Fate and Bioavailability of Polychlorinated Dibenzo-p-dioxins in Aquatic Environments

by

Mark Roy Servos

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ABSTRACT

The environmental fate and bioavailability of 1,3,6,8-tetra- (T_4CDD) and octachlorodibenzo-p-dioxin (O_8CDD) were studied in large (40 m^3) lake enclosures at the Experimental Lakes Area in Northwestern Ontario. PCDDs were added to replicate enclosures as a sediment slurry at a nominal concentration of 58-59 ng/L. Both congeners partitioned rapidly to the surficial sediments where they persisted over the two years of the study. Although initially the total concentrations of T_4CDD in water were higher than O_8CDD, the concentrations of the T_4CDD in the water column declined more rapidly ($t_{1/2}$ 4.0±0.3 and 2.6±0.2 d respectively). Under laboratory conditions, sorption of PCDDs to dissolved organic carbon (DOC) increased the apparent solubility and decreased the apparent sediment to water partition coefficient (K_p) and bioconcentration factor (BCF). Sorption of PCDDs to organic matter (Aldrich humic acid and natural lake waters) reduced the truly dissolved concentrations in water determined using reverse-phase cartridges or gas sparging relative to organic matter free waters (i.e., <0.24 mg/L DOC). In lake enclosures 10-15% and <1% of the total T_4CDD and O_8CDD, respectively, were determined to be truly dissolved in the water column. Although the more highly chlorinated dioxins (i.e., O_8CDD) are more lipophilic, they partitioned rapidly to organic matter in the water column and sediments which limited their availability and therefore dictated their environmental fate (sorption, volatilization, bioconcentration, etc.). The T_4CDD was more bioavailable to caged benthic invertebrates and fish (white sucker) than O_8CDD immediately after the addition to the enclosures, but by day 24, the bioavailability of the T_4CDD had declined to a level similar to that of O_8CDD. Accumulation of PCDDs (particularly the T_4CDD) appeared to have shifted from direct equilibrium

partitioning from the water column during the first few days to a detrital based food chain transfer as the truly dissolved concentrations in the water column declined. Because PCDDs partition rapidly to sediment where they are very persistent, the sediments in aquatic environments (i.e., the Great Lakes) may act as a source of PCDDs to biota long after inputs have been reduced or eliminated.

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Finally I wish to thank my wife, Maria, for her understanding, support and patience.

For Daniel.

To go fishing is the chance to wash one's soul with pure air, with the rush of the brook, or with the shimmer of the sun on blue water. It brings meekness and inspiration from the decency of nature, charity toward tackle-makers, patience toward fish, a mockery of profits and egos, a quieting of hate, a rejoicing that you do not have to decide a darn thing until next week. And it is discipline in the equality of men - for all men are equal before fish.

Herbert Hoover

PREFACE

This work is part of an ongoing research effort at the Pesticide Research Laboratory, University of Manitoba, to examine the factors which control the environmental fate, bioavailability and toxicology of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF). To date this effort has included determination of physical/chemical properties for environmental fate modelling and field studies in soils and small ponds on selected congeners. This thesis augments this research effort and represents a series of experiments designed to further the understanding of the environmental dynamics of PCDDs in aquatic systems. The main body of the thesis is composed of five separate chapters each representing an independent series of experiments. Because of the format there is some repetition between chapters, but this repetition is minor and is intended to clarify the objectives, methods and conclusions in each section. Each chapter deals with specific aspects of the environmental chemistry of PCDDs:

I. the effect of dissolved organic matter on the determination of the sediment to water partition coefficient (Kp) for 1,3,6,8-tetrachloro-dibenzo-p-dioxin (T_4CDD),

II. the effect of particulate and dissolved organic matter on the determination of bioconcentration factors (BCFs) of several PCDDs,

III. the effect of dissolved organic matter from natural sources on the uptake kinetics of T_4CDD by an invertebrate,

IV. the environmental fate of T_4CDD and octachlorodibenzo-p-dioxin (O_8CDD) in natural environments, i.e., large lake enclosures at the Experimental Lakes Area,

V. the bioavailability of T_4CDD and O_8CDD in lake enclosures.

Although the topics of the chapters are very different (i.e., laboratory studies on Kp and BCF and field studies on the fate and bioavailability), all five chapters are tied together by the central theme of the importance of measuring "truly dissolved" water concentrations to understanding the dynamics of PCDDs in aquatic environments. Each chapter advances our understanding of the ecotoxicology of PCDDs and will contribute to the risk assessment and eventual abatement of these environmental contaminants.

