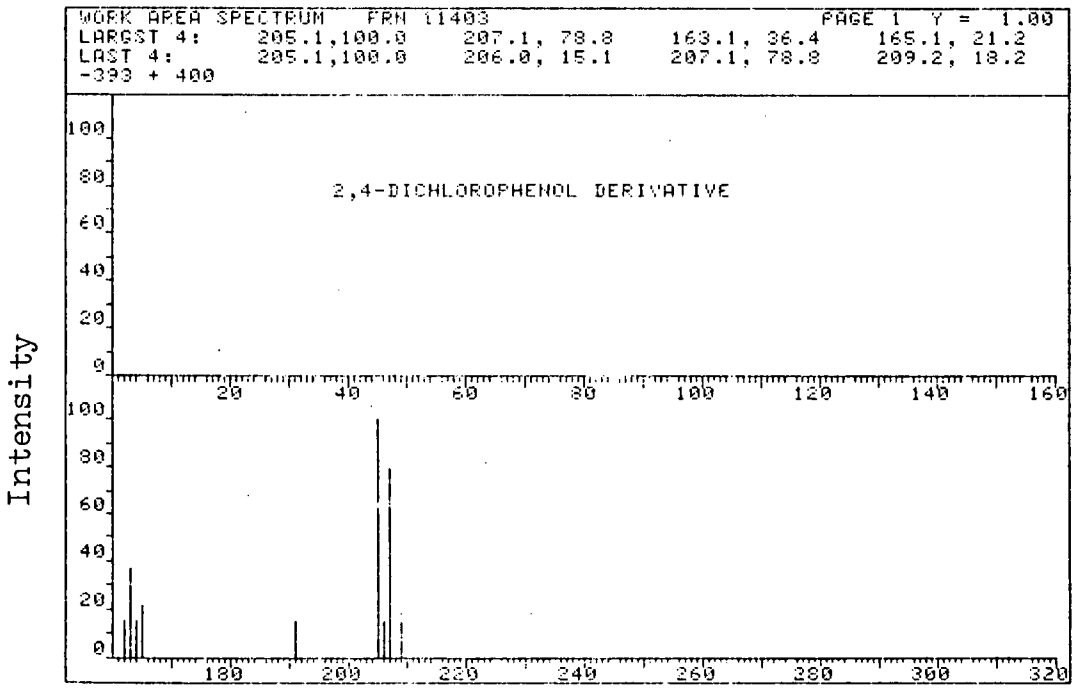

Fig. II.10. Mass spectra of 2,4-DCP acetate, obtained by chemical ionization.

