THE BIOAVAILABILITY OF POLYCHLORINATED DIBENZO-*P*-DIOXINS TO MUSSELS AND CRAYFISH IN AQUATIC ECOSYSTEMS

by

Mark David Segstro

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Master of Science
in the
Department of Soil Science
(Pesticide Research Lab)

Winnipeg, Manitoba, 1991

ISBN 0-315-76803-7

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ABSTRACT

The long-term environmental fate and bioavailability of 1,3,6,8-tetrachlorodibenzo-p-dioxin (1368-TCDD), 1,3,7,9-tetrachlorodibenzo-*p*-dioxin (1379-TCDD), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) were studied in limnological corrals at the Experimental Lakes Area, east of Kenora, Northwestern Ontario. On June 12, 1985, as part of a previous study (Servos, 1988) the PCDDs (1368-TCDD, HpCDD and OCDD) were added to the corrals as a sediment slurry, to simulate input from a runoff source. The 1379-TCDD congener, a contaminant in the stock 1368-TCDD, was also found in the sediment of the treated corrals from time t = 0. The PCDDs reached their maximum concentrations in the sediment by March 18, 1986. Following this, the concentrations in the sediment declined with half-lives of 4.4, 4.6, 6.2 and 6.0 years for the 1368-TCDD, 1379-TCDD, HpCDD and OCDD, respectively. The most likely pathways of disappearance of the PCDDs from sediment are export via emerging insects and degradation. Volatilization, burial and transport via the water column may play a minor role.

The bioavailability of 1368-TCDD, 1379-TCDD, HpCDD and OCDD from sediment, was studied in the limnocorrals using freshwater mussel, *Anodonta grandis* and crayfish, *Orconectes virilis* caged on the sediment during the summer of 1989 and the following winter. The PCDDs remained bioavailable even 5 years after their addition to the corrals. Both species accumulated PCDDs from the sediment into their tissues, with the crayfish generally showing higher residue levels than the mussels. Both species showed higher bioavailability indices for the

1368-TCDD and the 1379-TCDD than for the HpCDD and the OCDD. The bioavailability of all four PCDDs varied directly with the amount of sediment disturbance.

This study has shown that persistent, hydrophobic compounds such as chlorinated dioxins, are still available to the organisms present in our aquatic ecosystems years after the input into the system is believed to have ended. Assumptions that these chemicals are "safely out of the way" in the sediment of our rivers and lakes is fallacious, and needs to be addressed. Sediments will remain a source of these contaminants for years to come, even after inputs of these contaminants have ended.

ACKNOWLEDGEMENTS

I thank my supervisor Dr. G.R. Barrie Webster for the opportunity to perform this research and for his contributions to this project. I also thank Dr. Derek C.G. Muir for teaching me all the environmental chemistry that I know, for his patience, and for his other contributions to this project. I also thank the other members of my committee, Drs. Ken Friesen and Diane Malley, for their input to this project.

I thank Dr. Mark R. Servos for suggesting this study to me, just prior to his departure from the project with his Ph.D. The work I did for Mark on his part of the project was an excellent introduction to the field and lab aspects of environmental chemistry.

I thank the staff of the Freshwater Institute and the Experimental Lakes Area for their cooperation and suggestions. The contributions of Brian Billeck, I. Davies, D. Cruikshank, J. Shearer, B. Townsend, C. Baron and R. Boychuk are especially appreciated.

I thank the staff of the Dept. of Soil Science and the Manitoba Provincial Soil Testing Laboratory for their patience and support. In particular, I acknowledge the support of Len Sarna, M. Cerrato, J. Hicks and Drs. G. Racz and T.B. Goh.

Without continual encouragement from my very dear friend, Thea Kenny, and her excellent help in the lab and in the field, this project would not have been completed.

Several people have proof read all or part of this thesis, including Dr. G. Rawn, T. Kenny and C.A. Ford.

This project was financially supported by the Natural Sciences and Engineering Research Council of Canada.

Finally, I thank my wife, Cathy, for her support, patience and understanding during this entire project.

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ABBREVIATIONS

12347-PCDD	1,2,3,4,7-pentachlorodibenzo-*p*-dioxin
1234-TCDD	1,2,3,4-tetrachlorodibenzo-*p*-dioxin
1368-TCDD	1,3,6,8-tetrachlorodibenzo-*p*-dioxin
1379-TCDD	1,3,7,9-tetrachlorodibenzo-*p*-dioxin
2378-TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
2378-TCDF	2,3,7,8-tetrachlorodibenzofuran
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
AHH	aryl hydrocarbon hydroxylase
ALA	alanine
BaP	benzo(*a*)pyrene
BCF	bioconcentration factor
BI	bioavailability index
DCM	dichloromethane
DOM	dissolved organic matter
ELA	Experimental Lakes Area
EROD	7-ethoxyresorufin-*O*-deethylase
GC	gas chromatography
GC/MSD	gas chromatography with mass selective detector
GPC	gel permeation chromatography
HCB	2,4,5,2',4',5'-hexachlorobiphenyl
HpCDD	1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin
HPLC	high pressure liquid chromatography
LD_{50}	dose required to kill 50% of the test subjects
m/z	mass to charge ratio

MFO	mixed function oxidase
OC	organic carbon
OCDD	1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin
OM	organic matter
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCN	polychlorinated naphthalenes
POC	particulate organic carbon
POM	particulate organic matter
RBF	round bottom flask
R_f	relative retention time
SCUBA	self-contained underwater breathing apparatus
SIM	selected ion monitoring
TEF	toxic equivalents factor (based on 2378-TCDD)
THF	tetrahydrofuran
TLC	thin layer chromatography
U.S.E.P.A.	United States Environmental Protection Agency
UDP	uridine diphosphate

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FIGURES

Introduction

Polychlorinated dibenzo-p-dioxins are a family of 75 chlorinated hydrocarbons (Buser *et al.*, 1978), consisting of a dioxin nucleus (two benzene rings joined by two oxygen atoms) and up to eight chlorine substituents (Fig. 1). The different congeners have been found to have quite different biological activities, depending on their chlorine substitution pattern and number (Kociba and Cabey, 1985; Barnes *et al.*, 1986). The 2,3,7,8-substituted dioxins are generally considered to be the most toxic, with toxicity decreasing with increasing chlorination (Esposito *et al.*, 1980). 2378-TCDD is believed to be the most toxic of all anthropogenic chemicals. LD_{50}s for the guinea pig and the mouse are 0.6 to 2 and 114 to 284 µg 2378-TCDD/kg body weight, respectively (Kociba and Cabey, 1985). For comparison, LD_{50}'s of 1368-TCDD, HpCDD and OCDD (congeners under investigation in this study) are provided in Table 1.

PCDDs can have many biochemical, toxicological and physiological effects on the organisms in which they are found. Van der Weiden *et al.* (1990) have shown that doses of 2378-TCDD as low as 0.5 µg/kg caused both EROD induction in the liver and histopathological changes in the spleen of rainbow trout. Fish dosed with 5 µg/kg showed increases in liver weight and the first signs of growth inhibition. Lans *et al.* (1990) have shown a marked decrease in liver Vitamin A levels in female rats fed 0.5 µg 2378-TCDD/kg body weight and a decrease also in their neonates. All the treated females and their neonates showed an increase in liver size. The exposed neonates showed a decrease in birth weight as compared to controls. Serum thyroxin (T_4, a thyroid hormone) levels

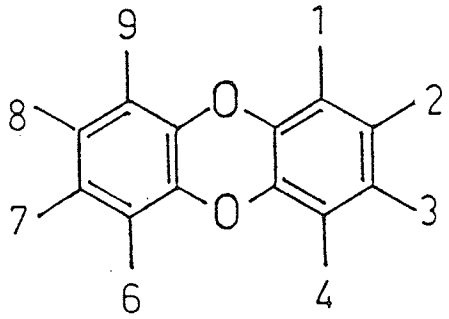


Figure 1. The structure of chlorinated dibenzo-*p*-dioxins. Chlorine atoms may be substituted at one or all of the numbered positions.

Table 1. Comparative acute oral toxicity of four chlorinated dioxins (from Kociba and Cabey, 1985).

Congener	LD_{50}(µg/kg) mouse	guinea pig
1368-TCDD	>3,000,000	>15,000,000
HpCDD	>14,000[1]	>600
OCDD	>4,000,000	>170,000[1]
2378-TCDD	114 - 284	0.6 - 2.0

[1] my estimate from Table 1, Kociba and Cabey, 1985

in the females decreased to 51% of the control level. Studies have also shown that the 2,3,7,8-substituted PCDDs are potent inducers of other enzymes, such as AHH, hepatic cytochrome P-450/448, UDP-glucuronyl-transferase, ALA-synthetase, and epidermal transglutaminase (Esposito *et al.*, 1980; Puhvel *et al.*, 1984; Kociba and Cabey, 1985; Ahlborg *et al.*, 1989). Other effects of PCDDs include teratogenicity, embryotoxicity and carcinogenicity (Courtney, 1976; Esposito *et al.*, 1980).

The pathways that introduce PCDDs into the environment have been documented extensively in the literature, and include the liquid effluent from the pulp and paper industry (Södergren, 1989; Kitunen and Salkinoja-Salonen, 1990; Whittemore *et al.*, 1990), flue gases and fly and bottom ash from municipal and industrial waste incineration (Czuczwa and Hites, 1986a; Addink *et al.*, 1990; Barton *et al.*, 1990; Düwel *et al.*, 1990), the use of chlorophenol-based compounds such as pentachlorophenol, 2,4-D and 2,4,5-T (Fishbein, 1973; Cochrane *et al.*, 1980; Bellward *et al.*, 1983), and other combustion sources, such as cigarettes and automobile exhaust (Esposito *et al.*, 1980; Marklund *et al.*, 1987).

Since PCDDs rapidly partition to suspended particulates (Servos and Muir, 1989; Servos *et al.*, 1989), the sediments of rivers and lakes become sinks for these and other persistent organic chemicals (Rice and White, 1987; McCarthy and Black, 1988). Levels of PCDDs in the sediment of lakes and rivers vary greatly with geographical location, and can range in value from less than 1 pg/g to greater than 104 ng/g. In general, as the number of chlorine atoms per dioxin molecule increases, there is a corresponding increase in the concentration of those

congeners in the sediment (Table 2).

According to Connell (1988), lipophilic compounds are the organics most likely to accumulate in living organisms. Equations relating **bioconcentration factors** (BCF) and **1-octanol/water partition coefficients** (K_{OW}) are of the form (Mackay, 1982; Hawker and Connell, 1985):

$$\log BCF = \log K_{OW} - 1.32 \qquad \text{Eqn. 1.}$$

where K_{OW} is the ratio of the concentration of the contaminant in 1-octanol to the concentration of the contaminant in water, and

$$BCF = C_t/C_w \qquad \text{Eqn. 2.}$$

where Ct = pollutant concentration in organism
Cw = pollutant concentration in water (freely dissolved)

Equation 1, however, is only valid for compounds with log K_{OW} between 2 and 6 (Connell, 1988). For compounds with log K_{OW} greater than 6 (the PCDDs for example), the bioconcentration factors decrease as the log K_{OW} increases (Muir *et al.*, 1985a). This may be due to a lack of, or to a low rate of membrane permeation due to the large molecular size (>9.5 Å) of these compounds (Opperhuizen *et al.*, 1985; Opperhuizen and Sijm, 1990).

In order to calculate the BCF, the pollutant concentration in the water must be the freely dissolved concentration. If this concentration is determined by extraction of the water as a whole, any organic material present in the water that the pollutant can sorb to, such as DOM or POM, will tend to increase the apparent water concentration. The freely dissolved concentration can only be measured if, 1) the effect of

Table 2. Concentrations of dioxins in the sediment of rivers and lakes (pg/g dry weight).

Location	ΣTCDD	ΣPCDD	ΣHxCDD	ΣHpCDD	OCDD	reference
Niagara R.[1]	99000	84500	170000	88500	104000	Kraus and Steele 1987
Hudson R.[1]	26	130	710	9600	8700	Petty *et al.* 1983
Rhine R.	-[2]	-	-	100	600	Hagenmaier *et al.* 1986
Neckar R.[1]	-	-	50	400	800	"
R. Danube[1]	-	100	100	350	1400	"
Dala R.[1]	56	59	300	1450	3700	Kjeller *et al.* 1990
Red R. Viet Nam[1]	-	-	1	8	230	Schecter *et al.* 1989
Dong Nai R.[1]	-	4	21	112	980	"
Saigon R.	-	87	220	940	4530	"
Bothnian Sea	200	500	1100	700	850	Södergren 1989
Baltic Sea (middle)	35	100	170	210	250	Rappe *et al.* 1989
Siskiwit L.	26	12	10	32	560	Czuczwa *et al.* 1984
Pentenwell Res.	187	377	1926	6910	20560	Kuehl *et al.* 1987
L. Vänern[1]	2	14	61	1100	8500	Kjeller *et al.* 1990
L. Vättern[1]	17	51	180	630	1500	"
L. Erie[3]	-	-	10	100	2000	Czuczwa and Hites 1986b
L. Ontario[3]	-	-	20	400	4800	"
L. Michigan[3]	15	25	60	160	780	"

[1] averaged value of range
[2] not detected
[3] my estimate from Figure 3

pollutant associated with POM is removed by centrifugation of the water at 20,000 g for 30 min (Servos, 1988) and 2) the pollutant associated with DOM is removed by passing the supernatant through a reverse-phase cartridge (C_{18} Sep-Pak®, Waters Scientific, Milford, MA, U.S.A.). The DOM (and the pollutants associated with the DOM) will partition to the C_{18}, while the freely dissolved phase will pass through with the eluate. The greater the hydrophobicity of the compound, the more difficult it is to measure the free concentration in the water.

Since PCDDs are extremely hydrophobic, their true solubilities in water are very low, and therefore the concentration of these compounds in water are very difficult to measure. To avoid the difficulties of measuring these very low water solubilities, a BAF, or **bioaccumulation factor**, can be calculated in place of the BCF (Ferraro *et al.*, 1990):

$$BAF = C_t/C_s \qquad \text{Eqn. 3.}$$

where BAF = bioaccumulation factor,
C_t = pollutant concentration in tissue,
C_s = pollutant concentration in sediment.

For hydrophobic pollutants the concentration in the sediment will be much greater than the concentration in the water because of the tendency of these compounds to partition to POM and DOM (Servos, 1988). Concentrations in the sediment are, therefore, much easier to measure than concentrations in the water. Sediment concentrations can be used to calculate accumulation factors because of the relationship between the sediment/water and the 1-octanol/water partition coefficients (Karickhoff *et al.*, 1979):

$$K_{OC} = 0.41K_{OW} \qquad \text{Eqn. 4.}$$

where K_{OC} = sediment/water partition coefficient.

If the BAFs are normalized to the lipid content of the organism and to the OC content of the sediment (the compartments where these compounds are most likely to be found), the partition coefficient becomes a **bioavailability index**, or BI (Foster *et al.*, 1987; Connell, 1988; McElroy and Means, 1988; Fairchild *et al.*, 1990):

$$BI = (C_t/f_L)/(C_s/f_{OC}) \qquad \text{Eqn. 5.}$$

where f_L = fraction lipid,
f_{OC} = fraction organic carbon in sediment.

Since PCDDs in the aquatic environment are mainly associated with particulates and sediment and are so highly hydrophobic, the BI appears to be a more useful factor than the BCF.

Dioxins have been found in the tissues of biota across the world. There is less geographical variation in the levels of dioxins seen in biota than in sediment, however. The values range from less than 1 pg/g to greater than 400 pg/g, but in most organisms there appears to be little or no distinct trend of increasing concentration with increasing chlorine substitution. Nevertheless, in humans this trend is observed (Table 3).

Although 2378-TCDD is considered to be the most toxic of the 75 PCDD congeners and is the most studied, the four congeners in this study, 1368-TCDD, 1379-TCDD, HpCDD and OCDD are important for several reasons. Cochrane *et al.* (1980) showed that 1368-TCDD and 1379-TCDD were the dominant congeners in 2,4-D, a widely used herbicide. Hutzinger *et al.* (1985) and Miyata *et al.* (1989) have shown that 1368-TCDD and 1379-TCDD are the dominant tetra-chlorinated congeners in incinerator fly ash and stack effluents in Europe and Japan (20% 1368-

Table 3. Concentrations of 2378-substituted dioxins in biota (pg/g wet weight).

Species and location	ΣTCDD	ΣPCDD	ΣHxCDD	ΣHpCDD	OCDD	reference
Human (adipose tissue)						
Umeä, Sweden[1]	5	18	9	56	308	Rappe *et al.* 1984
Human (milk)						
Germany[1]	2	14	22	70	396	"
Hector's dolphin						
New Zealand[1]	8	9	3	3	11	Buckland *et al.* 1990
Harbour seal						
North Sea[1]	3	4	4	2	7	Beck *et al.* 1990
Harbour porpoise						
North Sea	-[2]	-	1	22	97	"
Guillemot						
Karlsö	7	22	16	-	-	Rappe *et al.* 1984
Eagle						
Poland	-	12	6	-	-	"
Pike						
L. Vänern	78	39	17	-	22	Kjeller *et al.* 1990
L. Vättern[1]	437	160	53	-	14	"
Spottail shiner						
Niagara R.	245	210	395	215	260	Kraus and Steele 1987

Table 3. (continued)

Perch						
Bothnian Sea[1]	11	1	1	-	1	Södergren 1989
Carp						
Wisconsin R.	370	13	24	30	38	Kuehl *et al.* 1986
Lobster (digestive gland)						
Sydney Harbour	-	-	-	9	2	Clement *et al.* 1987
Miramichi R.	-	-	6	14	6	"
Chaleur Bay	-	-	22	4	5	"
Crab (meat)						
Hoh Chi Minh City	1	2	1	5	13	Olie *et al.* 1989
Crayfish						
Spring R. MO	9	-	-	-	-	Crunkilton *et al.* 1987
Clams						
Niagara R.	275	125	41	13	22	Kraus and Steele 1987
Blue mussel						
Rishiri	2	2	3	-	-	Miyata *et al.* 1987
Funka Bay	9	4	4	6	6	"
Misaka	77	15	14	20	31	"
Hokko	160	20	28	22	29	"

[1] averaged value of range
[2] not detected

TCDD, 12% 1379-TCDD and 5% 2378-TCDD). Corbet (1983) suggested that 1368-TCDD might be a good surrogate for 2378-TCDD in bioaccumulation studies. HpCDD and OCDD are important since they are the major congeners found in incinerator fly ash, stack effluents, air particulates and sediments (Czuczwa and Hites, 1984; Hutzinger *et al.*, 1985; Rappe and Kjeller, 1987; Yasuhara *et al.*, 1987).