LIST OF ABREVIATIONS

BCF	bioconcentration factor
DCM	dichloromethane
DOC	dissolved organic carbon
DOM	dissolved organic matter
ELA	Experimental Lakes Area
f	fraction organic carbon
free	truly dissolved
g	gravity
H_6CDD	1,2,3,4,7,8-hexachlorodibenzo-p-dioxin
H_7CDD	1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin
HLC	Henry's Law Constant
HPLC	high performance liquid chromatography
K_d	depuration rate constant
K_{doc}	dissolved organic carbon partition coefficient
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol to water partition coefficient
K_p	sediment to water partition coefficient
K_u	uptake rate constant
LSC	liquid scintillation counting
nd	not detectable
NRC	National Research Council
O_8CDD	octachlorodibenzo-p-dioxin
PCDD	polychlorinated dibenzo-p-dioxin
POC	particulate organic carbon
POM	particulate organic matter

SS	suspended sediments
T_4CDD	1,3,6,8-tetrachlorodibenzo-p-dioxin
2,3,7,8-T_4CDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TSS	total suspended solids

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BACKGROUND

Polychlorinated dibenzo-p-dioxins (PCDDs) are extremely toxic compounds which have become ubiquitous trace contaminants in aquatic environments (Czuczwa and Hites 1986; Rappe et al. 1987). 2,3,7,8-Tetrachloro-dibenzo-p-dioxin (2,3,7,8-T_4CDD) is one of the most toxic anthropogenic compounds known. The LC_{50} (single oral dose) for 2,3,7,8-T_4CDD in the guinea pig is only 0.6-2 µg/kg (Table 1). Twenty-eight day LC_{50}s for 2,3,7,8-T_4CDD in fathead minnows is less than 63 ng/L and exposures as short as 24 h to only 82 ng/L results in >90% mortality within 60 days (Adams et al. 1986). Exposure of rainbow trout to 107 ng/L 2,3,7,8-T_4CDD for only 6 h caused mortality several weeks after the initial exposure and caused liver enlargement, fin rot, hemorrhaging and reduced growth rates after 64 days. Miller et al. (1973) also reported delayed motality and reduced growth of fish exposed to 2,3,7,8-T_4CDD through both water and food. Exposure of rainbow trout eggs to only 0.1 ng/L caused significant growth reduction for 72 d and exposure to 1 ng/L caused generalized edema, teratologic defects and increased mortality relative to controls (Helder 1981). Mehrle et al. (1988) determined a 56 day (28 d continuous exposure 28 d depuration) LC_{50} for rainbow trout of 46 pg/L. Exposure of rainbow trout to 38 pg/L caused growth reduction and abnormal behaviour such as lethargic swimming and feeding inhibition (Mehrle et al. 1988).

PCDDs which are not chlorinated in all of the 2,3,7,8 positions (Fig. 1) are considerably less toxic, e.g., the LC_{50} for 1,3,6,8-T_4CDD in the guinea pig is $>15 \times 10^6$ µg/kg (Table 1). The toxicity of PCDDs (including 2,3,7,8-substituted congeners) also declines with increasing degree of chlorination (Table 1). Although PCDDs other than 2,3,7,8-T_4CDD are less

Table 1. Comparative toxicity and biological activity of chlorinated dibenzo-p-dioxins.

congener	LD_{50}(μg/kg)[1] guinea pig	LD_{50}(μg/kg)[1] mouse	Relative AHH[1] activity in rat hepatoma cells	2,3,7,8-T_4CDD TEF[2]
2,3,7,8-T_4CDD	0.6-2	114-284	1	1
1,3,6,8-T_4CDD	$15x10^6$	$3.0x10^6$	0.017-0.004	0.01
1,2,3,7,8-P_5CDD	3.1	337.5	0.2-0.019	0.20
1,2,3,4,7,8-H_6CDD	72.5	825	0.1-0.05	0.04
1,2,3,4,6,7,8-H_7CDD	>600		0.0035-0.0027	0.001
O_8CDD		$>4x10^6$	$1.8x10^{-5}$	0

1. from Kociba and Cabey (1985)

2. toxicity equivalence factor; from Barnes et al. (1986)