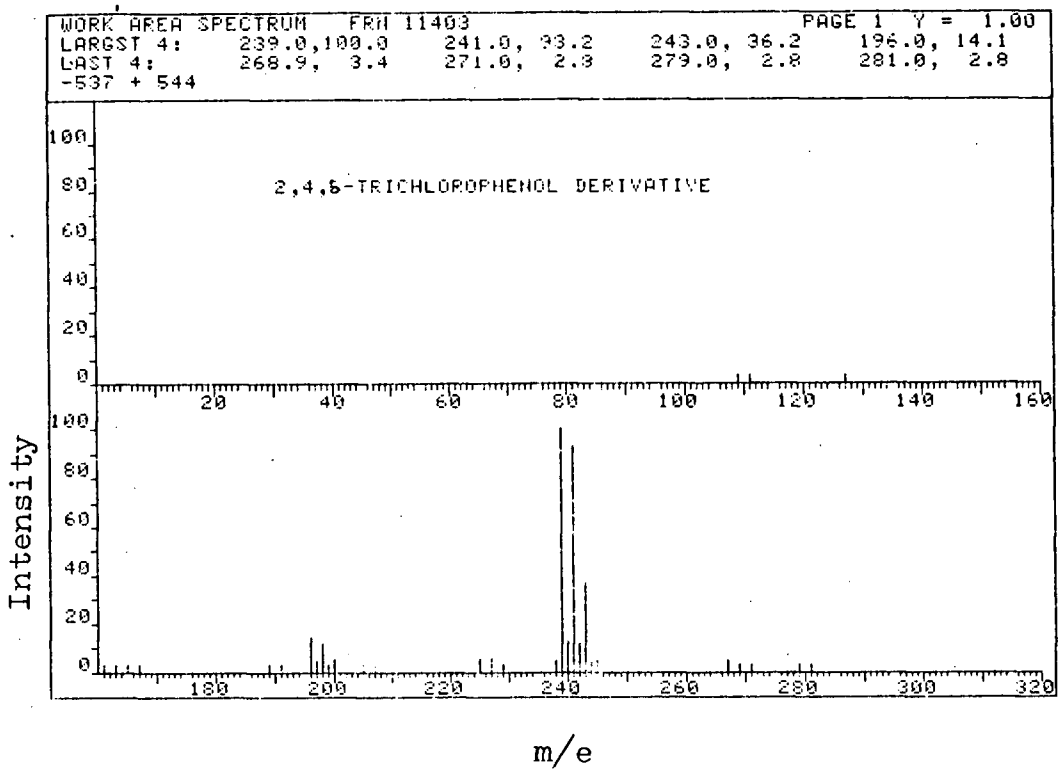

Fig. II.11. Mass spectra of 2,4,6-TCP acetate, obtained by chemical ionization.

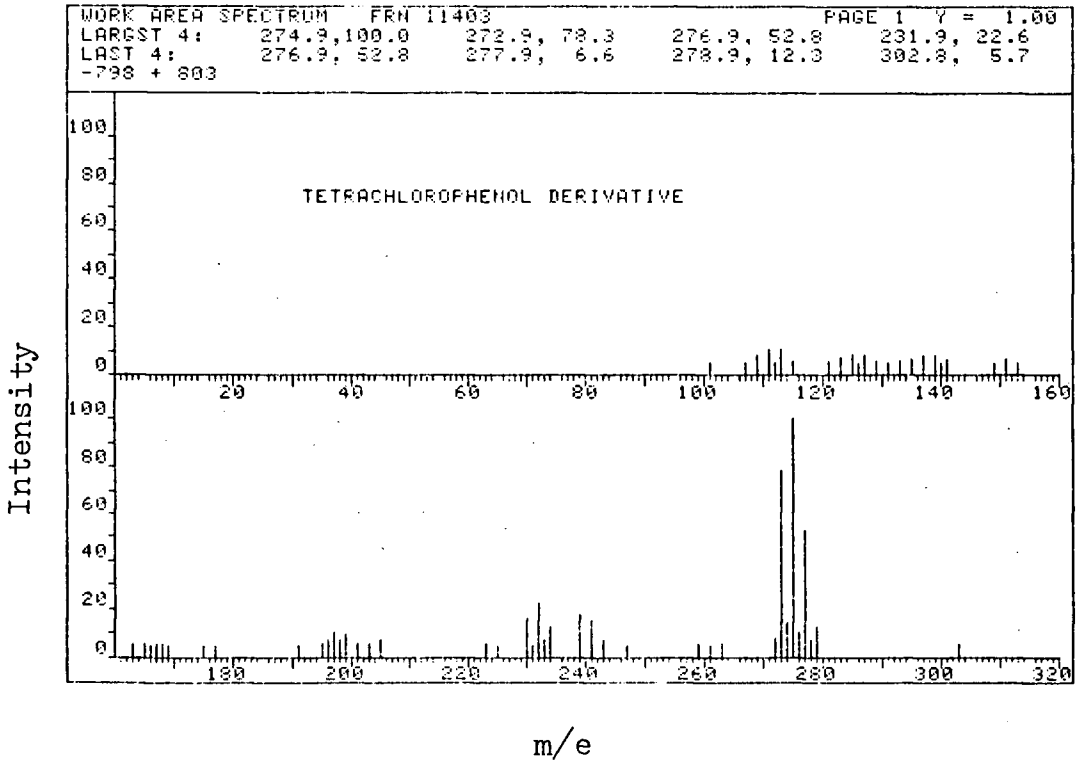

Fig. II.12. Mass spectra of 2,3,4,6-TTCP acetate, obtained by chemical ionization.