The lack of information concerning the bioaccumulation of these four compounds (in particular, the bioaccumulation directly from the sediment) is striking. While their individual toxicities are low compared to 2378-TCDD, the combination of their toxicity, as measured by their TEF, and their levels in the environment give us reason for concern and make them and other PCDDs important for continued study. TEFs are usually expressed in terms of some biological endpoint such as MFO or other enzyme induction. The risk posed by these and other toxic organic compounds to humans and other animals is a combined function of their toxicity and the exposure of the organism to these compounds in the environment (Kimbrough, 1990). A contaminant present in high concentrations but exhibiting low toxicity may actually pose a greater risk than a more toxic compound present in lower exposure concentrations.

Several marine and freshwater organisms such as clams and mussels (Boese *et al.*, 1990; Osibanjo and Bamgbose, 1990; Ramesh *et al.*, 1990), have been shown to be capable of accumulating chlorinated organic chemicals (Langston, 1978; Tanabe *et al.*, 1989; Metcalfe and Charlton, 1990) and therefore, are good biomonitors for this type of pollution. Another reason to study bioaccumulation with these organisms is that

many of them are economically important species, and can give us an idea as to the levels of contaminants being consumed by the general human population when they eat these and similar species. Year-round monitoring with these species may provide information on local discharges and on the seasonal variation in concentration of contaminants (Ramesh *et al.*, 1990).

To assess the potential for bioaccumulation from dredged materials or contaminated sediment, present U.S.E.P.A. regulations (since 1977) require the analysis of the concentrations of organic and inorganic contaminants in an infaunal polychaete worm, an infaunal mollusc and a crustacean following exposure to the test sediment (Lake *et al.*, 1990). While we in Canada are not governed by these E.P.A. regulations, it is important that there be some continuity between studies of this sort done in any country, considering the ubiquity of these and other contaminants in the environment. The use of the freshwater mussel, *Anodonta grandis* (a mollusc) and the crayfish, *Orconectes virilis* (a crustacean) in this study of the bioavailability of chlorinated dioxins from sediment, is one small step towards that continuity.

CHAPTER 1

THE LONG TERM FATE OF FOUR CHLORINATED DIBENZO-*P*-DIOXINS IN THE SEDIMENT OF LAKE ENCLOSURES

Introduction

Polychlorinated dibenzo-*p*-dioxins are persistent, xenobiotic chemicals that tend to accumulate in the environment. In an aquatic environment, PCDDs quickly partition to dissolved and particulate organic matter (Servos and Muir, 1989; Servos *et al.*, 1989). Therefore, the sediments of lakes and rivers become sinks for these and other organic chemicals (Marcheterre *et al.*, 1985; Foster *et al.*, 1987; Reuber *et al.*, 1987; Rice and White, 1987; Corbet *et al.*, 1988; McCarthy and Black, 1988). These sediments are thus a useful medium for the determination of global or local pollution levels (Hagenmaier *et al.*, 1986). PCDDs have been found in the sediments of relatively remote lakes (Czuczwa and Hites, 1986b) and in arctic biota (Norstrom *et al.*, 1990), indicating the widespread nature of PCDD contamination.

Once the PCDDs are present in the aquatic environment, several processes can occur that will ultimately affect the environmental fate of these compounds. These may include burial by sedimentation processes occurring in the lake or river (Curtis, 1990), volatilization from the water column into the air mass above the water (Friesen, 1988), or degradation, whether by microorganisms (Ward and Matsumura, 1978) or by larger organisms present in the water or sediment. Transportation processes may also occur, including resuspension of surficial sediments

(Wetzel, 1983) and their associated contaminants, physical movement of the water column and its suspended materials further downstream or out of the lake via the outflow, or accumulation by aquatic insect larvae and subsequent removal from the lake following their emergence from the water column as adults (Menzie, 1980).

The long-term fate of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin, 1,3,7,9-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin and octachlorodibenzo-*p*-dioxin was investigated in aquatic mesocosms over a period of five years. This study is directly concerned with the samples collected during the last two years. These congeners were chosen because they are dominant congeners in incinerator fly ash, air particulates, sediment and chlorophenol-based compounds such as 2,4-D (Cochrane *et al.*, 1980; Bellward *et al.*, 1983; Czuczwa and Hites, 1986a; Czuczwa and Hites, 1986b; Nakano *et al.*, 1987; Buck and Kirschmer, 1988). The structure and characteristics of 1368-TCDD, as well as its low toxicity, made it a suitable surrogate for 2378-TCDD in these mesocosm studies (Servos, 1988). The results generated (including sediment half-lives) give us a better understanding of the long-term fate of these important compounds.

Experimental

A. Chemicals

Universally diphenyl-ring-labelled (^{14}C)-OCDD and nonlabelled 1368-TCDD and HpCDD were purchased from Pathfinder Labs, Inc., St Louis, MO, U.S.A. The OCDD had a specific activity of 20.58 mCi/mmol and was >99.8% radiochemically pure by reverse-phase HPLC (Servos, 1988). Universally diphenyl-ring-labelled (^{13}C)-HpCDD and (^{13}C)-1234-TCDD (used as internal standards) were also purchased from Pathfinder Labs. Additional nonlabelled 1368-TCDD and 1379-TCDD, HpCDD and OCDD were purchased from Wellington Laboratories, Inc., Guelph, ON.

All solvents used in the workup and analysis of samples, including hexane, DCM, toluene, methanol, THF, and acetone, were "distilled in glass" quality and were purchased from Burdick & Jackson Co. via Baxter-Canlab Ltd., Winnipeg, MB.

Purified copper metal (electrolytic powder) used in the removal of sulphur from the sediment samples was obtained from Fisher Scientific Co., Winnipeg, MB. Concentrated HNO_3 and HPLC grade water used in the cleanup of the copper powder was supplied by Burdick & Jackson Co. via Baxter-Canlab Ltd., Winnipeg, MB.

Potassium dichromate ($K_2Cr_2O_7$), ferrous sulphate ($FeSO_4 \cdot 7H_2O$), concentrated H_2SO_4, Mohr's solution (156.8 g ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$] in 100 mL conc. H_2SO_4, diluted to 2000 mL with distilled water), indicator solution (0.1 g *N*-phenylanthranilic acid and 0.1 g Na_2CO_3 in 100 mL distilled water), all reagent grade chemicals (Baxter-Canlab Ltd., Winnipeg, MB), were used in the sediment organic

carbon and organic matter determinations.

Gel permeation beads (Bio-Beads S-X3), for lipid removal, were purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A.

Nitrogen gas to concentrate samples, and prepurified helium for GC analyses, were supplied by Welders Supply Co., Winnipeg, MB.

B. Site Preparation

During May 8-11, 1985, five 5 m diameter x 2 m deep, nylon-reinforced polyethylene limnological corrals were installed by M.R. Servos in the littoral zone of Lake 304 at the Experimental Lakes Area, in Northwestern Ontario (Johnson and Vallentyne, 1971; Servos, 1988). Approximately 30 cm of the corral curtain was pushed into the sediment at the base of the corral and anchored in place with sand bags. Large polystyrene floats were placed in a pocket surrounding the top of each corral in order that the corral remain upright.

On June 12, 1985, the 1368-TCDD, HpCDD and OCDD were added to the limnocorrals as a sediment slurry in order to simulate their input from a runoff source. A detailed description of the site, the experimental design and the fate of the PCDDs added, can be found in Servos (1988). Table 4 summarizes the amounts of each congener added to each corral. At the end of Servos' experiment in May, 1987 (day 710), the top 1 m of each of the corral curtains was removed.

C. Sampling

Using SCUBA, a diver collected duplicate sediment cores with a 5

Table 4. PCDD congeners added to the limnocorrals of Lake 304, ELA in May, 1985[1].

Corral	Congeners Added
A	2.0 mg 1368-TCDD[2]
	2.1 mg HpCDD
	4.3 mg (^{14}C)-OCDD
B	no PCDDs added
C[3]	3.8 mg (^{14}C)-1368-TCDD
	2.3 mg OCDD
D	2.0 mg 1368-TCDD[2]
	2.5 mg HpCDD
	4.3 mg (^{14}C)-OCDD
E[3]	3.9 mg (^{14}C)-1368-TCDD
	2.3 mg OCDD

[1] M.R. Servos, Dept of Fisheries and Oceans, Burlington, ON, pers. comm.

[2] Shown in this study to contain a 2:1 ratio of 1368-TCDD:1379-TCDD

[3] Corrals C and E were not used in this experiment since they were not spiked with HpCDD

cm internal diameter KB corer from corrals A, B and D on days 1499, 1562 and 1807 post "spike". The coring tube was pushed into the sediment and a rubber stopper placed into the top opening. Upon removal from the sediment, but while the coring tube was still below the surface of the water, a second rubber stopper was placed into the bottom opening. On shore, the tube was held vertically, the water was siphoned off and the core pushed up the coring tube into a short plexiglas cylinder. The 0-6 cm layer was cut with a thin sheet of galvanized steel, washed into a 500 mL Mason jar and frozen at -32°C until analyzed.

An Ekman dredge sample of the sediment at the center of Lake 304 was taken on day 1562, and frozen at -32°C until needed for a recovery study.

Frozen, 0-6 cm sediment cores from five sampling periods between June 20, 1985 and May 18, 1987 (day 8 to day 697, post "spike") were gratefully received from M.R. Servos.

D. Analysis

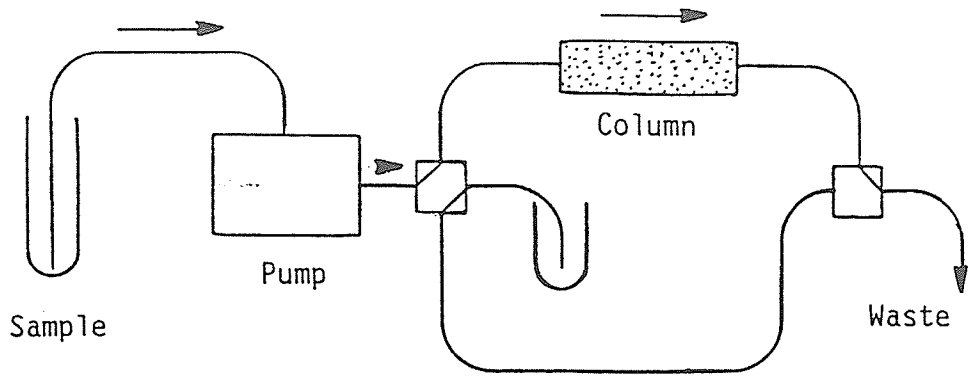
All sediment cores were lyophilized on a Lab Con Co. Freeze Dry 5 freeze drier (Fisher Scientific Co., Winnipeg, MB) at -68°C to -75°C and a pressure of 0.5 to 1 Pa for at least 72 h. After being thoroughly mixed, the dried sediments were weighed into 500 mL round bottom flasks, spiked with 12 ng (^{13}C)-1234-TCDD and 20 ng (^{13}C)-HpCDD and refluxed overnight (≈16 h) in 270 mL of 1:1 DCM/hexane. The mean dry weight of sediment refluxed was 3.810 ± 1.204 g. The sediments were filtered through Millipore GV 0.22 μm filters (Millipore (Canada) Ltd., Mississauga, ON) and washed with 3 x 50 mL of 1:1 DCM/hexane. The

extract was reduced to ≈2 mL by rotary evaporation and transferred to 20 mL screw cap test tubes.

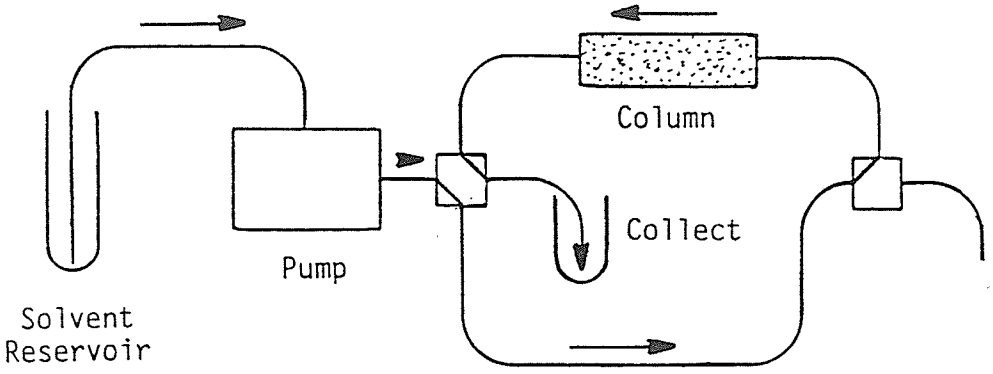
In order to be free of interferences from sulphur, the extracts were pretreated with copper powder, prepared as follows. The copper powder was washed in ≈50 mL 10% (v/v) HNO_3 for ≈1 h. The acid was poured off and the copper washed with five 50 mL rinses of distilled water, 5 of acetone, 5 of hexane and was stored under hexane.

After ≈1 g of copper had been added to each test tube, the tubes were mixed and allowed to stand for 1 h. The extracts were transferred to the head of a 60 g GPC column (2.5 cm i.d.) and the test tube washed with two 2 mL washes of 1:1 DCM/hexane. Once the sample was entirely on the column, 300 mL of 1:1 DCM/hexane was added to the column. The first 150 mL, containing lipids and pigments, was discarded. The second 150 mL, containing the PCDDs, was collected in a 500 mL round bottom flask, reduced to 2 mL by rotary evaporation, transferred to a 15 mL graduated centrifuge tube and made up to 15 mL with 1:1 DCM/hexane.

The PCDDs were isolated on an activated carbon/glass fibre column (Fig. 2). The isolation of the PCDDs by GPC and an activated carbon/glass fibre column is based on the method of Norstrom and Simon (1990). The column was made up as follows. An 0.8000 g aliquot of 2.7 μm retention Whatman GF/D glass microfibre filter paper (Baxter-Canlab Ltd., Winnipeg, MB) was ground in 80 mL DCM using a model PT 10-35 Polytron homogenizer (Brinkman Instruments (CANADA) Ltd., Rexdale, ON) set at high speed. An 0.0800 g aliquot of Amoco Super A Activated Carbon AX-21 (#610202-30) was added and the slurry was ground until well mixed. The slurry was packed into an Anspec Omnifit pyrex tube #H8416 (Anspec Company, Inc., Ann Arbor, MI, U.S.A.) to a length of 20.0 mm.



Load Sample (forward)



Elute PCDDs (reverse)

Figure 2. Diagramatic representation of the carbon/glass fibre column and pump apparatus.

The ends of the column were also from the Anspec Co. and were 1) fixed length endpiece #H8424 and 2) variable length endpiece #H8425, with a 2 μm Whatman HPLC column frit (Baxter-Canlab Ltd., Winnipeg, MB) at each end. All tubing connecting the column to the pump, the waste and the collection vessels was 3.2 mm o.d. teflon tubing. Valves used were Hamilton Mininert valves with Hamilton fittings (Baxter-Canlab Ltd., Winnipeg, MB). The pump was a Masterflex 1-100 rpm peristaltic pump with a model 7021-20 QuickLoad pump head fitted with 30 cm of size 14 (6.2 mm o.d., 1.6 mm i.d.) Viton tubing (Cole Parmer Instrument Co., Chicago, IL, U.S.A.).

The sample was pumped onto the column at 5 mL/min, followed by a 45 mL wash of 75:20:5 (v/v) DCM/methanol/toluene, both in forward flow, and discarded. The wash removed any compounds such as lipids and pigments not removed by the GPC, as well as other nonplanar compounds present in the extract. This was followed by 110 mL of toluene in reverse flow, which was collected. This back-elution with toluene is sufficient to desorb the PCDDs from the carbon column. The column was then regenerated in reverse flow with 150 mL toluene, 150 mL MeOH, 150 mL toluene and 25 mL DCM. The collected toluene was evaporated to 2 mL, transferred to a 15 mL graduated centrifuge tube, further concentrated to ≈100 μL using a gentle stream of nitrogen, and transferred to 600 μL Reacti-Vials (Chromatographic Specialties Ltd., Brockville, ON). The extracts were then evaporated to a pre-calibrated, 50 μL mark on the Reacti-Vial. A 2 μL aliquot was then injected onto a 30 m DB-5 column (J&W Scientific, Folsom, CA, U.S.A.) in a Hewlett-Packard 5890 gas chromatograph with a Hewlett-Packard 5970 series mass selective detector (see Table 5 for conditions). The ions used in the SIM analysis were:

Table 5. GC/MSD conditions for the determination of PCDDs.

Temperature program	100°C for 3 min, 20°C/min to 180°C, 5°C/min to 300°C, hold 2 min
Injector temperature	250°C
Detector temperature	280°C
Carrier gas	Helium
Flow rate	1.5 mL/min
Ion energy	70 eV
Multiplier voltage	2000 V
Dwell time	100 msec

m/z 259, 320, 322 and 324 (from 13.5 to 21.5 min) for the 1368-TCDD and 1379-TCDD congeners, m/z 363, 424, 426 and 428 (from 21.5 to 29.5 min) for the HpCDD and m/z 397, 458, 460 and 462 (from 29.5 to 33.0 min) for the OCDD. The three ions of greatest abundance in the parent cluster as well as the ion of greatest abundance in the M^+-COCl cluster were chosen since they are characteristic ions for the PCDDs. If the ion ratios observed for the samples are the same as for the standards, one can be confident that there are no other compounds interfering with the quantitation of the PCDDs. The base peak in the spectrum of each of the PCDDs was the peak used for quantitation. The ions monitored for the internal standards were m/z 334 for the (^{13}C)-1234-TCDD and m/z 438 for the (^{13}C)-HpCDD.

The equation used to calculate the concentrations of the PCDDs in the sediment was:

$$\frac{\text{pg PCDD}}{\text{g sediment}} = \frac{(\text{area PCDD}_{samp})*(\text{area IS}_{std})*(50)}{(\text{area PCDD}_{std})*(\text{area IS}_{samp})*(wt)} \qquad \text{Eqn. 6.}$$

where area PCDD_{samp} = area of PCDD peak in sample,
area IS_{std} = area of internal standard in standard,
area PCDD_{std} = area of PCDD peak in standard,
area IS_{samp} = area of internal standard in sample,
50 = pg standard injected,
wt = dry weight of sediment in g.