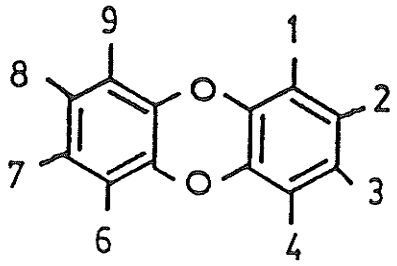

Figure 1. The structure of polychlorinated dibenzo-p-dioxins.

toxic, they are produced and found in relatively large quantities (Sheffield 1985; National Research Council of Canada 1981) and therefore represent a potential hazard to the environment. Higher chlorinated PCDDs are also the dominant congeners found in human adipose tissue and milk (Rappe et al. 1987). O_8CDD accounts for 46 to 81% of the PCDD residues detected in human adipose tissue (Rappe et al. 1987). The majority of literature has focused on only the 2,3,7,8-T_4CDD congener and relatively little effort has been spent on understanding the environmental dynamics of the less toxic congeners, especially in aquatic environments.

Numerous sources of PCDDs have been identified including incineration of municipal and industrial waste, chlorophenols (e.g., pentachlorophenol), pesticides derived from chlorophenols (e.g., 2,4,5-T) and automobile exhaust (Table 2, 3). Although release of PCDD from incinerators and the use of pentachlorophenol are generally considered to be the major sources of PCDDs, recent evidence indicates that automobile exhausts may be a major source, at least in urban areas (Ballschmiter et al. 1986a; Buchert and Ballschmiter 1986; Marklund et al. 1987). Any incomplete combustion as well as any thermal treatment of organic material above 400-500^{o}C in combination with a chlorine source can lead to the formation of PCDDs (Ballschmiter et al. 1986a; Ballschmiter et al. 1986b). In general the concentration of PCDD homologues in the various sources increase with increasing chlorine substitution (Table 3). For example, the concentration of total tetrachloro congeners found in pentachlorophenol is only 0.4-2 µg/kg compared to 733-790 x 10^3 µg/kg of O_8CDD (Hagenmaier and Brunner 1987). This general pattern is also seen in air particulates and aquatic sediments in most environments (Tables 3, 4). Environmental samples (e.g., sediments) show a shift toward the O_8CDD congener relative to combustion sources (Czuczwa and Hites 1986). The less chlorinated

Table 2. Sources of polychlorinated dioxins in the environment ranked from the greatest to the least amounts (taken from the Report of the Ministers Expert Advisory Committee on Dioxins; Health and Welfare Canada, Environment Canada 1983).

1.	municipal and industrial incineration sources*
2.	chlorophenols, used in wood treatment and pesticides*
3.	landfills containing organic wastes and incinerator fly ash
4.	other combustion sources, such as wood burning, fires involving electrical devices containing chlorinated organics, cigarettes, automobile exhausts.
5.	pesticides derived from chlorophenols, including 2,4-D, 2,4,5-T and others
6.	parmaceutical and certain domestic products

* approximately equal

Table 3. The homologue pattern found in selected polychlorinated dioxin sources.

substrate	2,3,7,8 T_4CDD	total T_4CDD	P_5CDD	H_6CDD	H_7CDD	O_8CDD	reference
Flyash (µg/kg)							
	<1	62	5	4	1010	1700	Yasuhara et al. 1987
	<1	25	2	2	81	84	"
	<1	43		104	78	52	Kuehl et al. 1985
	2	224		166	107	95	"
	<1	62	79	121	186	249	Asada et al. 1987
Flue gas (ng/m^3)							
	<1	54	66	101	148	114	Asada et al. 1987
Pentachlorophenol (mg/kg)							
	3E-5	2E-3	6E-3	2	154	733	Hagenmaier and Brunner 1987
	5E-5	4E-4	2E-2	3	198	790	"
Waste oil (µg/kg)							
		nd	<1	3	5	10	Rotard et al. 1987
Air particulates (pg/m^3)							
Gothenburg	3	150	200	100	380	290	Rappe and Kjeller 1987
"	9	350	840	520	2900	1900	"
Rorvik	<1	9	31	32	140	64	"
"	5	130	280	190	1000	540	"
Air particulates (µg/kg)							
Washington		<1	6	2	21	200	Czuczwa and Hites 1984
St. Louis		1	<1	1	24	170	"

Table 4. The concentration of polychlorinated dioxins reported in sediments (ng/kg).