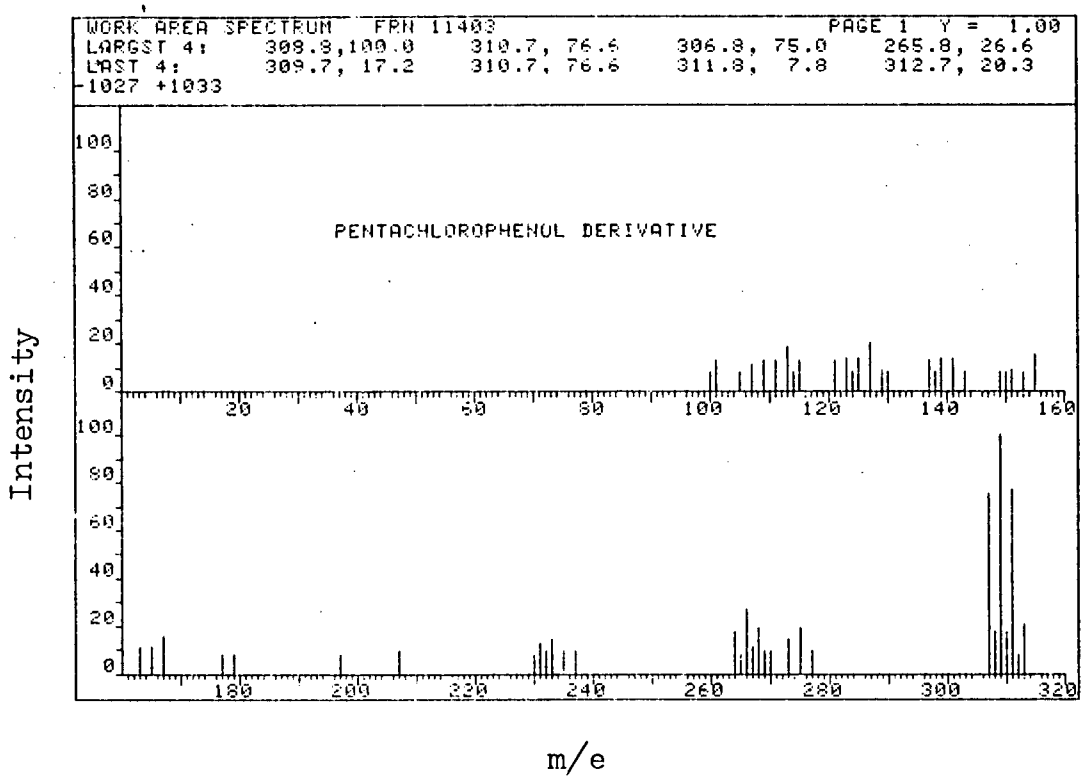

Fig. II.13. Mass spectra of PCP acetate, obtained by chemical ionization.