Sediment percent organic matter content was determined on two randomly chosen sediment samples (Ekman dredge sample and corral B, day 1562), by the Manitoba Provincial Soil Testing Laboratory. The first method was a slow, overnight ignition in a muffle furnace and re-weighing in the morning. The % OM was calculated from the difference in weights. The second method involves a conc. H_2SO_4 digestion (20 mL) in the presence of 1 N $K_2Cr_2O_7$ (10 mL). After cooling, 250 mL of distilled

water was added and the sample was auto-titrated with 0.5 N $FeSO_4 \cdot 7H_2O$.

For comparison, the sediment percent organic carbon content was determined for the corral B, day 1562 sample by the method of Yeomans and Bremner, 1988. A preweighed sample was digested for 30 min at 170°C in 7.5 mL conc. H_2SO_4 and 5.0 mL 1.00 N $K_2Cr_2O_7$. After cooling, the digest was diluted with 80 mL distilled water. After 0.3 mL of the indicator solution (0.1 g *N*-phenylanthranilic acid and 0.1 g Na_2CO_3 in 100 mL distilled water) was added, the sample was titrated with 0.2 N Mohr's solution on a Mettler DL21 titrator with a Mettler DM140 electrode (Fisher Scientific Co., Winnipeg, MB).

The **% OC** was calculated by the following equation:

$$\% \text{ OC} = \frac{(A)(0.2)(0.003)(100\%)}{(\text{sample wt in g})} \qquad \text{Eqn. 7.}$$

where $A = [(B - S)(\frac{U - B}{U})] + (B - S)$,

B = mL titrant for boiled control,
U = mL titrant for nonboiled control,
S = mL titrant for sample,
0.2 = the Normality of the Mohr's solution,
0.003 = a constant.

To be able to calculate the amount of dichromate lost by boiling in the abscence of sample, at least one boiled control and one nonboiled control with 5 mL of 1.00 N $K_2Cr_2O_7$ and 7.5 mL of conc. H_2SO_4 should be included in each series of analyses (Yeomans and Bremner, 1988).

Results and Discussion

A. Sediment Characteristics

The composition of the surficial sediments (0-6 cm) of Lake 304 at the 2 m depth contour was 51% clay, 37% silt and 12% sand (Servos, 1988). The mean experimental percent organic matter by digestion was 46.2% ± 2.9%, whereas it was 50.8% ± 1.1% when determined by ignition. Results obtained by ignition might be expected to be higher than by digestion because ignition oxidizes all the organic matter to CO_2, even that which is tightly bound to the sediment (total OM), while digestion determines only the easily extractable OM. Organic matter that is tightly bound to the sediment particles will not be determined by this method. Lyman (1982) reports that the percent OM in a soil can be converted to the percent OC in a soil by dividing the percent OM value by 1.724. It is not stated whether this relationship also holds true for sediments. Servos (1988) reported an organic carbon content in the sediment of 25.2%, which compares favourably with a mean of 25.9% ± 1.4% for the more recent sediment samples. Therefore, for the Lake 304 sediments, the percent OM is equal to the percent OC multiplied by 1.96. The water content of the sediment (0-6 cm) was estimated to be 0.85-0.92 mL H_2O/mL of sediment (Servos, 1988).

Curtis (1990) reports the sedimentation rate at the center of Lake 304 to be ≈0.6 g/m^2/d. Most sedimentation occurs in the 6 months from May to October, i.e., over ≈180 days (G. Brunskill, Freshwater Institute, Dept of Fisheries and Oceans, Winnipeg, MB, pers. comm.). Therefore, the yearly sedimentation rate for Lake 304 is 108 g/m^2/y.

Since the corrals have an area of 19.6 m^2, 2120 g of sediment is deposited per corral per year. The sediment has a mean density of 1.9 g/cm^3 (Servos, 1988), which translates into 1100 cm^3 of sediment deposited/corral/year, or 5500 cm^3 in 5 years. Since this deposited sediment is spread over 19.6 m^2, ≈0.28 cm of sediment thickness is deposited in 5 years. Taking into account the amount of water per mL of sediment, 1.9 to 3.5 cm of sediment thickness was deposited in the 5 year period since the addition of the PCDDs. These calculations do not take into account any compression of the lower sediments as more sediment accumulates at the sediment/water interface, any resuspension of surficial sediment particles into the water column, or the fact that sedimentation nearer the shore is less than at the center of the lake. However, they do show that a 0-6 cm sediment core is sufficient to sample the layer in which the PCDDs would be expected to be deposited.

B. Dioxin Analysis

Initially, only the 1368-TCDD, the HpCDD and the OCDD congeners were to be analyzed, since these were the congeners added to the corrals in 1985. However, upon examination of the GC/MSD chromatograms, a peak with the correct ion ratios for a tetra-chlorinated dioxin was detected at a retention time of 18.27 min, approximately 12 seconds after the 1368-TCDD peak (RT = 18.07 min). With the knowledge that 1379-TCDD is a contaminant in 1368-TCDD, the identity of the new peak was confirmed to be 1379-TCDD after analyzing a combined 1368-TCDD/1379-TCDD standard on the GC/MSD and comparing retention times and ratios of m/z 259, 320, 322 and 324, in both the sample and the standard (see Appendix 1). The

retention times of the HpCDD and OCDD congeners were 28.46 and 31.11 min, respectively.

The expected ion ratios (from standards) and observed ion ratios (from samples) for the ions collected in the SIM analysis of the PCDDs, can be found in Appendix 1.

Dioxin concentrations in the sediment of all corrals, prior to the addition of the PCDDs (Servos, 1988), and in the control corral (B) during the five years of sampling, were below the detectable limits for the congeners. Detection limits were 2.2 pg for the 1368-TCDD and 1379-TCDD congeners, 3.6 pg for the HpCDD and 5.4 pg for the OCDD (based on a 3:1 signal to noise ratio for an injected amount). Percent recoveries of internal standards were 85 ± 6 and 78 ± 4 for the (^{13}C)-1234-TCDD and the (^{13}C)-HpCDD, respectively (n=22). The mean percent recovery of 1368-TCDD, HpCDD and OCDD spiked onto "clean" sediment at 1.0 and 100 ng PCDD/g dry weight of sediment was 68 ± 4, 76 ± 6 and 75 ± 7 percent, respectively (n=3, for each treatment level).

Since the ratio of the mass per gram dry weight of sediment of the 1368-TCDD:1379-TCDD remained constant at 2:1 over the five years of sampling, and since the 1379-TCDD was present in the day 8 sample, this suggests that the 1379-TCDD was present in the original 1368-TCDD stock standard (>98.6% pure, according to Pathfinder Labs, St. Louis, MO, U.S.A.). Therefore, of the 2 mg of 1368-TCDD added to corrals A and D, one third or 0.67 mg appears to have been the 1379-TCDD congener. One way that the 1379-TCDD might have been formed during the production of the 1368-TCDD was by a Smiles rearrangement of the 1368-TCDD predioxin (Esposito *et al.*, 1980). In a Smiles rearrangement, one of the rings spontaneously reverses to its mirror image at the moment of ring closure

(Gray *et al.*, 1976; Shine, 1973) (Fig. 3).

The ratios of the mass of 1368-TCDD:1379-TCDD:HpCDD:OCDD per gram dry weight of sediment remained relatively constant over five years at ≈2:1:6:3. The constancy of this ratio dispels any suggestion that the OCDD may degrade to the HpCDD congener in the sediment over time.

The mean concentrations of each congener at each sampling time were compared by a two-way analysis of variance (SAS Institute Inc., 1989c) using the least significant difference (LSD) method, with $p<0.05$, T-crit = 2.31 (Appendix 4). In general, it can be seen that the means used in the calculation of the half-lives (see below) are not significantly different from each other, while the means of the first two sampling dates are significantly different from the last six dates. However, because of the small number of samples at each time period (n=2 for days 8-697 and n=4 for days 1499-1807) the value of these statistical tests is limited.

In corrals A and D, the mean concentrations of the four congeners increased to a maximum of 118, 57.6, 346 and 155 ng/g dry weight of sediment, averaged over the entire 0-6 cm core, for the 1368-TCDD, 1379-TCDD, HpCDD and OCDD, respectively, by day 271. By the final sampling date (day 1807), these concentrations had decreased to 46.0, 22.3, 141 and 65.0 ng/g dry weight of sediment, respectively (Fig. 4). The vertical bars in Fig. 4 represent one standard deviation. The means ± s.d. for the data in Fig. 4 can be found in Appendix 2.

The concentrations of the PCDDs in the sediment on days 271, 348 and 697, agree with the data of Servos (1988), who reports a concentration range for 1368-TCDD of 48-112 ng/g dry weight and of 52-70 ng/g dry weight for OCDD over those same days. The values from days

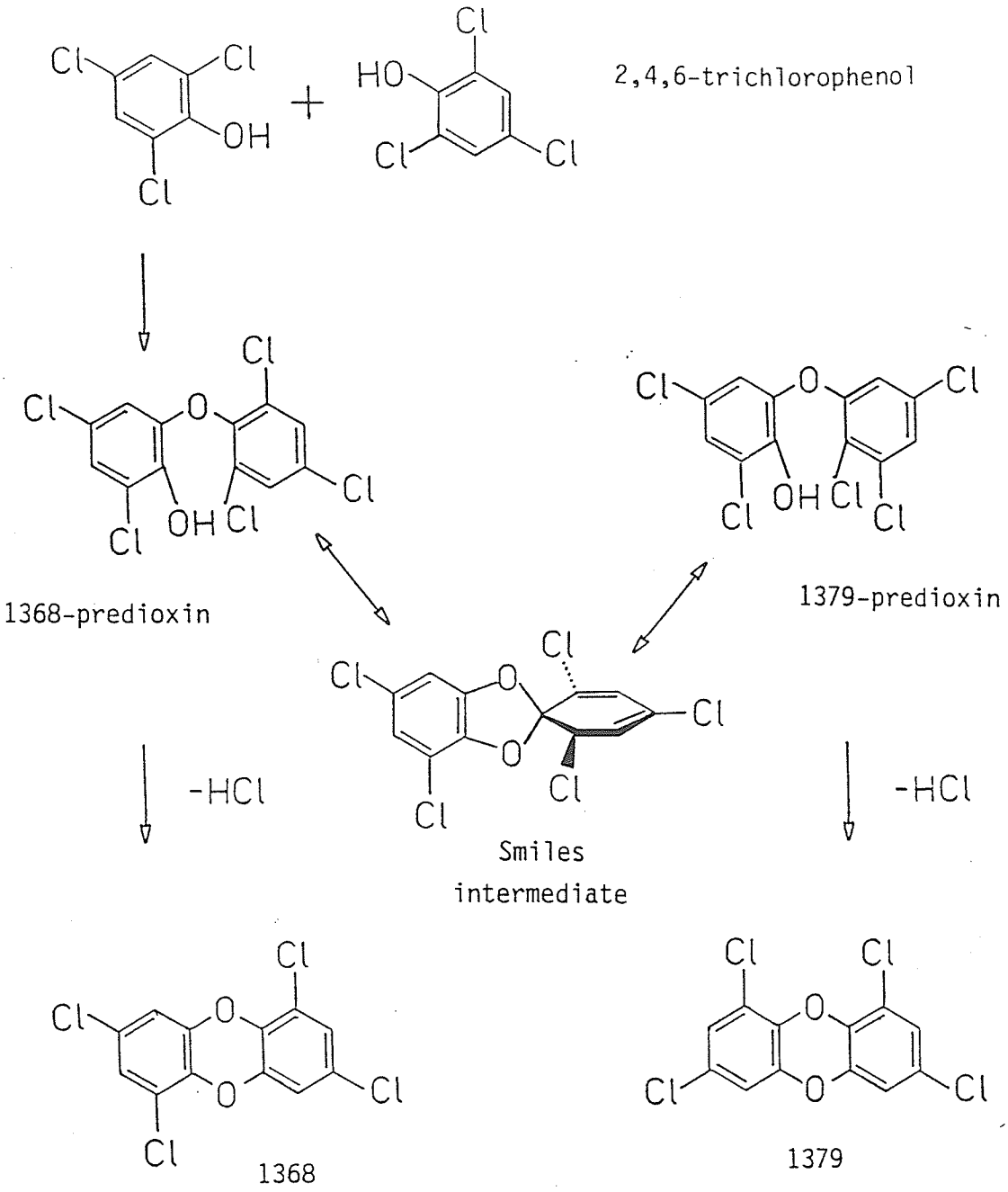


Figure 3. Formation of 1368 and 1379 from a 1368-predioxin, via a Smiles rearrangement intermediate (from Kende and DeCamp, 1975 and Gray *et al.*, 1975).

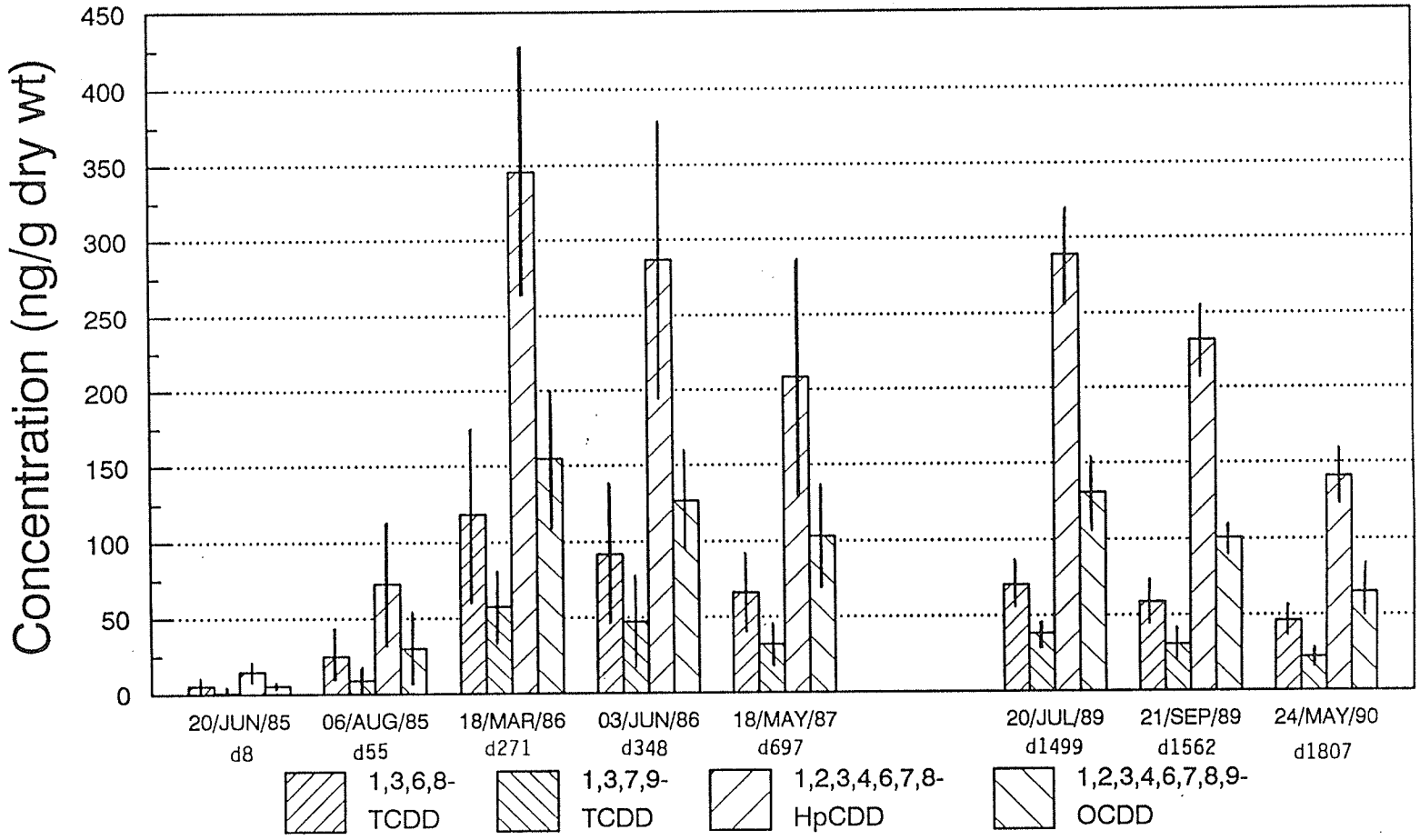


Figure 4. Concentrations of four dioxin congeners in the 0-6 cm layer of the sediment of corrals A and D in Lake 304, ELA between June, 1985 and May, 1990 (days post PCDD spike)

8 and 55, however, are approximately $\frac{1}{2}$ to 1 order of magnitude lower than the corresponding data of Servos (1988). Servos (1988) reported 1368-TCDD concentrations in the sediment to be 44 and 112 ng/g dry weight of sediment on days 8 and 55, respectively, and OCDD concentrations to be 42 and 88 ng/g dry weight of sediment, respectively. No analytical anomalies in this study can account for this difference. M. Servos (Dept of Fisheries and Oceans, Burlington, ON, pers. comm.) is of the opinion that his values would be more suspect than those of this study since he analyzed small subsamples (≈0.1 g) of the sediment cores; whereas in the present study, the rest of the core (≈2.6 g) was analyzed in its entirety. Marcheterre et al. (1985) report that the maximum measured concentration of OCDD in their pond sediment occurred 382 days post "spike", at approximately 1.5 times the concentration measured on day 51. This compares favourably with the sediment concentration in this study which reached a maximum by day 271.