site	2,3,7,8 T_4CDD	total T_4CDD	P_5CDD	H_6CDD	H_7CDD	O_8CDD	reference
Siskiwit Lake		26	12	10	32	560	Czuczwa et al. 1985a
Lake Zurich[1]		170	40	130	260	1700	Czuczwa et al. 1985b
Lake Buldegg[1]		10	35	60	200	1000	"
Lake Lugano[1]		7	55	65	230	1300	"
Saginaw Bay[1]				300	600	42000	Czuczwa and Hites 1986
Lake Huron[1]			50	300	400	1300	"
Lake Michigan[1]		15	25	60	160	780	Czuczwa and Hites 1984
Lake Erie[1]				10	100	2000	"
Lake Ontario[1]				20	400	4800	"
Lake Constance[1]		50	50	170	330	750	Hagenmaier et al. 1986
Stockholm archipelago							
inner	2.4	69	230	49	5700	3100	Rappe & Kjeller 1987
middle	2.0	21	99	16	1200	510	"
outer	<2.0	23	86	19	880	26	"
Varo Mill	120	230	170	92	31	87	Rappe et al. 1987
R. Viskan (mouth)	0.2	6.4	13	64	190	900	"
Petenwell Res.	170	187	377	1926	6910	20560	Kuehl et al. 1987
Japan	nd	1.6			3.3	11.5	Yasuhara et al. 1987

1. Approximated from figures.

dioxins have higher vapour pressures and water solubilities than the higher chlorinated congeners, e.g., O_8CDD (Table 5). Increased sorption of the higher chlorinated PCDDs (e.g., O_8CDD) to particulates in the air or water may influence their environmental fate (e.g., increased sedimentation) which could lead to the relatively higher concentrations in environmental samples compared to combustion sources. The greater proportion of the more chlorinated PCDDs bound to particulates in the air or water could possibly reduce their availability to undergo chemical (e.g., photolysis) or biological transformations leading to a relative enrichment of the higher chlorinated congeners in the environment.

Only a small fraction of the total tetrachloro congeners found in various sources is made up of the toxic 2,3,7,8-T_4CDD congener (Table 3). 2,3,7,8-T_4CDD usually represents less than 5% of the tetrachloro congeners found in fly ash, stack effluents, and air particulates (Hutzinger et al. 1985; Rappe and Kjeller 1987; Table 3). 2,3,7,8-T_4CDD also generally represents less than 5% of the tetrachloro congeners in lake sediments (Table 4). However, in some environments the proportion of 2,3,7,8-T_4CDD is much higher, e.g., Petenwell Reservoir (Table 4).

This general pattern in sources and lake sediments is in sharp contrast to the homologue profiles found in biota in which tetra- and not octachloro congeners predominate (Table 6). 2,3,7,8-T_4CDD is usually the only tetrachloro congener found in biota in the environment (Kuehl et al. 1987b; Rappe et al. 1987). This difference can be at least partly explained by the rapid transformation of non 2,3,7,8-substituted congeners in biota (Muir et al. 1985d; 1986). However, the higher chlorinated congeners, such as O_8CDD, which are fully chlorinated at the 2,3,7,8 positions, are not readily metabolized (Muir et al. 1985d; 1986). Based on the high octanol to water

Table 5. The physical/chemical properties of polychlorinated dibenzo-p-dioxins.

congener	water solubility[1] ng/L (20°C)	log K_{ow}[2]	vapour[3] pressure Pa (25°C)	quantum[4] yield	BCF[5]	predicted BCF[6]
2,3,7,8-T_4CDD	19.2 (22)	7.02	2.0×10^{-7}	2.2×10^{-3}	39000	180000
1,3,6,8-T_4CDD	317	7.13	7.0×10^{-7}	2.2×10^{-3}	2100	230000
1,2,3,4,7-P_5CDD	120	7.44	8.8×10^{-8}	9.8×10^{-3}	810	420000
1,2,3,4,6,7-H_6CDD	56	7.79	5.1×10^{-9}	1.1×10^{-4}	2278	830000
1,2,3,4,6,7,8-H_7CDD	2.4	8.20	7.5×10^{-10}	1.5×10^{-5}	1425	1860000
O_8CDD	0.4	8.60	1.1×10^{-10}	2.3×10^{-5}	85	4070000

1. Freisen et al. (1985); Marple et al. (1986)
2. Burkhard and Kuehl (1986); Marple et al. (1986)
3. Rordorf et al. (1986)
4. Choudhry and Webster (1988)
5. Muir et al. (1985a), Mehrle et al. (1988)
6. Estimated from Veith et al. (1979); log BCF = 0.85 log K_{ow} - 0.70