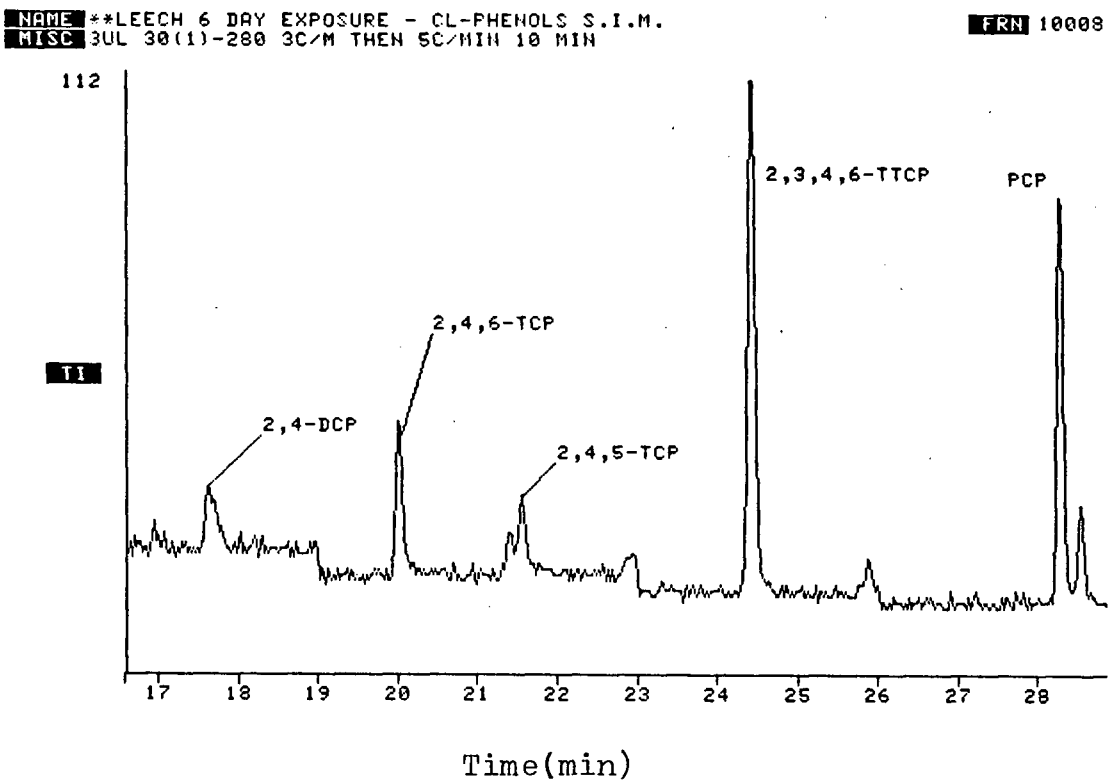

Fig. II.14a. Total ion chromatogram of a leech extract. The leech was exposed to the Fraser River water for 6 days. The chlorophenols were further identified and semiquantitated by single ion monitoring.

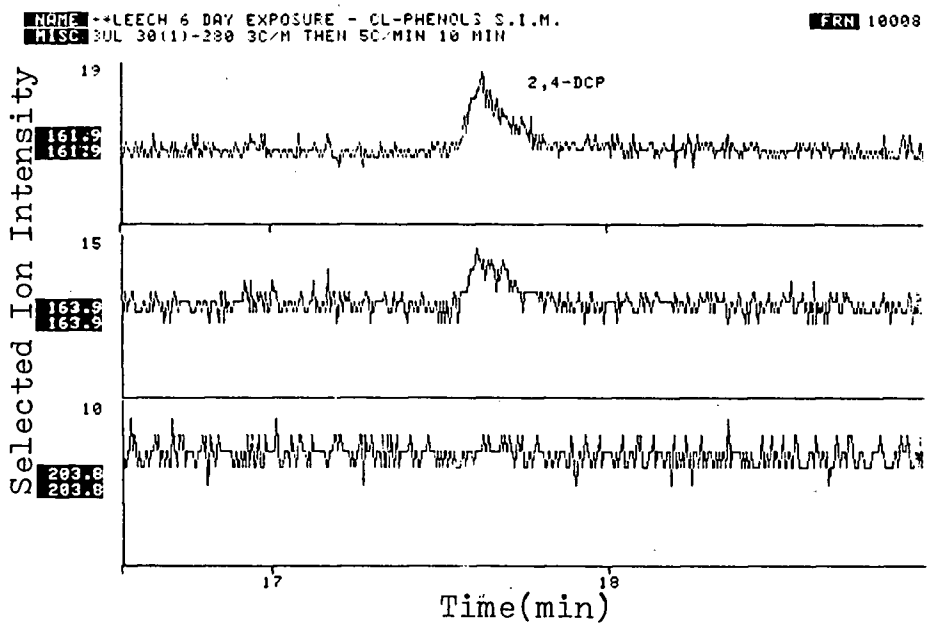

Fig. II.14b. Selected ion chromatogram of 2,4-DCP acetate, obtained by single ion monitoring.