When the concentrations of the PCDDs are log-transformed and plotted against time (Fig. 5), a straight line results, indicating that the disappearance seems to follow first-order kinetics. It is not a true first-order disappearance for, by definition, first-order reactions are those in which the reaction depends only on the first power of the concentration of a single reacting species, at a given temperature (Barrow, 1966). While the temperature of the water in Lake 304 does not remain constant over time, it is convenient to view the disappearance as following first-order kinetics. Disappearance rate constants can be calculated as the slopes of the lines (for linear regression analysis of the slopes see Appendix 3). The constants can then be used to calculate **disappearance half-lives** for the congeners in the sediment by the

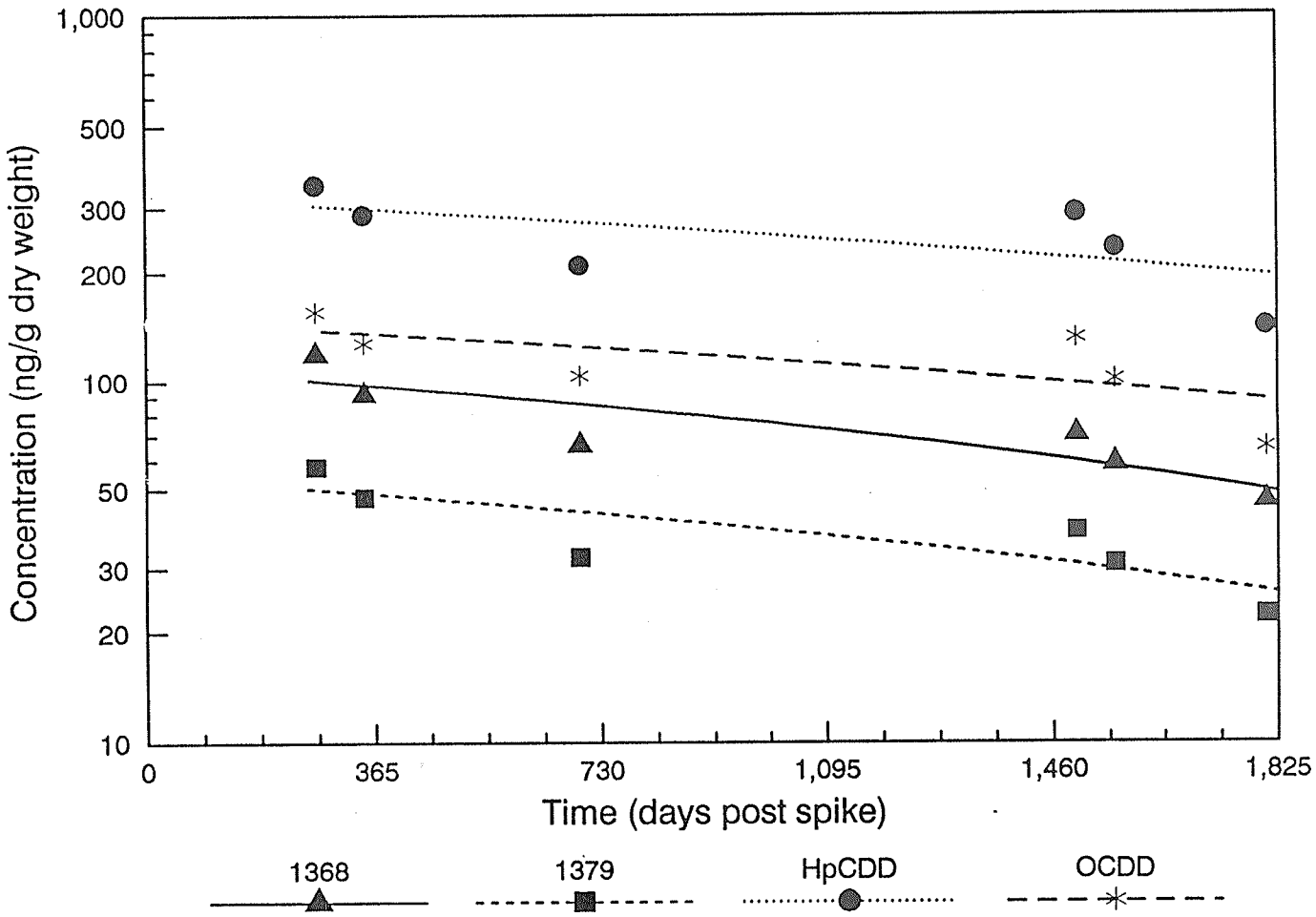


Figure 5. Disappearance of four dioxin congeners from the 0-6 cm layer of the sediment of corrals A and D in Lake 304, ELA since 1985

equation:

$$t_{\frac{1}{2}} = \frac{-0.693}{slope} \qquad \text{Eqn. 8.}$$

The disappearance half-lives for the 1368-TCDD, 1379-TCDD, HpCDD and OCDD are calculated to be 4.4 ± 1.7, 4.6 ± 1.9, 6.2 ± 3.1 and 6.0 ± 2.8 y, respectively. The data from days 8 and 55 is not used in the half-life calculations since the concentrations of the PCDDs in the sediment are still increasing during these times. The results of a linear regression analysis of the slopes (SAS Institute Inc, 1989a) can be found in Appendix 3. When the slopes (and their resultant half-lives) are analyzed using a General Linear Model (the homogeneity-of-slopes model, SAS Institute Inc, 1989b) they are not significantly different at $p<0.05$ (F value = 0.17, P>F = 0.9178). However, the apparent difference in the half-lives of the 1368-TCDD and 1379-TCDD compared to those of the HpCDD and the OCDD, is supported by the work of Marcheterre (1985) and Corbet (1983). Marcheterre (1985) showed that the retention of OCDD in small, 0.5 m deep ponds was longer than the retention of 1368-TCDD under similar conditions in the same ponds (Corbet, 1983). Less than 14.2% of the 1368-TCDD remained in the sediment of the ponds after 426 days, whereas 62-88% of the OCDD remained in the sediment after 652 days. This difference is consistent with the lower availability of the HpCDD and the OCDD to the water column (Servos, 1988) and hence to removal processes occurring in the water column.

The disappearance of the PCDDs may be due to a number of factors, such as burial, degradation, volatilization, or transport out of the corrals. As shown above, loss due to burial over the five year period

of the experiment was accomodated by sampling the 0-6 cm layer.

If transport out of the corrals via physical movement of the water column were a major removal pathway, we would not expect to see any major decrease in PCDD concentrations until after the removal of the top 1 m of the corral curtain in late May, 1987 (day 710). However, the disappearance of all congeners appears to be fairly steady from day 271 onward (Fig. 4).

Another possible pathway for the removal of PCDDs and other contaminants from the sediments is via emerging insects. Menzie (1980) has calculated that in lakes of high productivity (100 $g/m^2/y$), emerging insects could remove 50% of chemicals with high BCFs (>10,000 X), in ≈1.2 y. Lower productivities and lower BCFs would decrease the rate of removal. Fairchild *et al.* (1990) report that 0.6 to 2.1% of the total sediment load of 2378-TCDF was exported from their limnocorrals by insects of the Order Diptera (flies). Export via this pathway was probably greater, since this does not include export by other groups, such as Odonata (dragonflies) or Ephemeroptera (mayflies).

Volatilization should not have a major effect, since Servos (1988) reports that >97% of the PCDDs are found in the sediment compartment after day 24 and are not available for volatilization. However, the data from the present study show that the majority of the PCDDs are not in the sediment until some time between day 55 and day 271. In any case, it can be seen that volatilization would only be important near the beginning of the experiment.

Degradation of PCDDs can occur via several different pathways, including photolysis, microbial degradation, or possibly, non-photolytic reductive dechlorination. In our system, photolysis would not have

played a large role, in view of the low PCDD concentrations in the water column after day 10 (Servos, 1988), and the attenuation of light at depths greater than several cm (Friesen, 1988). Degradation of PCDDs by microorganisms has been observed or postulated in several studies (Matsumura and Benezet, 1973; Klecka and Gibson, 1980; Quensen and Matsumura, 1983; Muir *et al.*, 1985b). In a sediment/water incubation, Ward and Matsumura (1978) estimate the degradation of the 2378-TCDD congener to be between 1 and 4% over 588 days. They believe that degradation of 2378-TCDD probably involves a monooxygenase, since all possible sites of attack by a dioxygenase are blocked by chlorine. This would also be true for the HpCDD and the OCDD, but not for the 1368-TCDD or the 1379-TCDD congeners, which have two positions open for dioxygenase attack. Muir *et al.* (1985b) showed that between 0.1 and 7.0% of the 1368-TCDD in pond and lake sediment was degraded over 675 days. Reductive dechlorination of 1368-TCDD in sediment/water systems was ruled out as a possible degradative pathway by Muir *et al.* (1985b), since those degradative products would have had far greater R_f (by reverse-phase TLC) than those actually isolated from the soil and sediment. The R_f would be greater because the more polar degradation products are less strongly adsorbed to the reverse-phase TLC plates than are the less polar parent compounds. The same would also be true for the congeners in this study.

Estimates of sediment/soil half-lives in the literature, range from less than one year to greater than 10 years (di Domenico *et al.*, 1980, Young, 1980; Muir *et al.*, 1985b; Miller and Zepp, 1987; Friesen *et al.*, 1990), and are highly dependent on the system being studied. For example, half-lives can be quite variable depending on whether the

PCDDs are surface applied, or are found in the deeper layers of the soil or sediment (Friesen *et al.*, 1990). The large range in these estimates indicates the need for long term studies (greater than 5 years), to get a better idea of the long term fate of these compounds.

Conclusions

An investigation into the long term aquatic fate of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin, 1,3,7,9-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin in limnological corrals placed in the littoral zone of a Canadian Shield lake, was extended to a period of five years.

The ratio of the concentrations of the four congeners to each other in the sediment remained essentially constant over the time of the experiment (5 years), showing that the congeners did not degrade over time to one of the other congeners being studied or did not degrade at all since it is unlikely that all four congeners would degrade at the same rate in soil or sediment. No other tetra- or hepta-chlorinated congeners were detected within the time windows analyzed.

In the sediment, the half-lives of all congeners under study were between 4-6 years, and fell in the range of half-life values for other PCDDs reported in the literature.

The disappearance data led to questions on the pathways of removal. Other studies have shown that the most likely pathways of disappearance of the PCDDs from sediment are export via emerging insects and degradation. Fairchild *et al.* (1990) has shown that export by one Order of insects (Diptera) can account for up to 2% of the loss of 2,3,7,8-tetrachlorodibenzofuran from lake sediments per year. The loss of contaminants via emerging insects would be greater than the 2% per year if the export by other common insect Orders, such as the Ephemeropterans and Odonates, were to be taken into account. Ward and

Matsumura (1978) showed that between 1 and 4% of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in sediment was microbially degraded over 588 days, while Muir *et al.* (1985b) have reported that up to 7% of the 1,3,6,8-tetrachlorodibenzo-*p*-dioxin in sediment was degraded over 675 days.

Transport via physical movement of the water column and volatilization may have played a very minor role in the disappearance of the PCDDs from the sediment.

Fate studies of this sort are important in that they give us a better idea as to the long-term fate of these and other hydrophobic xenobiotics in the environment. These data do not, however, give us any indication as to the percent of these compounds which are bioavailable, information which is of greater practical significance.

CHAPTER 2

THE BIOAVAILABILITY OF FOUR CHLORINATED DIBENZO-P-DIOXINS TO FRESHWATER MUSSELS (*Anodonta grandis*) AND CRAYFISH (*Orconectes virilis*) FROM THE SEDIMENT OF LAKE ENCLOSURES

Introduction

Polychlorinated dibenzodioxins are persistent, hydrophobic chemicals that tend to be associated with sediment organic carbon in an aquatic system (Servos and Muir, 1989; Servos *et al.*, 1989; Chapter 1). Accumulation of sediment-sorbed, hydrophobic organic contaminants by benthic organisms has been shown to be an important point of entry for these chemicals into the aquatic food chain (Langston, 1978; Roesijadi *et al.*, 1978; McElroy and Means, 1988).

McElroy and Means (1988) have shown that properties of the organism, as well as properties of the sediment, need to be taken into account when investigating accumulation of contaminants from sediment. Mechanisms of compound/particulate associations, the sediment OC content and type, the presence and levels of other toxic contaminants, the life-style, anatomy and metabolism of the organism, and the composition and availability of lipid pools within the organism can all contribute to observed differences in uptake of contaminants by different organisms.

PCDDs have been shown to accumulate in fish and other organisms (Table 3) with the 2,3,7,8-substituted PCDDs (especially 2378-TCDD) showing the greatest bioaccumulation potential (Muir *et al.*, 1983; Muir

and Yarechewski, 1988; Bergqvist *et al.*, 1989; Koistinen *et al.*, 1989; Miyata *et al.*, 1989; Rappe *et al.*, 1989).

Bioaccumulation of pollutants from sediment may be simply described by a **partition coefficient** (Ferraro *et al.*, 1990):

$$BAF = C_t/C_s \qquad \text{Eqn. 9.}$$

where BAF = bioaccumulation factor,
C_t = pollutant concentration in tissue,
C_s = pollutant concentration in sediment.

When BAFs are normalized to the lipid content of the organism and to the OC content of the sediment, the partition coefficient becomes the **bioavailability index** (Foster *et al.*, 1987; Connell, 1988; McElroy and Means, 1988; Fairchild *et al.*, 1990):

$$BI = (C_t/f_L)/(C_s/f_{OC}) \qquad \text{Eqn. 10.}$$

where BI = bioavailability index,
f_L = fraction lipid,
f_{OC} = fraction organic carbon.

The BI is a better measure of accumulation (and hence of the predictability of tissue concentrations from sediment concentrations) than is the BAF because BIs are normalized to the highly variable lipid contents of the individual organisms. There is therefore less variation in the BI than in the BAF (Lake *et al.*, 1990).

Another approach is to view accumulation as proceeding by a food chain based route, in which organisms ingesting sediments or filtering suspended sediments from the water, would accumulate the contaminants associated with the sediments. Landrum (1989) has modelled accumulation in this manner, using a two compartment model:

$$C_a = \frac{[K_s C_s (e^{-\lambda t} - e^{-Kt})]}{[K - \lambda]} \qquad \text{Eqn. 11.}$$

where C_a = concentration in organism (ng/g wet weight),
C_s = concentration in sediment (ng/g),
K_s = uptake constant (g sediment/g organism/hr),
K = elimination constant (hr^{-1}),
λ = constant for compound to become biologically unavailable,
t = time.

However, for the higher K_{ow} compounds tested (HCB, log K_{ow} = 6.7 and BaP, log K_{ow} = 6.5), Landrum (1989) found that the equation could be simplified to:

$$C_a = K_s C_s t \qquad \text{Eqn. 12.}$$

This occurred because the $e^{-\lambda t}$ approached 1 owing to a negligible value, and because there was no appreciable elimination because of a constant concentration of contaminant in the sediment. This approach (Eqn. 11 and 12) is preferable to the partitioning approach (Landrum, 1989; Landrum and Robbins, 1990) because it does not assume that steady state conditions exist between the organism and the sediment, and it is able to incorporate multiple sources in the kinetic calculations, thereby permitting the modelling of field situations where contaminant accumulation results from multiple sources.

Bivalves have been shown to be important biomonitors for xenobiotics in the aquatic environment (Boehm and Quinn, 1977; Langston, 1978; Kraus and Hamdy, 1985; Foster *et al.*, 1987; Wade *et al.*, 1989; Ferraro *et al.*, 1990). Most of these data, however, have been collected in the marine environment. The importance of freshwater bivalves in accumulation studies is coming to the fore with the

increasing concern over PCDD and other chlorinated organic contamination from bleached Kraft pulp mills (Amendola *et al.*, 1989; Clement *et al.*; 1989; Södergren, 1989; Trudel, 1991). Miyata *et al.* (1987) have reported that the freshwater bivalve, *Corbicula* was an excellent biomonitor for low-chlorinated dioxins, particularly the 1368-TCDD congener.

Farrington *et al.* (1983) have given several reasons why bivalves (especially mussels and oysters) are excellent sentinel species: 1) they are widely distributed geographically, 2) they are sedentary and therefore serve better than mobile species as biomonitors in a given area, 3) bivalves, in contrast to fish and crustaceans, exhibit low activity of those enzymes that are capable of metabolizing xenobiotics, 4) they are capable of surviving very polluted conditions, 5) they can be successfully transplanted to intertidal shallows where normal populations would not grow, and 6) many are commercially valuable seafood species on a worldwide scale. Crayfish and other crustaceans are also important organisms since they are more sensitive in toxicological studies than are molluscs (Swartz, 1987) and are also commercially important worldwide.

Mussels, crayfish and other invertebrates are important organisms in aquatic ecosystems for several reasons. Hargrave (1976) reported that their egested faecal material is rapidly colonized by successions of microorganisms whose oxygen demand is maximized within 2 to 3 days. Following this, the microbial populations fall off to a level equivalent to that found in uningested sediments. Therefore, the reworking of sediment and detritus by benthic organisms provides space for microbial growth, and in this way benthic invertebrates enhance their food supply

and stimulate the decomposition of organic matter in the sediment.

The conversion of detrital organic matter through the decomposer food chain into the tissues of organisms at higher food chain levels may, therefore, be of primary importance in determining overall productivity in many aquatic and terrestrial ecosystems (Hargrave, 1976).

Adams (1987), in his review of the bioavailability of sediment-associated neutral lipophilic compounds contained on sediment, observed that in contrast to their marine counterparts, freshwater benthic invertebrates are not sediment ingesters and that the primary food source for these organisms is associated with particulates and organic matter of various types. Discriminate feeders should consume particles with higher OC content and, therefore, higher contaminant content than indiscriminate feeders. Whether these organisms are filter feeders, like the mussel or are shredders and scavengers, like the crayfish, the effect of their contribution to the detrital pool through their faeces and other undigested particles is much greater than the effect of their dead bodies and exuviae (Berrie, 1976; Adams, 1987). Therefore, benthic organisms contribute much more to the energy cycle of the ecosystem when they are alive, than when they die and are themselves decomposed.

Experimental

A. Chemicals

Universally diphenyl-ring-labelled (^{14}C)-OCDD and nonlabelled 1368-TCDD and HpCDD were purchased from Pathfinder Labs, Inc., St Louis, MO, U.S.A. The OCDD had a specific activity of 20.58 mCi/mM and was >99.8% radiochemically pure by HPLC (Servos, 1988). Universally diphenyl-ring-labelled (^{13}C)-HpCDD and (^{13}C)-1234-TCDD (used as internal standards) were also purchased from Pathfinder Labs. Additioal nonlabelled 1368-TCDD and 1379-TCDD, HpCDD and OCDD were purchased from Wellington Laboratories, Inc., Guelph, ON.

All solvents used in the workup and analysis of samples, including hexane, DCM, toluene, methanol, THF and acetone, were "distilled in glass" quality and were purchased from Burdick & Jackson, Co. through Baxter-Canlab Ltd., Winnipeg, MB.

Gel permeation beads (Bio-Beads S-X3), for lipid removal, were purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A..

Nitrogen gas to concentrate samples and prepurified helium for GC analyses, were supplied by Welders Supply Co., Winnipeg, MB.

B. Site Preparation

Eight cages were built in Winnipeg, 2 weeks prior to collecting the biota needed for this experiment. The cages were constructed from 3.8 cm x 3.8 cm x 2.4 m lengths of spruce (known commercially as "2 x 2"), cut and nailed together to form a flattened-cube frame, 90 cm x 90

cm x 50 cm in height. The 6 sides were covered with galvanized steel hardware cloth (1.25 cm mesh) and stapled in place. A 20 cm x 30 cm opening was cut in the centre of the top panel and made into a wood-frame, mesh-covered door with brass hinges and sliding locks.