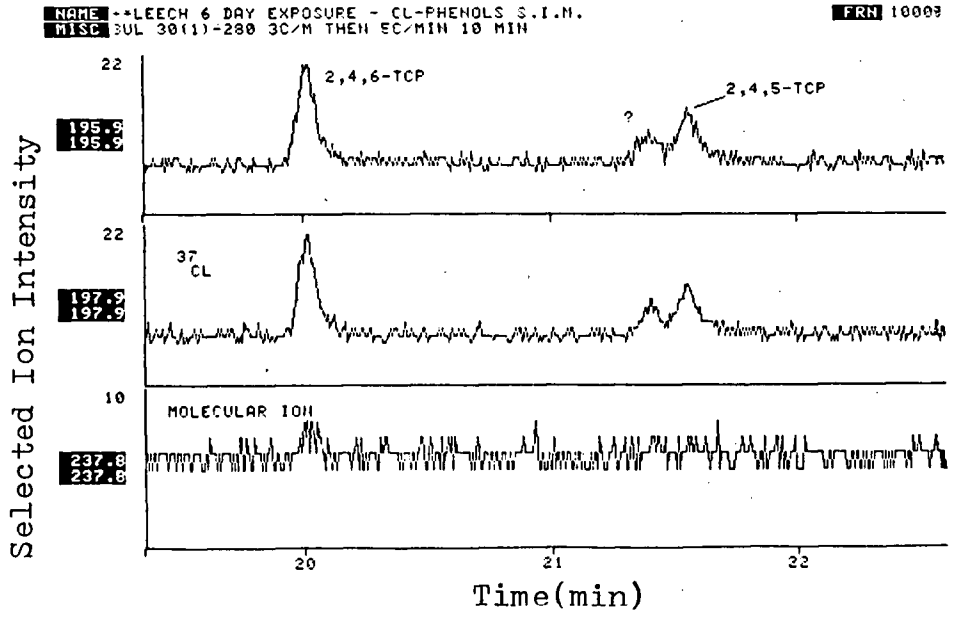

Fig. II.14c. Selected ion chromatogram of 2,4,6-TCP acetate, obtained by single ion monitoring.

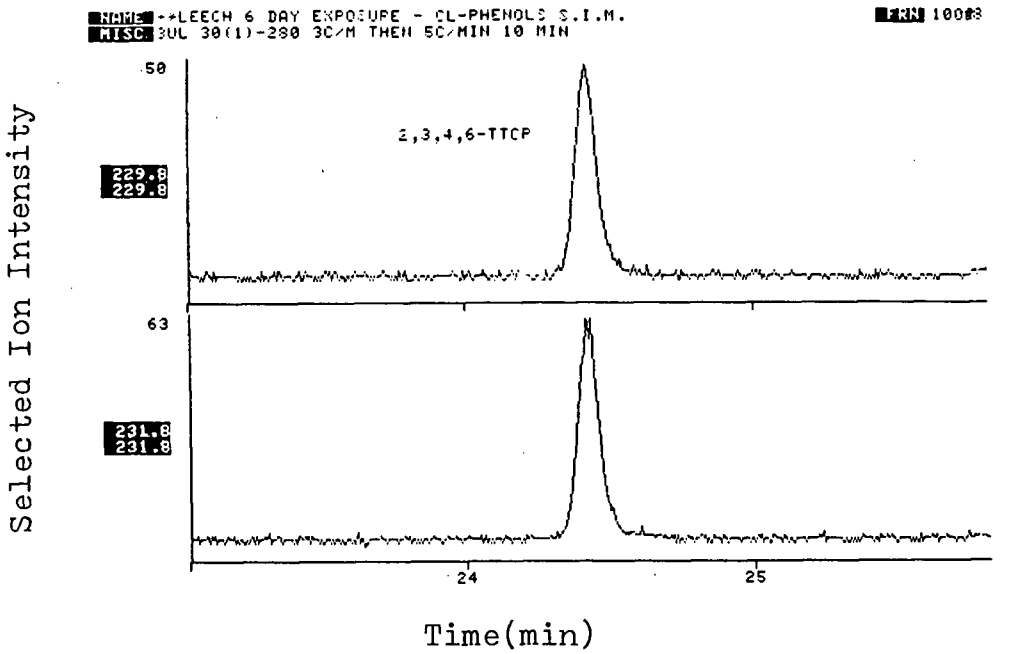

Fig. II.14d. Selected ion chromatogram of 2,3,4,6-TTCP acetate, obtained by single ion monitoring.

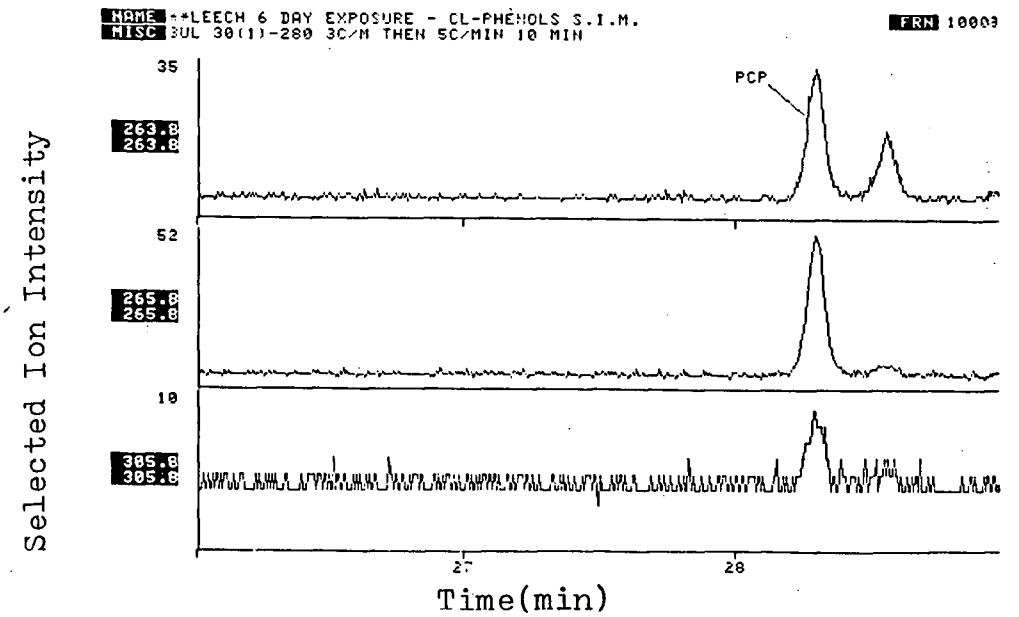

Fig. II.14e. Selected ion chromatogram of PCP acetate, obtained by single ion monitoring.

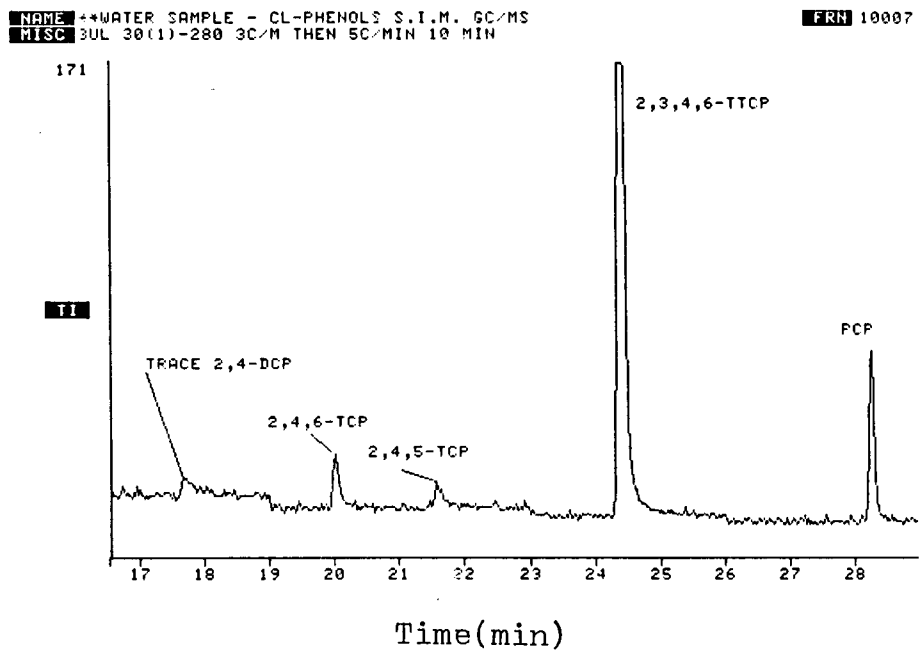

Fig. II.15a. Total ion chromatogram of a Fraser River water sample. The CPs were identified and semiquantitated by single ion monitoring.