On June 5, 1989 the cages and all other required materials were transported to ELA, carried in to Lake 304 and ferried across the lake. For each cage, two 3 m lengths of 1 cm diameter iron bars (Rebar) were driven into the sediment at the bottom of the cut-off corrals in order to have a firm anchor for the cages. A 5 cm dia. float was attached with string to each of the 16 bars to mark their position.

After several unsuccessful attempts at collecting freshwater mussels (*Anodonta grandis*) from Lake 377, 90 specimens were successfully collected from Lake 938, on June 12, 1989. The mussels were held in a cage just offshore in Lake 304 until they were introduced into the corrals.

Attempts to collect crayfish (*Orconectes virilis*) in modified minnow traps from Lakes 239, 240 and Roddy Lake, and by hand in the stream flowing from Lake 149 into Lake 150, between June 7 and June 13, 1989, were unsuccessful. However, during the night of July 2-3, 1989, a team of divers succeeded in collecting 79 male crayfish along the shore of Lake 938. Male crayfish were used in order to avoid introducing a population into a lake in which they are not usually found. After consultation with Ian Davies (Dept. of Fisheries and Oceans, Winnipeg, MB), the crayfish cages were also covered with a nylon window screen (≈1 mm mesh) in order to prevent escapes. The possibility of escapes had to be taken seriously for crayfish are known to be able to escape easily from coarse-mesh covered cages.

On July 5, 1989, 20 mussels were placed into each of the 4 mussel cages (2 control and 2 experimental), 17 crayfish into each of 2 control crayfish cages and 18 crayfish into each of 2 experimental crayfish cages. Using SCUBA, a diver pushed the bottom of each cage several cm into the sediment and secured the cage to the Rebar with locking wire. Two mussel cages were placed into the control corral and one cage into each experimental corral. The SCUBA diver had sufficient air supply to anchor crayfish cages only into the experimental corrals. The control cages were anchored to the sediment in about 60 cm of water, approximately 5 m from the corrals.

The small mesh size of the crayfish cages required that the crayfish be fed approximately every two weeks with two hot dogs, split along their length for every cage (≈0.6 g hot dog/g crayfish).

C. Sampling

By the use of SCUBA and a small dip net, 2 mussels were removed from each cage and placed into Whirl-Pak bags (Baxter-Canlab, Winnipeg, MB) 15, 29, 49, 78 and 103 days after the introduction of the cages into the corrals. In total, 4 experimental and 4 control animals were removed per sampling day. On day 323, the last surviving mussel sample was taken. To convert time to days "post PCDD-spike", 1484 is added to these day numbers. All Whirl-Pak bags were frozen at -32°C until the contents could be analyzed.

Crayfish were sampled in the same manner as were the mussels. However, mortality was high in all crayfish cages, and only 3 experimental crayfish could be sampled on day 78, all from corral A, and

none on day 103.

D. Analysis

The mussels and the crayfish were freeze-dried under the same conditions as were the sediments (Chapter 1).

The crayfish were extracted using a ball mill apparatus. A crayfish was placed into a 50 mL stainless steel centrifuge tube with two 1 cm diameter steel ball bearings. After it was spiked with 12 ng (^{13}C)-1234-TCDD and 20 ng (^{13}C)-HpCDD and had received 20.00 mL of 1:1 DCM/hexane, the tube was capped and shaken on a wrist action shaker (Burrell Corp., Pittsburgh, PA, U.S.A.) for 1 h and allowed to stand overnight. The sample was transferred to a 50 mL Corex tube with four 5 mL washes of DCM/hexane (1:1) and centrifuged at 3000 rpm for 20 min.

The supernatant was transferred to a 250 mL round bottom flask and the pellet washed with four 5 mL washes of 1:1 DCM/hexane. The sample was centrifuged at 3000 rpm for 20 min and the supernatant added to the extract between each wash. The sample was reduced in volume to ≈1 mL by rotary evaporation and transferred to a 20 mL screw cap test tube. The flask was washed with four 2 mL rinses of 1:1 DCM/hexane and 1 mL of DCM was added to the test tube to ensure a 1:1 ratio of DCM and hexane for GPC.

The mussels were finely cut up, placed into 250 mL round bottom flasks and spiked with 12 ng (^{13}C)-1234-TCDD and 20 ng (^{13}C)-HpCDD. Each sample was ground in 130 mL of 1:1 DCM/hexane, using a model PT 10-35 Polytron homogenizer (Brinkman Instruments (CANADA) Ltd., Rexdale, ON) at high speed. The samples were filtered using 1.5 μm retention

Whatman 934-AH glass microfibre filter papers. The residuum was washed three times with 25.0 mL of 1:1 DCM/hexane. The filtrate was rotary evaporated to ≈1 mL, transferred to a screw cap test tube and the flask washed with four 2 mL washes of 1:1 DCM/hexane. One mL of DCM was also added to these samples.

The biota extracts were loaded onto a 60 g GPC column (as described in Chapter 1) and the PCDD-containing fraction collected in a 500 mL round bottom flask. After evaporation and transferral to 15 mL graduated centrifuge tubes, the PCDDs were isolated on an activated carbon/glass fibre column as in Chapter 1. The toluene extracts were reduced in volume, transferred to 600 µL Reacti-Vials (Chromatographic Specialties, Brockville, ON) and evaporated to a pre-calibrated, 50 µL mark on the Reacti-Vial. A 2 µL aliquot was analyzed by GC/MSD (see Table 5) in the SIM mode as in Chapter 1.

E. Lipid determination

In order to determine the percent lipid present in each of the individual biota samples, an aliquot equal to 5% of the volume of extract was taken from the extract prior to the rotary evaporation step (Konemann and van Leeuwen, 1980). The aliquot was pipetted into a tared scintillation vial and the solvent allowed to evaporate. The vial was then placed in a dessicator overnight, reweighed in the morning and the weight of lipid determined by difference.

Results and Discussion

A. Lipid Determination

A problem with the method of lipid determination became evident once the calculations were complete. A number of samples were calculated to contain 0 percent or even negative percent lipid! During consultation with K. Cooper (Rutgers U., Piscataway, NJ, U.S.A.), it was confirmed that the results of the Konemann and van Leeuwen (1980) method of lipid determination were intrinsically, highly variable for samples with low lipid contents, and therefore of questionable value. The mean percent lipid was then determined for the remaining 8 mussel and 5 crayfish. These biota were extracted as described above and the weight of lipid in the entire extract determined by difference, after the solvent had been evaporated. The mean percent lipid in the mussel and crayfish (based on wet weight) was determined to be 0.34 and 0.57 percent, respectively. Based on their dry weights, they were 4.30 and 1.87 percent lipid, respectively.

B. Dioxin Analysis

B.1. Control Biota

Dioxin concentrations in all pre-treatment biota and in the control biota (corral B) during the eleven months of sampling, were below the detectable limits for all congeners. Detection limits were 2.2 pg for the 1368-TCDD and 1379-TCDD congeners, 3.6 pg for the HpCDD

and 5.4 pg for the OCDD (based on a 3:1 signal to noise ratio for an injected amount). Recoveries of the (^{13}C)-1234-TCDD and the (^{13}C)-HpCDD from the mussel were 84 ± 6 and 76 ± 7 percent, respectively, (n=40), while from the crayfish they were 81 ± 6 and 75 ± 5 percent, respectively, (n=30). Mean percent recoveries of 1368-TCDD, HpCDD and OCDD spiked onto "clean" mussel at 50 and 500 pg PCDD/g wet weight of mussel were 67 ± 4, 73 ± 5 and 71 ± 5 percent, respectively, while for the crayfish they were 69 ± 5, 75 ± 7 and 74 ± 8 percent, respectively (n=3 for each treatment level, for each species).

B.2. Treated Biota

The four congeners that were detected in the sediment (1368-TCDD, 1379-TCDD, HpCDD and OCDD), were detected in all mussel and crayfish samples from corrals A and D.

The expected ion ratios (from standards) and observed ion ratios (from samples) for the ions collected in the SIM analysis of the PCDDs, can be found in Appendix 1.

The ratios of the mass of 1368-TCDD:1379-TCDD:HpCDD:OCDD per gram wet weight of mussel tissue was highly variable over time, and ranged from ≈5:1:4:6 to ≈3:1:1:1. This variability was also seen in the congener ratios in the crayfish tissue (≈6:2:1:1 to ≈4:1:1:1) and is considered to be due to the natural variability in uptake and depuration rates, metabolism, ventilation rates and feeding rates between different individuals, even of the same species (B. Boese, U.S.E.P.A., Newport, OR, U.S.A., pers. comm.). The ratios of the concentrations of PCDDs in the tissue of the mussel or crayfish did not mirror the ratios in the

sediment (≈2:1:6:3, respectively). This difference indicates that the concentrations seen in the biota were due to bioaccumulation from food and water, and were not biased to any great extent by sediment that may have been present in the gut of the organisms.

The mean concentrations of each congener at each sampling time were compared by a two-way analysis of variance (SAS Institute Inc., 1989c) using the least significant difference (LSD) method, with $p<0.05$, $T\text{-}crit_{mussel} = 2.57$ and $T\text{-}crit_{crayfish} = 2.78$ (Appendix 4). Although the statistical analysis indicates that there is no significant difference between the concentrations of the congeners at each sampling time, a trend (as described below, i.e., a rapid increase in concentration, followed by a decrease and another increase) can be seen in the graphical representations of the data (Figs. 6 and 7). If the sample size had been larger for each time period, the apparent difference might have been borne out in the statistical analysis.

As shown in Fig. 6, the concentrations of the PCDDs in the mussel were at a maximum of 169, 50, 276 and 171 pg/g wet weight for the 1368-TCDD, 1379-TCDD, HpCDD and OCDD, respectively, on day 15. The concentrations of the 1368-TCDD and 1379-TCDD decreased to a minimum of 54 and 11 pg/g wet weight, respectively, on day 78, whereas the concentrations of the HpCDD and OCDD reached a minimum of 23 and 29 pg/g wet weight, on day 49. By day 103, the concentrations of the 1368-TCDD, 1379-TCDD, HpCDD and OCDD had increased to 133, 41, 78 and 85 pg/g wet weight, respectively. The single mussel sampled on May 24, 1990 (day 323) had 1368-TCDD, 1379-TCDD, HpCDD and OCDD concentrations of 59, 29, 157 and 138 pg/g wet weight, respectively.

A similar trend can be seen in the PCDD levels in the tissue of

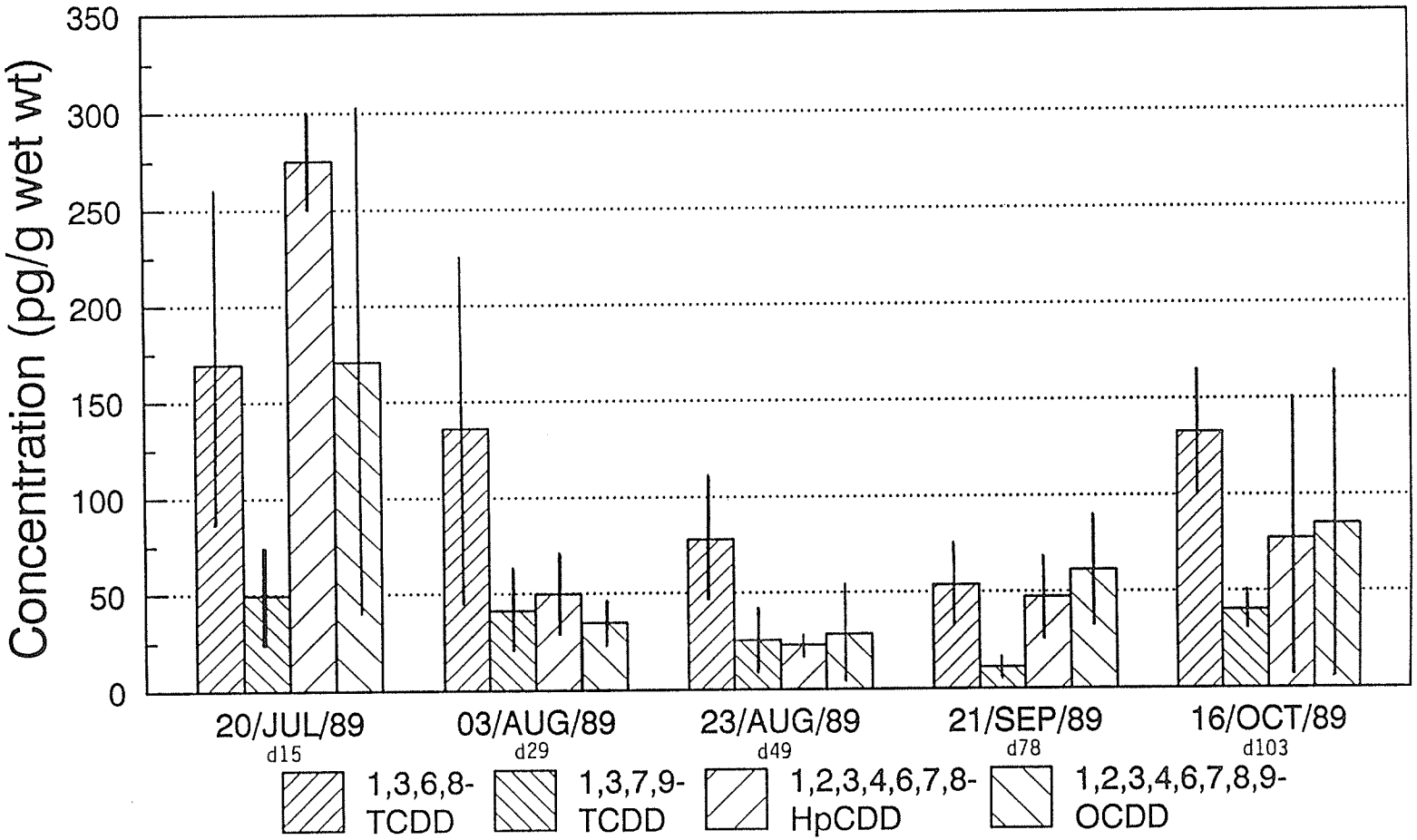


Figure 6. Concentrations of four dioxin congeners in the freshwater mussel, *Anodonta grandis* in corrals A and D of Lake 304, ELA during 1989 (days post introduction into corrals)

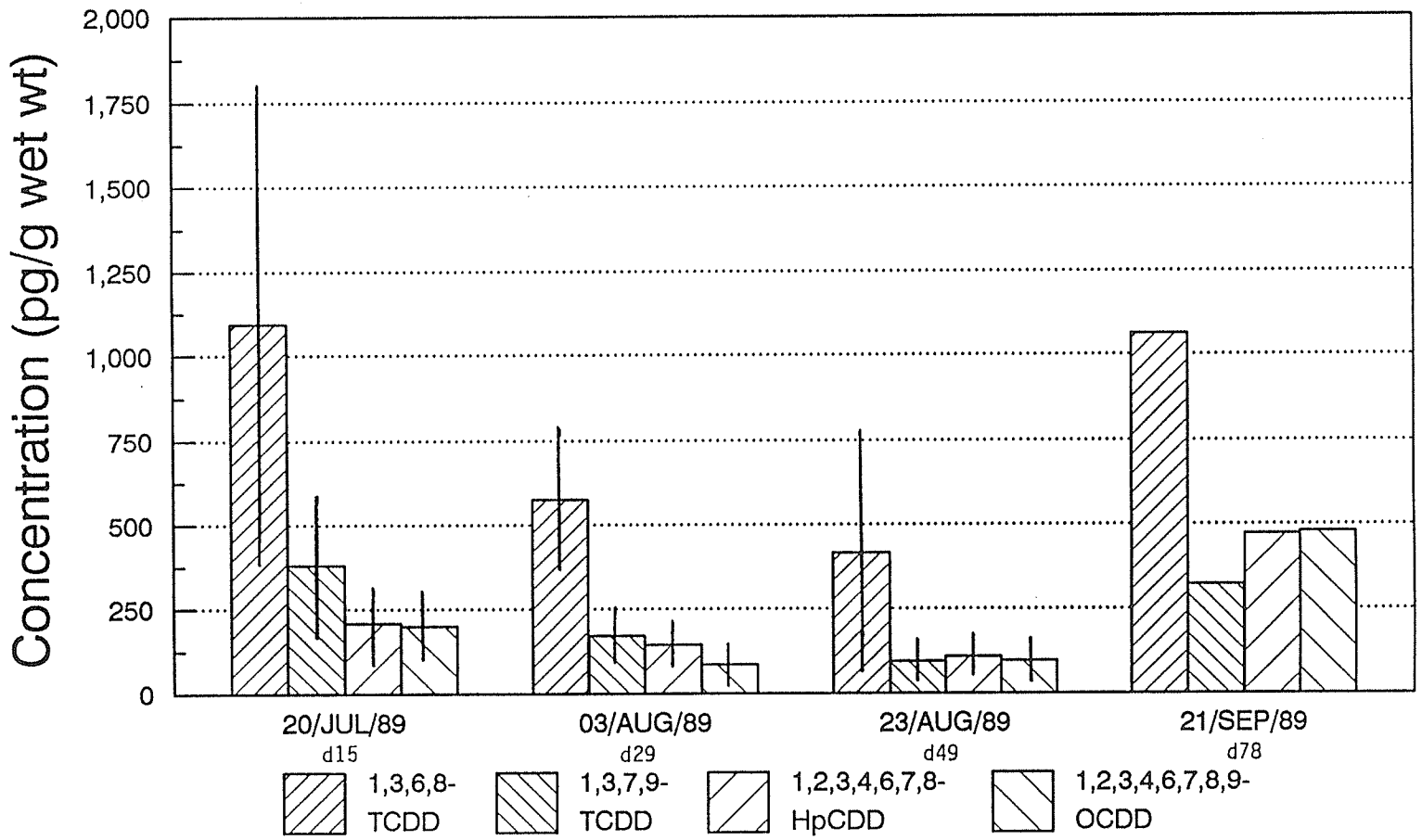


Figure 7. Concentrations of four dioxin congeners in crayfish, *Orconectes virilis* in corrals A and D of Lake 304, ELA during 1989 (days post introduction into corrals)

the crayfish (Fig. 7). On day 15, the concentrations of the 1368-TCDD, 1379-TCDD, HpCDD and OCDD were 1,090, 381, 209 and 201 pg/g wet weight, respectively, decreasing to 416, 96, 110 and 98 pg/g wet weight by day 49. By day 78, the concentrations had increased to 1,060, 322, 473 and 481 pg/g wet weight for the 1368-TCDD, 1379-TCDD, HpCDD and OCDD, respectively. The concentrations for day 78 in Fig. 7, do not have bars signifying the standard deviation, since these were crayfish only from corral A. Crayfish suffered high mortality in corral D.