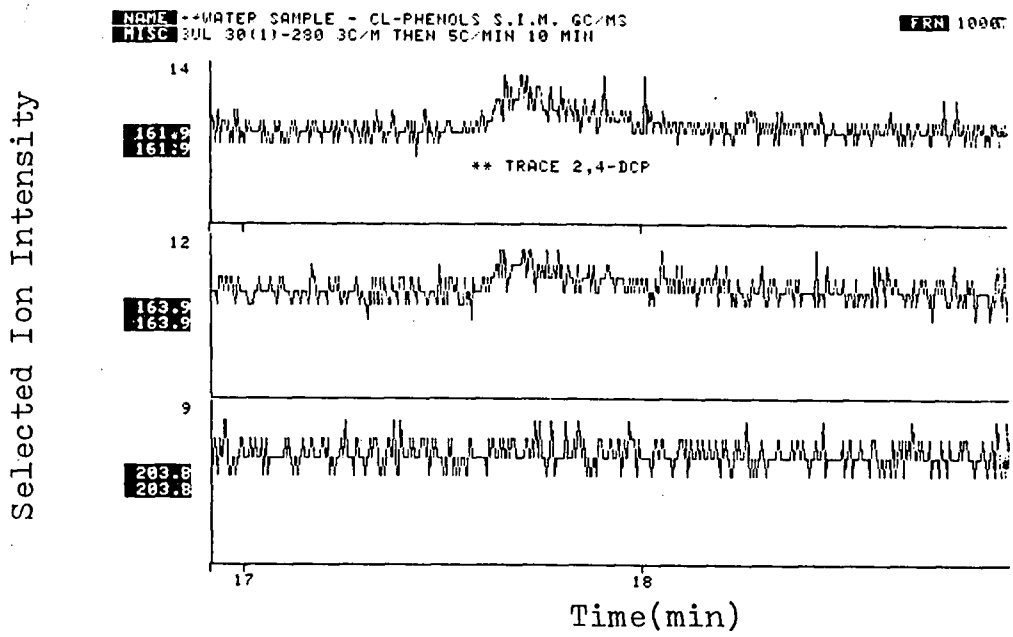

Fig.II.15b. Selected ion chromatogram of 2,4-DCP acetate from a derivatized Fraser River water sample.

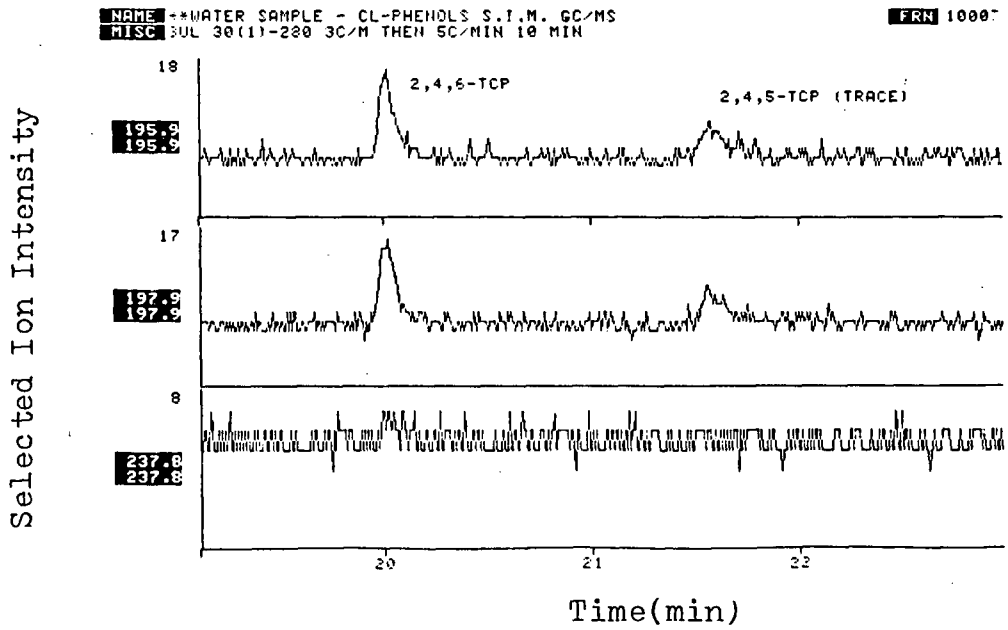

Fig.II.15c. Selected ion chromatogram of 2,4,6 and 2,4,5-TCP acetates from a derivatized Fraser River water sample.