These results are quite different from the ones expected on the basis of a "classical" uptake curve. In such a curve, the concentrations rise at a specific rate (the uptake rate minus the depuration rate) and eventually plateau at a level in which the concentration in the organism is in equilibrium with the concentration in its surroundings, and the uptake rate equals the depuration rate (Hawker and Connell, 1985). This sort of curve is seen in many studies involving the uptake of hydrophobic organics from food, water, food and water, sediment, sediment and water, etc. (McLeese *et al.*, 1980; Bruggeman *et al.*, 1981; Muir *et al.*, 1983; Rubinstein *et al.*, 1984; Muir and Yarechewski, 1988).

In our study, however, there was a rapid uptake of all PCDDs, followed by a decrease in tissue levels and an increase again near the end of the study. The changes in the availability of the PCDDs correlate inversely with changes in the visibility observed in the water column of Lake 304 (i.e., with changes in the suspended sediment concentration) at the time of the introduction of the cages into the corrals, and during subsequent sampling. When the cages (containing biota) were introduced into the corrals, the sediment was greatly

disturbed by movements of the divers' flippers and by the action of pushing the cages into the sediment. Visibility at this time was low, *viz.*, ≈0.5 to 1 m. As the summer progressed and the sediments settled, the visibility increased to ≈2 to 3 m. Visibility decreased once again near the end of the sampling season (to ≈0.5 to 1 m), because of inclement fall weather and the phenomenon of fall turnover. During turnover, a lake becomes isothermal and no longer has a density gradient (thermocline) separating the upper and lower layers. With the density gradient absent, the water in the lake is free to mix from top to bottom (Wetzel, 1983). With the water moving above it, the sediment once again is disturbed. The 1989 fall turnover date for Lake 304 is not known exactly, but was estimated from previous years data to be between 23 September and 7 October, 1989 (D. Cruikshank, Dept. of Fisheries and Oceans, Winnipeg, MB, pers. comm.). The correlations with visibility are qualitative because no direct measurements of visibility (e.g., Secchi disc readings) were taken.

With more sediment in the water column during times of low visibility, there would be more sediment-associated PCDDs present in the water column. It is these PCDDs that appear to have been available to the crayfish and mussels in the cages and to have caused the rapid increase in tissue levels. Increased availability of PCBs and other organic chemicals due to the disturbance of sediment by dredging, has been documented by Seelye *et al.* (1982), Hartley and Johnston (1983), Seelye and Mac (1984), and Rice and White (1987). As the sediment-associated PCDDs sank to the bottom of the corrals they would have become less available to the biota, whose tissue levels fell, only to rise again when the sediment was redisturbed.

A similar increase and decrease in PCDD concentrations in fathead minnows (*Pimephelas promelas*) and crayfish (*Procambarus* spp.) was observed by Friesen (1988). In his study, 1,2,3,4,7-pentachlorodibenzo-*p*-dioxin (12347-PCDD) was added to small ponds as a sediment slurry or as a sprayover. Resulting from both methods of introduction, the 12347-PCDD concentration in the biota increased rapidly (to a maximum on day 2 for the fish and on day 8 for the crayfish) and was followed by a gradual decrease over the next 30 to 60 days. These results may indicate that the main route of uptake is from the water column and not the particulates. However, the biota in Friesen (1988) were present in the ponds from time, t = 0, while the biota in the present study were introduced 1484 days after the original spike. This is important, for Servos (1988) reports that the contribution of the water compartment to the uptake of the PCDDs decreases dramatically at greater than 10-20 days post-spike, and that uptake then becomes mainly food driven.

Rice and White (1987), however, show the route of uptake for clams to be quite different than for fish. Fingernail clams (*Sphaerium striatinum*) appeared to integrate locally-occurring, sediment-associated PCBs at the sediment/water interface, but were unable to accumulate the bulk of the PCBs in the water column. Fathead minnows showed the opposite tendency. Rice and White (1987) conclude that the accumulation for the clams was mainly from the sediment (i.e., from suspended particles filtered by the clams), while for the fish it was water-based.

Rubinstein *et al.* (1984) report that the dietary contribution of PCBs accounted for 53% of the total body burden of fish fed for 20 days, and the percentage appeared to be increasing with time. They also

report that fish isolated from direct contact with contaminated sediment accumulated less PCB than did those fish allowed direct contact.

Langston (1978) shows that clams (*Cerastoderma edule*) accumulate twice as much Aroclor 1254 in a high density suspension of alumina particles (3.6×10^6 parts/mL) than in a low density suspension (1×10^6 parts/mL), concluding that PCB concentrations in the bivalves directly reflect the density of suspended, contaminated particles to which they are exposed. Langston (1978) also reports that the areas of greatest marine bivalve contamination would be the shallow, coastal areas with their high particle loads, which would allow for increased adsorption and scavenging of PCBs from the water column. The greater the density of suspended particulates (and hence, the lower the visibility in the water column), the greater the accumulation of sediment-associated contaminants.

In Lake 304, the results for the mussels appear to agree with those of Rice and White (1987) and Langston (1978), while the crayfish results were not as conclusive. There are several possible reasons for this. First, crayfish are very mobile creatures and were constantly stirring up the sediment around them with their movements. Since their local sediment is being continually disturbed, they should not show as great a difference between high and low concentrations. Second, when a crayfish molts, the new exoskeleton requires time to harden, making it very vulnerable to attack by its companions. Crayfish cannibalism was quite evident in the cages. When crayfish eat each other, they most likely take up PCDDs from their victims, as well as from the sediment. One way to prevent this would be to place each crayfish in its own separate, smaller cage. This would, however, introduce another biasing

factor, *viz.*, stress, resulting from the extreme constriction of their natural, free-ranging mode of existence and of their ability to feed (I. Davies, Dept. of Fisheries and Oceans, Winnipeg, MB, pers. comm.).

The mussel data in this study are consistent with the data of Miyata *et al.* (1987), in that the tetra-chlorinated congeners are accumulated to a greater extent than are the HpCDD or the OCDD, and that OCDD is accumulated more than HpCDD even though the sediment levels of OCDD are less than those of HpCDD. Miyata *et al.* (1987) do not speculate as to the reason for the increased uptake of OCDD as compared to HpCDD in their study. In contrast to the biota in most accumulation studies, the mussel in Miyata *et al.* (1987), show an increased affinity for the 1368-TCDD and 1379-TCDD congeners, and a surprising lack of affinity for the 2378-TCDD.

The bioavailability index (BI) of the PCDDs in the mussel and the crayfish are shown in Table 6. The **bioavailability index** is calculated as follows (Kuehl *et al.*, 1987):

$$BI = \frac{\text{ng PCDD/g lipid in the organism}}{\text{ng PCDD/g OC in the sediment}} \qquad \text{Eqn. 13.}$$

The bioavailability indices of the two tetra congeners in mussel are much less than the BI's for crayfish. This difference is also seen in the data of Muir *et al.* (1990) for 2378-TCDF (Table 6). For the HpCDD and the OCDD, however, the BI's for mussel are greater than the BI's for crayfish. The BI's for the 1368-TCDD, 1379-TCDD, HpCDD and the OCDD are all less than the BI for the 2378-TCDF of Muir *et al.* (1990), which is to be expected since the 2378-TCDF is more readily bioaccumulated than the PCDDs in this study (Esposito *et al.*, 1980;

Table 6. Bioavailability indices (BI) for PCDDs and PCDFs in mussels and crayfish in ELA limnocorrals.

Congener	BI	
	Mussel	Crayfish
1368-TCDD[1]	0.22	0.85
1379-TCDD[1]	0.13	0.58
HpCDD[1]	0.10	0.05
OCDD[1]	0.15	0.10
1368-TCDD[2]	29.4	
OCDD[2]	4.73	
1368-TCDD[3]	1.67	
OCDD[3]	0.93	
2378-TCDF[4]	18.6	24.6

[1] this study (1484-1499 days post "spike", 0-15 days after introduction into the corrals)

[2] calculated from mussels caged on the sediment, 0-10 days post "spike" (from Figs. 27 and 31, Servos, 1988)

[3] calculated from mussels caged on the sediment, 10-24 days post "spike" (from Figs. 27 and 31, Servos, 1988)

[4] Muir *et al.*, 1990

Kociba and Cabey, 1985). The 2,3,7,8-substituted PCDD/Fs accumulate to a greater extent than do the non-2,3,7,8-substituted PCDD/Fs because of the preference of enzymes for metabolic conversion on the lateral (the 2,3,7 and 8) positions in combination with the high binding affinities for the cytosolic receptor proteins (van den Berg *et al.*, 1986).

The BI's calculated from the data of Servos (1988) are much higher than those calculated from this study (Table 6). This difference may have occurred because the contribution of the water compartment to the tissue concentrations of 1368-TCDD and OCDD, was still fairly high at this point in his experiment (10 days post "spike") (Servos, 1988). However, as the importance of the water compartment decreases (24 days post "spike"), there is a decrease in the BI's for both congeners (and probably the 1379-TCDD and the HpCDD as well, although these were not analyzed by Servos). By day 1499 post "spike" (15 days after the introduction of the biota into the corrals), the BI's had decreased to a level where the contribution of the sediment compartment to the tissue residue levels far outweighed the contribution of the water compartment.

Although the concentrations of all the congeners in the water column should have been very low, the concentration of the 1368-TCDD and 1379-TCDD should have been greater than the concentrations of the HpCDD and the OCDD, since the log K_{ow} of the 1368-TCDD and the 1379-TCDD are less than the log K_{ow} of the HpCDD and the OCDD (Table 7). Since K_{ow}s are the ratio of the concentration in 1-octanol divided by the concentration in water, as the concentration in water decreases the K_{ow} value necessarily must increase. When the BI's are examined together with the K_{ow}s, it might be suggested that the crayfish receive their PCDDs from the water while the mussel accumulate them from the sediment.

Table 7. Log 1-octanol/water partition coefficients (Log K_{ow}) for the four PCDDs added to Lake 304, ELA

Congener	Log K_{ow}
1368-TCDD	6.29[1]
1379-TCDD	6.39[1]
HpCDD	8.00[2]
OCDD	8.20[2]

[1] Sijm *et al.*, 1989.

[2] Shiu *et al.*, 1988.

This view is supported by D.T.H.M. Sijm (U. of Amsterdam, Amsterdam, The Netherlands, pers. comm.) and by the data of Rice and White (1987), as discussed above. W. Fairchild (Dept. of Fisheries and Oceans, Winnipeg, MB, pers. comm.), however, is firm in his belief that his data (Fairchild *et al.*, 1990) show that the uptake of 2378-TCDF into crayfish, clams and other benthic invertebrates, is food chain based, rather than resulting from partitioning from the water. This reasoning is based on the fact that even though the log K_{ow} for the tetra congeners is less than the log K_{ow} for the HpCDD and the OCDD, all four congeners are so hydrophobic that the free concentrations in the water column are negligible. Servos (1988) has shown that in the first 48 hours "post-spike", less than 1% of the OCDD and 10 to 15% of the 1368-TCDD was freely dissolved in the water column, and that the concentrations in the water declined with a half-life of 4.0 days for the OCDD and 2.6 days for the 1368-TCDD. The data of Servos (1988), and his use of the food chain model of Thomann (1981) and Thomann and Connolly (1984), also show that the PCDDs studied tended to accumulate via the food chain.

Further evidence for food chain transfer is presented in Weston (1990). Weston found that the accumulation of benzo(*a*)pyrene (BaP) by the deposit-feeding polychaete worm, *Abarenicola pacifica*, correlated with feeding rate, suggesting that the source of the BaP was ingested sediment. Fowler *et al.* (1978), Klump *et al.* (1987) and Boese *et al.* (1990), have also shown that direct uptake from the sediment is important in the accumulation of PCBs and other organochlorine contaminants by polychaete and oligochaete worms and also by clams. These findings are in direct contrast to the work of Leversee

et al. (1983), Landrum *et al.* (1985) and Landrum *et al.* (1987) who show that uptake of PAHs occurs primarily from the water. These reseachers worked primarily with low K_{OW} PAHs, such as naphthalene (log K_{OW} = 3.35, Wasik *et al.*, 1983) which would be expected to have a much greater uptake from water than would PAHs such as BaP (log K_{OW} = 6.50, Yalkowsky *et al.*, 1983), or the PCBs, PCDFs and PCDDs (log K_{OW} > 6.0). However, interpreting the results of flow-through experiments, Landrum (1989) reports that although interstitial water is likely an important source for accumulation of most sediment-associated chemicals, ingestion can also play an important role. For example, in Landrum's experiment, the accumulation of BaP by ingestion could account for 100% of the accumulated body burden in the benthic amphipod, *Pontoporeia hoyi* (Landrum, 1989).

An explanation of the observation that the BIs of the tetra-chlorinated congeners in crayfish are greater than in mussels, while the BIs of the higher chlorinated congeners are less than in mussel may be that these two organisms preferentially feed on different particle sizes, i.e., at different trophic levels. Decapod crustaceans, like the crayfish, are tearers and chewers, are scavengers and are omnivorous, while the mussel is a filter feeder (Adams, 1987). Fairchild *et al.* (1990), have shown that > 88% of the 2378-TCDF in their sediment associates with particles smaller than 10 μm and that > 60% associates with particles between 0.22 and 1 μm in size. If the tetra-chlorinated congeners are associated with a different particle size range than are the hepta- and octa-chlorinated congeners, then the different BIs may be explained by the organisms feeding on different particle sizes. Another explanation may be that the uptake of the HpCDD and the OCDD is

inhibited by the size of the molecules. Opperhuizen *et al.* (1985) and Opperhuizen and Sijm (1990) have shown that low uptake efficiencies of higher chlorinated dioxins, furans and naphthalenes may be caused by inhibited membrane permeation due to the large cross-sectional area (>9.5 Å) of these molecules as compared to lower chlorinated PCDDs, PCDFs and PCNs. A cross sectional area of ≈9.5 Å appears to be the limiting size for easy permeation of chlorine-, bromine-, etc. substituted organic molecules because this is the largest size of pore that passes through the lipid bilayer of a cell (Opperhuizen *et al.*, 1985). However, accumulation of molecules with larger cross sectional areas, such as HpCDD and OCDD might be occurring by enzyme-mediated and/or micellar transport across cell membranes, in which case steric factors would have little or no effect (Opperhuizen *et al.*, 1985). Muir *et al.*, (1986) report the assimilation efficiency of 1368-TCDD from food by rainbow trout, *Salmo gairdneri* was 0.13 while it was 0.03 for OCDD. The lower assimilation efficiency of OCDD as compared to 1368-TCDD in conjunction with similar sediment concentrations of the two congeners in this study, would result in lower tissue concentrations in the biota if accumulation of these PCDDs is food chain based, as Servos (1988) has shown.

The presence of degradation products in the mussel or crayfish tissue was not investigated in this study. However, Servos (1988) reports less than 5% degradation products of 1368-TCDD present in mussel, *Anodonta grandis*, while Friesen (1988) reports 5 - 9% degradation products of 12347-PCDD present in crayfish, *Procambarus* spp. These findings are consistent with those of Wade *et al.*, (1989) and Lee *et al.*, (1972), who showed that bivalves have low or

undetectable enzyme activity for degrading xenobiotics compared to fish and crustaceans and should accumulate contaminants without alteration. Langston (1978) has shown that two bivalve molluscs (*Cerastoderma edule* and *Macoma balthica*) have the ability to degrade PCBs with less than 5 chlorine atoms, but are unable to degrade PCBs with higher chlorination patterns. Chlorine position was also shown to be an important factor controlling the biodegradability of PCBs by these two clams.

Conclusions

The bioavailability of sediment-associated 1,3,6,8-tetrachlorodibenzo-*p*-dioxin, 1,3,7,9-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,6,7,8-heptachloro-dibenzo-*p*-dioxin and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin to freshwater mussels, *Anodonta grandis* and crayfish, *Orconectes virilis*, was investigated in limnological corrals placed in the littoral zone of a Canadian Shield lake.

The results indicate that the PCDDs remained bioavailable up to 5 years after their introduction into the limnocorrals. Mussels and crayfish showed a propensity to accumulate all four PCDDs from the sediment into their tissues. The crayfish generally had higher concentrations of all congeners than did the mussels.

This study shows that the bioavailability of these PCDD congeners to mussel and crayfish appears to vary with the amount of sediment disturbance. The greater the sediment disturbance, whether by lake turnover, bioturbation or dredging, the greater the accumulation of the PCDDs.

The change in availability depending on the state of the sediments is important for future work in this field. A disturbance to the sediment prior to, or during, sampling can bias results in subsequent samplings. This is especially true in the case of monitoring studies, in which samples may only be taken a few times per year. If these samples are taken just after spring or fall turnover for example, the levels of contaminants in the biota may be higher than expected.

Bioavailability indices (concentration in lipid/concentration in sediment organic carbon) show that crayfish exhibited a greater tendency

to accumulate the tetra-chlorinated PCDDs from the sediment than did the mussels. The opposite was true for the hepta- and octa-chlorinated congeners. This result may be due to the species feeding on different trophic levels, or it may reflect the different lipid pool sizes in the two species. Alternatively, it may just be a property reflecting the life-style, anatomy and/or metabolism of the organisms (McElroy and Means, 1988). Both species showed higher BI values for the tetra-chlorinated congeners than for the hepta- and octa-chlorinated congeners. This may be due to decreased membrane permeability to HpCDD and OCDD as compared to the 1368-TCDD and 1379-TCDD (Opperhuizen and Sijm, 1990), or because the OCDD (and presumably the HpCDD) has a lesser assimilation efficiency than does the 1368-TCDD (and presumably the 1379-TCDD) (Muir *et al.*, 1986).

This study shows that mussels and crayfish can be used as biomonitoring agents. However there are problems associated with the use of both species. The size of mesh required to contain crayfish in their cages necessitates that the crayfish be fed every two weeks. Between these feedings, cannibalism occurs. The levels of PCDDs measured in the tissues of the crayfish were, therefore, a result of accumulation directly from the sediment as well as from the tissues of other crayfish.

References

Adams, W.J. 1987. Bioavailability of neutral lipophilic organic chemicals contained on sediments: A review. pp. 219-244. In Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. K.L. Dickson, A.W. Maki and W.A. Brungs, eds. Pergamon Press, New York, NY, U.S.A.

Addink, R., B. Van Bavel, R. Visser, H. Wever, P.Slot and K. Olie. 1990. Surface catalyzed formation of polychlorinated dibenzo-*p*-dioxins/dibenzofurans during municipal waste incineration. Chemosphere 20:1929-1934.

Ahlborg, U.G., F. Wærn, E. Manzoor and H. Håkansson. 1989. Effects of combinations of PCDDs/PCDFs given to Sprague-Dawley rats. Chemosphere 18:283-289.

Amendola, G., D. Blosser, L. Lafleur, A. McBride, F. Thames, T. Tiernon and R. Whittemore. 1989. The occurence and fate of PCDDs and PCDFs in five bleached kraft pulp and paper mills. Chemosphere 18:1181-1188.

Barnes, D.J., J. Bellin and D. Cleverly. 1986. Interim procedures for estimating risk associated with exposure to mixtures of chlorinated dibenzodioxins and dibenzofurans (CDDs and CDFs). Chemosphere 15:1895-1901.

Barton, R.G., W.D. Clark, W.S. Lanier and W.R. Seeker. 1990. Dioxin emissions from waste incinerators. Chemosphere 20:1825-1832.

Beck, H., E.M. Breuer, A. Droß and W. Mathar. 1990. Residues of PCDDs, PCDFs, PCBs and other organochlorine compounds in harbour seals and harbour porpoise. Chemosphere 20:1027-1034.

Bellward, G., J.-F. Boivin, F.C. Fraser, F.W. Karasek, R. Roberts, L. Shadoff, G.R.B. Webster and R.F. Willes. 1983. Report of the Joint Health and Welfare Canada/Environment Canada Expert Advisory Committee on Dioxins. November, 1983. 57 pp.

Bergqvist, P.-A., S. Bergek, H. Hallbäck, C. Rappe and S.A. Slorach. 1989. Dioxins in cod and herring from the seas around Sweden. Chemosphere 19:513-516.

Berrie, A.D. 1976. Detritus, micro-organisms and animals in fresh water. pp. 323-338. In The Role of Terrestrial and Aquatic Organisms in Decomposition Processes. J.M. Anderson and M. Macfadyen, eds. Blackwell Scientific Publications, Oxford.

Boehm, P.D. and J.G. Quinn. 1977. The persistence of chronically accumulated hydrocarbons in the hard shell clam *Mercenaria mercenaria*. Mar. Biol. 44:227-233.

Boese, B.L., H. Lee II, D.T. Specht, R.C. Randall and M.H. Winsor. 1990. Comparison of aqueous and solid-phase uptake for

hexachlorobenzene in the Tellinid clam *Macoma nasuta* (Conrad): A mass balance approach. Environ. Toxicol. Chem. 9:221-231.

Bruggeman, W.A., L.B.J.M. Martron, D. Kooiman and O. Hutzinger. 1981. Accumulation and elimination kinetics of di-, tri- and tetra chlorobiphenyls by goldfish after dietary and aqueous exposure. Chemosphere 10:811-832.

Buck, M. and P. Kirschmer. 1988. Concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in outdoor air of North-Rhine-Westphalia, Germany. Toxicol. Environ. Chem. 16:173-202.

Buckland, S.J., D.J. Hannah, J.A. Taucher, E. Slooten and S. Dawson. 1990. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in New Zealand's Hector's dolphin. Chemosphere 20:1035-1042.

Buser, H.R., H.P. Bosshardt and C. Rappe. 1978. Identification of polychlorinated dibenzo-*p*-dioxin isomers formed in fly ash. Chemosphere 7:165-172.

Clement, R.E., C. Tashiro, S. Suter and D. Hollinger. 1989. Chlorinated dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) in effluents and sludges from pulp and paper mills. Chemosphere 18:1189-1197.

Clement, R.E., H.M. Tosine, V. Taguchi, C.J. Musial and J. Uthe. 1987. Investigation of American lobster, *Homarus americanus*, for the

presence of chlorinated dibenzo-*p*-dioxin and dibenzofurans. Bull. Environ. Contam. Toxicol. 39:1069-1075.

Cochrane, W.P., J. Singh, W. Miles, B. Wakeford and J. Scott. 1980. Analysis of technical and formulated products of 2,4-dichlorophenoxy acetic acid for the presence of chlorinated dibenzo-*p*-dioxins. pp. 209-215. In Chlorinated Dioxins and Related Compounds. Impact on the Environment. O. Hutzinger, R.W. Frei, E. Merian and F. Pocchiari, eds. Pergamon Press, Oxford.

Connell, D.W. 1988. Bioaccumulation behavior of persistent organic chemicals with aquatic organisms. Rev. Environm. Contam. Toxicol. 102:117-154.

Corbet, R.L. 1983. The fate of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin, a trace contaminant in the herbicide 2,4-D, in a model aquatic system. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba. 146 pp.

Corbet, R.L., G.R.B. Webster and D.C.G. Muir. 1988. Fate of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin in an outdoor aquatic system. Environ. Toxicol. Chem. 7:167-180.

Courtney, K.D. 1976. Mouse teratology studies with chlorodibenzo-p-dioxins. Bull. Environ. Contam. Toxicol. 16:674-681.

Crunkilton, R.L., L.M. Smith, J.D. Petty and R.D. Kleopfer. 1987.

Residues of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Spring River, Missouri. Water Air Soil Pollut. 32:219-231.

Curtis, P.J. 1990. Biochemistry of Iron in Small Precambrian Shield Lakes at the Experimental Lakes Area, Northwestern Ontario. Ph.D. Thesis, University of Manitoba, Winnipeg, Manitoba.

Czuczwa, J.M. and R.A. Hites. 1984. Environmental fate of combustion-generated polychlorinated dioxins and furans. Environ. Sci. Technol. 18:444-450.

Czuczwa, J.M. and R.A. Hites. 1986a. Sources and fate of PCDD and PCDF. Chemosphere 15:1417-1420.

Czuczwa, J.M. and R.A. Hites. 1986b. Airborne dioxins and dibenzofurans: sources and fates. Environ. Sci. Technol. 20:195-200.

Czuczwa, J.M., F. Niessen and R.A. Hites. 1984. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediments from Siskiwit Lake, Isle Royale. Science 226:568-569.

di Domenico, A., V. Silano, G. Viviano and G. Zapponi. 1980. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Sèveso, Italy. Ecotoxicol. Environ. Safety 4:339-345.

Düwel, U., A. Nottrodt and K. Ballschmiter. 1990. Simultaneous

sampling of PCDD/PCDF inside the combustion chamber and on four boiler levels of a waste incineration plant. Chemosphere 20:1839-1846.

Esposito, M.P., T.O. Tiernan and F.E. Dryden. 1980. Dioxins. EPA-600/2-80-197. U.S. Environmental Protection Agency, Cincinnati, OH, U.S.A.

Fairchild, W.L., D.C.G. Muir, R.S. Currie and A.L. Yarechewski. 1990. Bioavailability of 2,3,7,8-TCDF to clams, crayfish and invertebrates in lake mesocosms. Presented at the Society of Environmental Toxicology and Chemistry (SETAC) 11th Annual Meeting, Nov. 11-15, 1990, Arlington, VA, U.S.A.

Farrington, J.W., E.D. Goldberg, R.W. Risebrough, J.H. Martin and V.T. Bowen. 1983. U.S. "Mussel Watch" 1976-1978: An overview of the trace-metal, DDE, PCB, hydrocarbon, and artificial radionuclide data. Environ. Sci. Technol. 17:490-496.

Ferraro, S.P., H. Lee II, R.J. Ozretich and D.T. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. Arch. Environ. Contam. Toxicol. 19:386-394.

Fishbein, L. 1973. Mutagens and potential mutagens in the biosphere. I. DDT and its metabolites, polchlorinated biphenyls, chlorodioxins, polycyclic aromatic hydrocarbons, haloethers. Sci. Tot. Environm. 4:305-340.

Foster, G.D., S.M. Baksi and J.C. Means. 1987. Bioaccumulation of trace organic contaminants from sediment by Baltic clams (*Macoma balthica*) and soft-shell clams (*Mya arenaria*). Environ. Toxicol. Chem. 6:969-976.

Fowler, S.W., G.G. Polikarpov, D.L. Elder, P. Parsi and J.-P. Villeneuve. 1978. Polychlorinated biphenyls: accumulation from contaminated sediments and water by the polychaete *Nereis diversicolor*. Mar. Biol. 48:303-465.

Friesen, K.J. 1988. Physical Properties, Sunlight Photolysis, and Environmental Fate of Polychlorinated Dibenzo-*p*-dioxins in Aquatic Ecosystems. Ph.D. Thesis, University of Manitoba, Winnipeg, Manitoba. 167 pp.

Friesen, K.J., M.D. Segstro, G.R.B. Webster and A.E. Smith. 1990. Environmental fate of 1,2,3,4,7-pentachlorodibenzo-*p*-dioxin in a Canadian prairie soil. Intern. J. Environ. Anal. Chem. 39:187-195.

Gray, A.P., S.P. Cepa and J.S. Cantrell. 1975. Intervention of the Smile's rearrangement in syntheses of dibenzo-*p*-dioxins. 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (HCDD). Tetr. Lett. 33:2873-2876.

Gray, A.P., S.P. Cepa, I.J. Solomon and O. Aniline. 1976. Synthesis of

specific polychlorinated dibenzo-*p*-dioxins. J. Org. Chem. 41:2435-2437.

Hagenmaier, H., H. Brunner, R. Haag and A. Berchtold. 1986. PCDDs and PCDFs in sewage sludge, river and lake sediments from south west Germany. Chemosphere 15:1421-1428.

Hargrave, B.T. 1976. The central role of invertebrate faeces in sediment decomposition. pp. 301-321. In The Role of Terrestrial and Aquatic Organisms in Decomposition Processes. J.M. Anderson and M. Macfadyen, eds. Blackwell Scientific Publications, Oxford.

Hartley, D.M. and J.B. Johnston. 1983. Use of the freshwater clam *Corbicula manilensis* as a monitor for organochlorine pesticides. Bull. Environ. Contam. Toxicol. 31:33-40.

Hawker, D.W. and C.W. Connell. 1985. Relationships between partition coefficient, uptake rate constant, clearance rate constant and time to equilibrium for bioaccumulation. Chemosphere 14:1205-1219.

Hutzinger, O., M.J. Blumich, M. van den Berg and K. Olie. 1985. Sources and fate of PCDDs and PCDFs: An overview. Chemosphere 14:581-600.

Johnson, W.E. and J.R. Vallentyne. 1971. Rationale, background, and development of experimental lakes studies in northwestern Ontario.

J. Fish. Res. Bd. Canada 28:123-128.

Karickhoff,S.W., D.S. Brown and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res. 13:241-248.

Kende, A.S. and M.R. DeCamp. 1975. Smiles rearrangement in the synthesis of hexachlorodibenzo-*p*-dioxins. Tetr. Lett. 33:2877-2880.

Kimbrough, R.D. 1990. Consumption of fish: benefits and perceived risk. In Dioxin and PCBs: Proceedings of the National Conference on Approaches to Address Human Health Risks and Aquatic Life Impacts. May 10-11, 1990, Washington, DC, U.S.A.

Kitunen, V.H. and M.S. Salkinoja-Salonen. 1990. PCDDs/PCDFs in unbleached pulp. Chemosphere 20:1663-1670.

Kjeller, L.-O., S.-E. Kulp, S. Bergek, M. Boström, P.-A. Bergqvist, C. Rappe, B. Jonsson, C. deWit, B. Jansson and M. Olsson. 1990. Levels and possible sources of PCDD/PCDF in sediment and pike samples from Swedish lakes and rivers. (Part One). Chemosphere 20:1489-1496.

Klecka, G.M. and D.T. Gibson. 1980. Metabolism of dibenzo-*p*-dioxin and chlorinated dibenzo-*p*-dioxins by a *Beijerinckia* species. Appl. Environ. Microbiol. 39:288-296.

Klump, J.V., J.R. Krezoski, M.E. Smith and J.L. Kaster. 1987. Dual tracer studies of the assimilation of an organic contaminant from sediments by deposit feeding oligochaetes. Can. J. Fish. Aquat. Sci. 44:1574-1583.

Kociba, R.J. and O. Cabey. 1985. Comparitive activity and biological activity of chlorinated dibenzo-*p*-dioxins and furans relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Chemosphere 14:649-660.

Koistinen, J., J. Paasivirta and P.J. Vuorinen. 1989. Dioxins and other planar polychloroaromatic compounds in Baltic, Finnish and Arctic fish samples. Chemosphere 19:527-530.

Konemann, H. and K. van Leeuwen. 1980. Toxicokinetics in fish: Accumulation and elimination of six chlorobenzenes by guppies. Chemosphere 9:3-19.

Kraus, P.B. and Y.S. Hamdy. 1985. Biological monitoring of organochlorine contaminants in the St. Clair and Detroit Rivers using introduced clams, *Elliptio complanatus*. J. Great Lakes Res. 11:247-263.

Kraus, P. and J. Steele. 1987. Environment ministry releases result of tests at mouth of Pettit Flume, Niagara River. Ontario Ministry of the Environment, February 9, 1987 Newsrelease/communiqué.

Kuehl, D.W., P.M. Cook and A.R. Batterman. 1986. Uptake and depuration studies of PCDDs and PCDFs in freshwater fish. Chemosphere 15:2023-2026.

Kuehl, D.W., P.M. Cook, A.R. Batterman, D. Lothenbach and B.C. Butterworth. 1987. Bioavailability of polychlorinated dibenzo-*p*-dioxins and dibenzofurans from contaminated Wisconsin River sediment to carp. Chemosphere 16:667-679.

Lake, J.L., N.I. Rubinstein, H. Lee II, C.A. Lake, J. Heltshe and S. Pavignano. 1990. Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. Environ. Toxicol. Chem. 9:1095-1106.

Landrum, P.F. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediment for the amphipod *Pontoporeia hoyi*. Environ. Sci. Technol. 23:588-595.

Landrum, P.F., S.R. Nihart, B.J. Eadie and L.R. Herche. 1987. Reduction in bioavailability of organic contaminants to the amphipod *Pontoporeia hoyi* by dissolved organic matter of sediment interstitial waters. Environ. Toxicol. Chem. 6:11-20.

Landrum, P.F., M.D. Reinhold, S.R. Nihart and B.J. Eadie. 1985. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials

and natural dissolved organic matter. Environ. Toxicol. Chem. 4:459-467.

Landrum, P.F. and J.A. Robbins. 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. pp. 237-263. In Sediments: Chemistry and Toxicity of In-Place Pollutants. R. Baudo, J. Geisy and H. Muntau, eds. Lewis Publ. Inc. Chelsea, MI, U.S.A.

Langston, W.J. 1978. Persistence of polychlorinated biphenyls in marine bivalves. Mar. Biol. 46:35-40.

Lans, M.C., A. Brouwer, J.G. Koppe and M. van den Berg. 1990. Enzyme induction and alterations in thyroid hormone, vitamin A and K levels by TCDD in neonatal and maternal rats. Chemosphere 20:1129-1134.

Lee, R.F., R. Sauerhaber and A.A. Benson. 1972. Petroleum hydrocarbons: Uptake and discharge by the marine mussel *Mytilus edulis*. Science 177:344-346.

Leversee, G.J., P.F. Landrum, J.P. Giesy and T. Fannin. 1983. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. Can. J. Fish. Aquat. Sci. 40(Suppl. 2):63-69.

Lyman, W.J. 1982. Adsorption coefficient for soils and sediments. In Handbook of Chemical Property Estimation Methods. Environmental

Behavior of Organic Compounds. pp. 4-1 - 4-33. W.J. Lyman, W.F. Reehl and D.H. Rosenblatt eds. McGraw-Hill, Inc, New York, NY, U.S.A.

Mackay, D. 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. 16:274-276.

Marcheterre, L. 1985. Fate of octachlorodibenzo-*p*-dioxin in small ponds. M.Sc. Thesis. University of Manitoba, Winnipeg, Manitoba. 80 pp.

Marcheterre, L., G.R.B. Webster, D.C.G. Muir and N.P. Grift. 1985. Fate of ^{14}C-octachlorodibenzo-*p*-dioxin in artificial outdoor ponds. Chemosphere 14:835-838.

Marklund, S., C. Rappe and M. Tysklind. 1987. Identification of polychlorinated dibenzofurans and dioxins in exhausts from cars run on leaded gasoline. Chemosphere 16:29-36.

Matsumura, F. and H.J. Benezet. 1973. Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ. Health Perspect., Exp. Issue No 5, pp. 253-258.

McCarthy, J.M. and M.C. Black. 1988. Partitioning between dissolved organic macromolecules and suspended particulates: Effects on bioavailability and transport of hydrophobic organic chemicals in aquatic systems. pp. 233-246. In Aquatic Toxicology and Hazard

Assessment: 10th Volume, ASTM STP 971. W.J. Adams, G.A. Chapman and W.G. Landis, eds. American Society for Testing and Materials, Philadelphia, PA, U.S.A.

McElroy, A.E. and J.C. Means. 1988. Factors affecting the bioavailability of hexachlorobiphenyls to benthic organisms. pp. 149-158. In Aquatic Toxicology and Hazard Assessment: 10th Volume, ASTM STP 971. W.J. Adams, G.A. Chapman and W.G. Landis, eds. American Society for Testing and Materials, Philadelphia, PA, U.S.A.

McLeese, D.W., C.D. Metcalfe and D.S. Pezzack. 1980. Uptake of PCBs from sediment by *Nereis virens* and *Crangon septemspinosa*. Arch. Environ. Contam. Toxicol. 9:507-518.

Menzie, C.A. 1980. Potential significance of insects in the removal of contaminants from aquatic sources. Water Air Soil Pollut. 13:473-480.

Metcalfe, J.L. and M.N. Charlton. 1990. Freshwater mussels as biomonitors for organic industrial contaminants and pesticides in the St. Lawrence River. Sci. Total Environm. 98:595-615.

Miller, G.C. and R.G. Zepp. 1987. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: environmental chemistry. pp. 82-93. In Solving Hazardous Waste Problems: Learning From Dioxins. J.H. Exner, ed. American Chemical Society. Washington, DC, U.S.A.

Miyata, H., K. Takayama, J. Ogaki, T. Kashimoto and S. Fukushima. 1987. Monitoring of PCDDs in Osaka Bay using blue mussel. Chemosphere 16:1817-1822.

Miyata, H., K. Takayama, M. Mimura and T. Kashimoto. 1989. Investigation on contamination sources of PCDDs and PCDFs in blue mussel in Osaka Bay in Japan. Chemosphere 19:517-520.