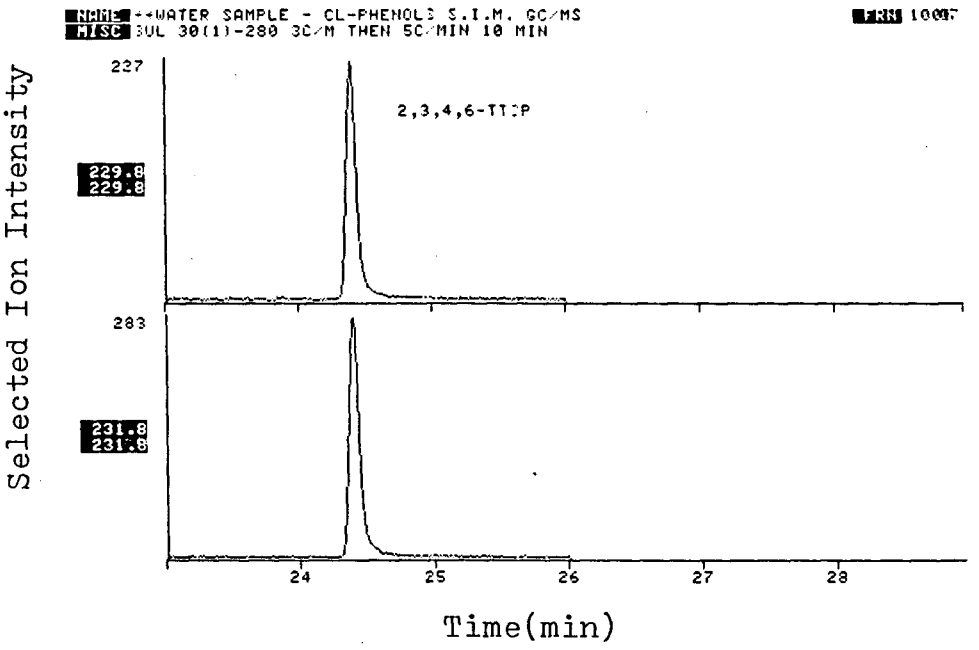

Fig.II.15d. Selected ion chromatogram of 2,3,4,6-TTCP acetate from a derivatized Fraser River water sample.

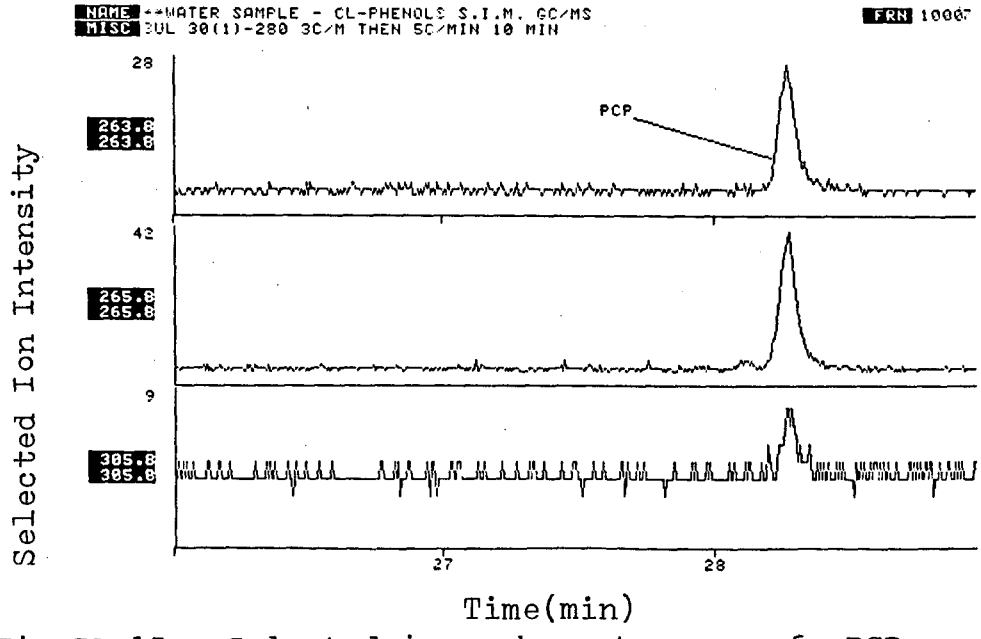

Fig.II.15e. Selected ion chromatogram of PCP acetate from a derivatized Fraser River water sample.

APPENDIX III

SYMBOLS

BF	: bioconcentration factor
CF	: concentration of chemical in fish
CW	: concentration of chemical in water
CP	: chlorophenol
DCP	: dichlorophenol
GC	: gas chromatograph, or gas chromatography
GC/MS	: GC-mass spectrometer or GC mass-spectrometry
K1	: uptake rate constant
K2	: depuration rate constant
Ka	: acid dissociation constant
Koc	: organic carbon:water partition coefficient
Kom	: organic matter:water partition coefficient
Kow	: octanol:water partition coefficient
Ks	: soil partition coefficient
nd	: not detected
PCP	: pentachlorophenol
Po/w	: octanol:water partition coefficient
QSAR	: quantitative structure-activity relationship
T	: temperature
TCP	: trichlorophenol
TIC	: total ion current
TTCP	: tetrachlorophenol

Note: TTCP symbol refers always to the 2,3,4,6-TTCP isomer, unless mentioned otherwise in text.