Muir, D.C.G., W.L. Fairchild and M. Whittle. 1990. Predicting bioaccumulation of chlorinated dioxins and furans in fish near Canadian bleached kraft mills. Submitted to Water Pollut. Res. J. Can.

Muir, D.C.G., B.E. Townsend and W.L. Lockhart. 1983. Bioavailability of six organic chemicals to *Chironomus tentans* larvae in sediment and water. Environ. Toxicol. Chem. 2:269-281.

Muir, D.C.G. and A.L. Yarechewski. 1988. Dietary accumulation of four chlorinated dioxin congeners by rainbow trout and fathead minnows. Environ. Toxicol. Chem. 7:227-236.

Muir, D.C.G., W.K. Marshall and G.R.B. Webster. 1985a. Bioconcentration of PCDDs by fish: effects of molecular structure and water chemistry. Chemosphere 14:829-833.

Muir, D.C.G., A.L. Yarechewski, R.L. Corbet, G.R.B. Webster and A.E.

Smith. 1985b. Laboratory and field studies on the fate of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin in soil and sediments. J. Ag. Food Chem. 33:518-523.

Nakano, T., M. Tsuji and T. Okuno. 1987. Levels of chlorinated organic compounds in the atmosphere. Chemosphere 16:1781-1786.

Norstrom, R.J. and M. Simon. 1990. Determination of specific polychlorinated dibenzo-*p*-dioxins and dibenzofurans in biological matrices by gel-permeation/carbon chromatography and gas chromatography-mass spectrometry. In Environmental Carcinogens - Methods of Analysis and Exposure Measurement. Volume 11 - Polychlorinated Dibenzodioxins, Dibenzofurans. C. Rappe and H.R. Buser, eds. World Health Organization, International Association for Research on Cancer, Lyon, France. (in press)

Norstrom, R.J., M. Simon and D.C.G. Muir. 1990. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in marine mammals in the Canadian north. Environ. Pollut. 66:1-19.

Olie, K., A. Schecter, J. Constable, R.M.M. Kooke, P. Serné, P.C. Slot and P. de Vries. 1989. Chlorinated dioxin and dibenzofuran levels in food and wildlife samples in the north and south of Vietnam. Chemosphere 19:493-496.

Opperhuizen, A., E.W.v.d. Velde, F.A.P.C. Gobas, D.A.K. Liem, J.M.D.v.d. Steen and O. Hutzinger. 1985. Relationship between

bioconcentration in fish and steric factors of hydrophobic chemicals. Chemosphere 14:1871-1896.

Opperhuizen, A. and D.T.H.M. Sijm. 1990. Bioaccumulation and biotransformation of polychlorinated dibenzo-p-dioxins and dibenzofurans in fish. Environ. Toxicol. Chem. 9:175-186.

Osibanjo, O. and O. Bamgbose. 1990. Chlorinated hydrocarbons in marine fish and shellfish of Nigeria. Mar. Pollut. Bull. 21:581-586.

Petty, J.D., L.M. Smith, P.-A. Bergqvist, J.L. Johnson, D.L. Stalling and C. Rappe. 1983. Composition of polychlorinated dibenzofuran and dibenzo-*p*-dioxin residues in sediments of the Hudson and Housatonic Rivers. pp. 203-207. In Chlorinated Dioxins and Dibenzofurans in the Total Environment. G. Choudhary, L.H. Keith and C. Rappe, eds. Butterworth Publ., Woburn, MA, U.S.A.

Puhvel, S.M., D.C. Ertl and C.A. Lynberg. 1984. Increased epidermal transglutaminase activity following 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: In vivo and in vitro studies with mouse skin. Toxicol. Appl. Pharmacol. 73:42-47.

Quensen, J.F. and F. Matsumura. 1983. Oxidative degradation of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin by microorganisms. Environ. Toxicol. Chem. 2:261-268.

Ramesh, A., S. Tanabe, A.N. Subramanian, D. Mohan, V.K. Venugopolan and

R. Tatsukawa. Persistent organochlorine residues from coastal waters of South India. Mar. Pollut. Bull. 21:587-590.

Rappe, C., P.-A. Bergqvist, M. Hansson, L.-O. Kjeller, G. Lindström, S. Marklund and M. Nygren. 1984. Chemistry and analysis of polychlorinated dioxins and dibenzofurans in biological samples. pp. 17-25. In Banbury Report 18: Biological Mechanisms of Dioxin Action. © Cold Spring Harbor Laboratory, Cold Spring Harbor, Long Island, NY, U.S.A.

Rappe, C., P.-A. Bergqvist and L.-O. Kjeller. 1989. Levels, trends and patterns of PCDDs and PCDFs in Scandinavian environmental samples. Chemosphere 18:651-658.

Rappe, C. and L.-O. Kjeller. 1987. PCDDs and PCDFs in environmental samples, air, particulates, sediments and soil. Chemosphere 16:1775-1780.

Reuber, B., D. Mackay, S. Paterson and P. Stokes. 1987. A discussion of chemical equilibria and transport at the sediment-water interface. Environ. Toxicol. Chem. 6:731-739.

Rice, C.P. and D.S. White. 1987. PCB availability assessment of river dredging using caged clams and fish. Environ. Toxicol. Chem. 6:259-274.

Roesijadi, G., J.W. Anderson and J.W. Blaylock. 1978. Uptake of

hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: Influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. J. Fish. Res. Bd. Can. 35:608-614.

Rubinstein, N.I., W.T. Gilliam and N.R. Gregory. 1984. Dietary accumulation of PCBs from a contaminated sediment source by a demersal fish (*Leiostomus xanthurus*). Aquat. Toxicol. 5:331-342.

SAS Institute Inc. 1989a. The REG procedure. pp. 1351-1456. In SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc., Cary, NC, U.S.A.

SAS Institute Inc. 1989b. The GLM procedure. pp. 891-996. In SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc., Cary, NC, U.S.A.

SAS Institute Inc. 1989c. The ANOVA procedure. pp. 209-244. In SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 1. SAS Institute Inc., Cary, NC, U.S.A.

Schecter, A., B.D. Eitzer and R.A. Hites. 1989. Chlorinated dioxin and dibenzofuran levels in sediments collected from rivers in Vietnam, 1984-6. Chemosphere 18:831-834.

Seelye, J.G., R.J. Hesselberg and M.J. Mac. 1982. Accumulation by fish

of contaminants released from dredged sediments. Environ. Sci. Technol. 16:459-463.

Seelye, J.G. and M.J. Mac. 1984. Bioaccumulation of toxic substances associated with dredging and dredged material disposal. EPA-905/3-84-005. U.S. Environmental Protection Agency, Gulf Breeze, FL, U.S.A.

Servos, M.R. 1988. Fate and Bioavailability of Polychlorinated Dibenzo Dioxins in Aquatic Environments. Ph.D. Thesis, University of Manitoba, Winnipeg, Manitoba. 181 pp.

Servos, M.R., and D.C.G. Muir. 1989. Effect of dissolved organic matter from Canadian Shield lakes on the bioavailability of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin to the amphipod *Crangonyx laurentianus*. Environ. Toxicol. Chem. 8:141-150.

Servos, M.R., D.C.G. Muir and G.R.B. Webster. 1989. The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-*p*-dioxins. Aquat. Toxicol. 14:169-184.

Shine, H.J. 1973. Aromatic Rearrangements. pp. 65-103. In Aromatic Compounds. Organic Chemistry, Series One, Vol. 3. MTP International Review of Science. H. Zollinger, ed. Butterworth & Co. (Publishers) Ltd. London.

Shiu, W.Y., W. Doucette, F.A.P.C. Gobas, A. Andren and D. Mackay. 1988.

Physical-chemical properties of chlorinated dibenzo-p-dioxins. Environ. Sci. Technol. 22:651-658.

Sijm, D.T.H.M., H. Wever, P.J. de Vries and A. Opperhuizen. 1989. Octan-1-ol/water partition coefficients of polychlorinated dibenzo-*p*-dioxins and dibenzofurans: experimental values determined with a stirring method. Chemosphere 19:263-266.

Sōdergren, A. 1989. Biological Effect of Bleached Pulp Mill Effluents. Report 3558 of the National Swedish Environmental Protection Board, Solna, Sweden. 139 pp.

Swartz, R.C. 1987. Toxicological methods for determining the effects of contaminated sediment on marine organisms. pp. 183-198. In Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems. K.L. Dickson, A.W. Maki and W.A. Brungs, eds. Pergamon Press, New York, NY, U.S.A.

Tanabe, S., N. Kannan, M. Fukishima, T. Okamoto, T. Wakimoto and R. Tatsukawa. 1989. Persistent organochlorines in Japanese waters: An introspective summary from a far east developed nation. Mar. Pollut. Bull. 20:344-352.

Thomann, R.V. 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can. J. Fish. Aquat. Sci. 38:280-296.

Thomann, R.V. and J.P. Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18:65-71.

Trudel, L. 1991. Dioxins and Furans in Bottom Sediments Near the 47 Canadian Pulp and Paper Mills Using Chlorine Bleaching. Report from the Water Quality Branch, Inland Water Directorate, Environment Canada, Ottawa. 290 pp.

van den Berg, M., F.W.M. van der Weilen and K. Olie. 1986. The presence of PCDDs and PCDFs in human breast milk from the Netherlands. Chemosphere 15:693-706.

van der Weiden, M.E.J., J. van der Kolk, A. Penninks, W. Seinen and M. van den Berg. 1990. A dose/response study with 2,3,7,8-TCDD in the rainbow trout (*Onchorhynchus mykiss*). Chemosphere 20:1053-1058.

van der Weiden, M.E.J., L.H.J. Craane, E.H.G. Evers, R.M.M. Kooke, K. Olie, W. Seinen and M. van den Berg. 1989. Bioavailability of PCDDs and PCDFs from bottom sediments and some associated biological effects in the carp (*Cyprinus carpio*). Chemosphere 19:1009-1016.

Wade, T.L., M.C. Kennicutt II and J.M. Brooks. 1989. Gulf of Mexico hydrocarbon seep communities: Part III. Aromatic hydrocarbon concentrations in organisms, sediments and water. Mar. Environm. Res. 27:19-30.

Ward, C.T. and F. Matsumura. 1978. Fate of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in a model aquatic environment. Arch. Environ. Contam. Toxicol. 7:349-357.

Wasik, S.P., M.M. Miller, Y.B. Tewari, W.E. May, W.J. Sonnefeld, H. DeVoe and W.H. Zoller. 1983. Determination of the vapour pressure, aqueous solubility, and octanol/water partition coefficient of hydrophobic substances by coupled generator column/liquid chromatographic methods. Res. Rev. 85:29-42.

Weston, D.P. 1990. Hydrocarbon accumulation from contaminated sediment by the deposit-feeding polychaete *Abarenicola pacifica*. Mar. Biol. 107:159-169.

Wetzel, R.G. 1983. Limnology. 2nd ed. pp. 73-79. Saunders College Publ. Philadelphia, PA, U.S.A.

Whittemore, R.C., L.E. LaFleur, W.J. Gillespie, G.A. Amendola and J. Helms. 1990. USEPA/paper industry cooperative dioxin study: The 104 mills. Chemosphere 20:1625-1632.

Yalkowsky, S.H., S.C. Valvani and D. Mackay. 1983. Estimation of the aqueous solubility of some aromatic compounds. Res. Rev. 85:43-55.

Yasuhara, A., H. Ito and M. Morita. 1987. Isomer-specific

determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in incinerator-related samples. Environ. Sci Technol. 21:971-979.

Yeomans, J.C. and J.M. Bremner. 1988. A rapid and precise method for routine determination of organic carbon in soil. Commun. In Soil Sci. Plant Anal. 19:1467-1476.

Young, A.L. 1980. The Chlorinated dibenzo-*p*-dioxins. pp. 133-205. In The Science of 2,4,5-T and Associated Phenoxy Herbicides. R.W. Bovey and A.L. Young, eds. John Wiley and Sons, Inc., New York, NY, U.S.A.

Appendix 1

Expected[1] and observed[2] ion ratios (as a percent of the base peak) during the SIM analysis of samples by GC/MSD.

Congener	Ion (m/z)	Expected	Observed		
			Sediment	Mussel	Crayfish
TCDDs	259	25.9	28.5	22.0	29.6
	320	79.1	76.9	77.4	78.5
	322	100.0	100.0	100.0	100.0
	324	47.9	50.8	52.9	46.3
HpCDD	363	23.2	20.6	31.7	29.3
	424	98.0	100.0	94.5	93.2
	426	100.0	97.3	100.0	100.0
	428	53.3	52.4	53.8	57.3
OCDD	397	13.4	20.9	18.4	17.5
	458	85.9	89.0	84.0	91.1
	460	100.0	100.0	100.0	100.0
	462	44.1	65.0	48.8	51.6

[1] Expected values are the averaged ratios of three standards (100, 200 and 400 pg/μL).

[2] Observed values are the averaged ratios of three samples from each sample type.

Appendix 2. Lake 304 sediment, mussel and crayfish data summary.

SEDIMENT DATA: 1985 – 1990 (ng/g dry weight).

DAY POST SPIKE	DAY POST INTRO TO CORRALS	1368 MEAN	1368 STD DEV	1379 MEAN	1379 STD DEV	HCDD MEAN	HCDD STD DEV	OCDD MEAN	OCDD STD DEV	n	DD/MMM/YY
8	−1476	5.5	2.8	1.0	0.4	14.7	6.8	5.4	2.2	2	20/JUN/85
55	−1429	24.8	16.0	9.1	9.9	72.8	39.8	30.1	21.1	2	06/AUG/85
271	−1213	118.3	57.6	57.6	25.5	346.0	87.9	155.2	43.8	2	18/MAR/86
348	−1136	91.7	39.5	47.3	21.6	286.8	85.9	126.9	36.4	2	03/JUN/86
697	−787	66.0	24.7	32.2	11.6	208.4	70.5	103.2	29.9	2	18/MAY/87
1499	15	70.4	11.4	38.2	7.8	289.0	31.4	130.9	21.4	4	20/JUL/89
1562	78	58.5	13.4	30.9	6.6	232.0	22.8	100.6	6.3	4	21/SEP/89
1807	323	46.0	9.9	22.3	5.4	140.9	16.7	65.0	10.9	4	24/MAY/90

MUSSEL DATA: 1989 (pg/g wet weight).

DAY POST SPIKE	DAY POST INTRO TO CORRALS	1368 MEAN	1368 STD DEV	1379 MEAN	1379 STD DEV	HCDD MEAN	HCDD STD DEV	OCDD MEAN	OCDD STD DEV	n	DD/MMM/YY
1499	15	169.6	88.6	49.7	24.3	275.6	23.0	170.8	133.6	4	20/JUL/89
1513	29	136.0	83.2	41.3	18.7	49.9	20.9	35.1	10.9	4	03/AUG/89
1533	49	78.3	31.9	25.5	15.1	23.1	1.7	28.6	21.5	4	23/AUG/89
1562	78	53.9	19.3	11.3	2.0	47.3	24.4	61.4	20.3	4	21/SEP/89
1587	103	132.8	33.9	40.5	6.8	77.7	74.2	85.1	87.2	4	16/OCT/89

CRAYFISH DATA: 1989 (pg/g wet weight).

DAY POST SPIKE	DAY POST INTRO TO CORRALS	1368 MEAN	1368 STD DEV	1379 MEAN	1379 STD DEV	HCDD MEAN	HCDD STD DEV	OCDD MEAN	OCDD STD DEV	n	DD/MMM/YY
1499	15	1093.8	703.2	381.0	218.2	209.0	103.1	200.5	130.8	4	20/JUL/89
1513	29	577.0	201.2	172.7	74.3	146.1	5.4	87.0	51.6	4	03/AUG/89
1533	49	416.3	368.1	96.1	72.0	109.6	69.1	97.8	76.5	4	23/AUG/89
1562	78	1061.9		321.6		473.0		480.6		3	21/SEP/89

Appendix 3. Regression analysis of the PCDD congener disappearance slopes (SAS Institute Inc, 1989a).

Congener	Slope	Standard Error	t-value	prob>|t|
1368-TCDD	-0.000431	± 0.000167	-2.566	0.0281
1379-TCDD	-0.000417	± 0.000169	-2.462	0.0336
HpCDD	-0.000306	± 0.000154	-1.988	0.0749
OCDD	-0.000315	± 0.000146	-2.160	0.0562

Appendix 4. Multiple comparisons of the mean concentrations of PCDD congeners in sediment, freshwater mussel and crayfish using least significant difference (LSD) method (SAS Institute Inc., 1989c).

Sediment

Day[1]	1368-TCDD	1379-TCDD	HpCDD	OCDD
8	5.5^{b}	1.0^{c}	14.7^{d}	5.4^{d}
55	24.8^{b}	9.2^{c}	72.8^{cd}	30.1^{cd}
271	118.3^{a}	57.6^{a}	346.0^{a}	155.2^{a}
348	91.7^{ab}	47.3^{ab}	286.8^{ab}	126.9^{ab}
697	66.0^{ab}	32.2^{abc}	208.4^{abc}	103.2^{abc}
1499	70.4^{ab}	38.2^{abc}	289.0^{ab}	130.9^{ab}
1562	58.5^{ab}	30.9^{abc}	232.0^{abc}	100.6^{abc}
1807	46.0^{ab}	22.3^{abc}	140.9^{bcd}	65.0^{bcd}

Means followed by the same symbol in each column are not significantly different ($p<0.05$).

[1] days post "spike"

Freshwater mussel

Day[1]	1368-TCDD	1379-TCDD	HpCDD	OCDD
15	169.6[a]	49.7[a]	275.6[a]	170.8[a]
29	136.0[a]	41.3[a]	49.9[b]	35.1[a]
49	78.3[a]	25.5[a]	23.1[b]	28.6[a]
78	53.9[a]	11.3[a]	47.3[b]	61.4[a]
103	132.8[a]	40.5[a]	77.6[b]	85.1[a]

Means followed by the same symbol in each column are not significantly different ($p<0.05$).

[1] days after introduction into the corrals

Crayfish

Day[1]	1368-TCDD	1379-TCDD	HpCDD	OCDD
15	1093.8[a]	381.0[a]	209.0[a]	200.5[ab]
29	577.0[a]	172.7[a]	146.1[a]	87.0[b]
49	416.3[a]	96.1[a]	109.6[a]	97.8[b]
78	1061.9[a]	321.6[a]	473.0[b]	480.6[a]

Means followed by the same symbol in each column are not significantly different ($p<0.05$).

[1] days after introduction into the corrals