Table 6. The concentration of polychlorinated dioxins reported in aquatic biota (ng/kg).

site and species	2,3,7,8 T_4CDD	total T_4CDD	P_5CDD	H_6CDD	H_7CDD	O_8CDD	reference
Blue mussels							
Hokko		140	20	23	26	42	Miyata et al. 1987a
Misaki-cho		68	14	14	33	61	"
Funka Bay		8.8	4.3	4.2	6.0	6.5	"
Rishiri		2.4	1.9	3.3	nd	nd	"
Crab							
Idefjorden	17	17	86	154	32	<1	Rappe et al. 1987
Crab (hepatopancreas)							
Grebbestad	17	17	76	170	30	<1	"
Varofjorden	170	170	270	465	85	<2	"
American lobster (digestive gland)							
Miramichi River		nd	nd	5.5	13.5	6	Clement et al. 1987
Chaleur Bay		nd	nd	21.5	3.5	5	"
Sydney Harbour		nd	nd	nd	8.5	2	"
Common carp							
Saginaw Bay		94	157	122	12	nd	Stalling et al. 1983
Bay Port		27	21	nd	31	32	"
Tittabawasee River		81	31	44	53	14	"
Lake Erie		nd	9	nd	11	50	"
Lake Huron	3	5	2	3	3	5	"

Table 6 continued

Common carp							
Wisconsin River[1]	120	nd	4.8	16	27	25	Kuehl et al. 1987
Alewife							
Lake Ontario	4		1	1	nd	nd	Norstrom pers. comm.
Herring							
Baltic Sea	nd		nd	nd	20	50	Burser et al. 1985
Kalskrona	<0.3		1.1	1.5	nd	nd	Rappe et al. 1987
Lulea	<0.6		4.7	8.1	nd	nd	"
Germany	4.7		12	8.0	3.6	19	W.Mathar pers.comm.
Cod							
Germany	23		1.3	22.2	10	83	W.Mathar pers. comm.
Salmon							
Ume River	1.9		8.8	4.6	nd	nd	Rappe et al. 1987
	1.3		4.3	2.3	nd	nd	"
Lake trout							
Lake Michigan		5	nd	nd	nd	5	Stalling et al. 1983
Siskiwit Lake		nd	nd	nd	nd	trace	"
Brown trout							
Lake Ontario		33	nd	nd	nd	nd	"

1. exposed in the laboratory

partition coefficients (K_{OW}), PCDDs, especially the higher chlorinated congeners, are expected to be rapidly bioconcentrated in aquatic biota (Table 6). A simple extrapolation of the relationship developed by Veith et al. (1979) between bioconcentration factor (BCF) and K_{OW} predicts extremely high BCFs ranging from 180,000 for 2,3,7,8-T_4CDD to 4,070,000 for O_8CDD. Adams et al. (1986) reported a BCF for 2,3,7,8-T_4CDD of only 7,900 and Mehrle at al. (1988) reported a somewhat higher BCF for 2,3,7,8-T_4CDD of 39,000. Muir et al. (1985a) and others (Bruggeman et al. 1984; Opperhuizen et al. 1985) have shown that the BCFs of PCDDs and other superlipophilic compounds decrease above a log K_{OW} of approximately 6.5 (Fig. 2). The reason for this decline has not been clearly demonstrated although sorption to dissolved organic matter (DOM), solubility factors, and steric hindrances have been suggested as possible explanations (Muir et al. 1985a; Opperhuizen et al. 1985; Gobas and Mackay 1987). DOM has been shown to reduce the "truly dissolved" water concentration in the exposure systems (Muir et al. 1985a; Landrum et al. 1985). Superlipophilic compounds such as PCDDs have a high affinity for organic matter in aquatic systems (Carter and Suffet 1982; McCarthy 1983; Muir et al. 1985a; Morehead et al. 1986; Chiou et al. 1987). An equilibrium will exist between the contaminant sorbed to particulate organic matter (POM), DOM and that truly dissolved (free) in solution (Fig. 3). Only the fraction which is truly dissolved in water is believed to be bioavailable to aquatic biota (Landrum et al. 1985; McCarthy et al. 1985). The high affinity of PCDDs for organic matter, especially the more lipophilic congeners (e.g., O_8CDD), may limit their truly dissolved concentrations in the water column and reduce their bioavailability in natural environments. The true BCFs for PCDDs including 2,3,7,8-T_4CDD may be considerably higher when the sorption to organic matter is taken into account.

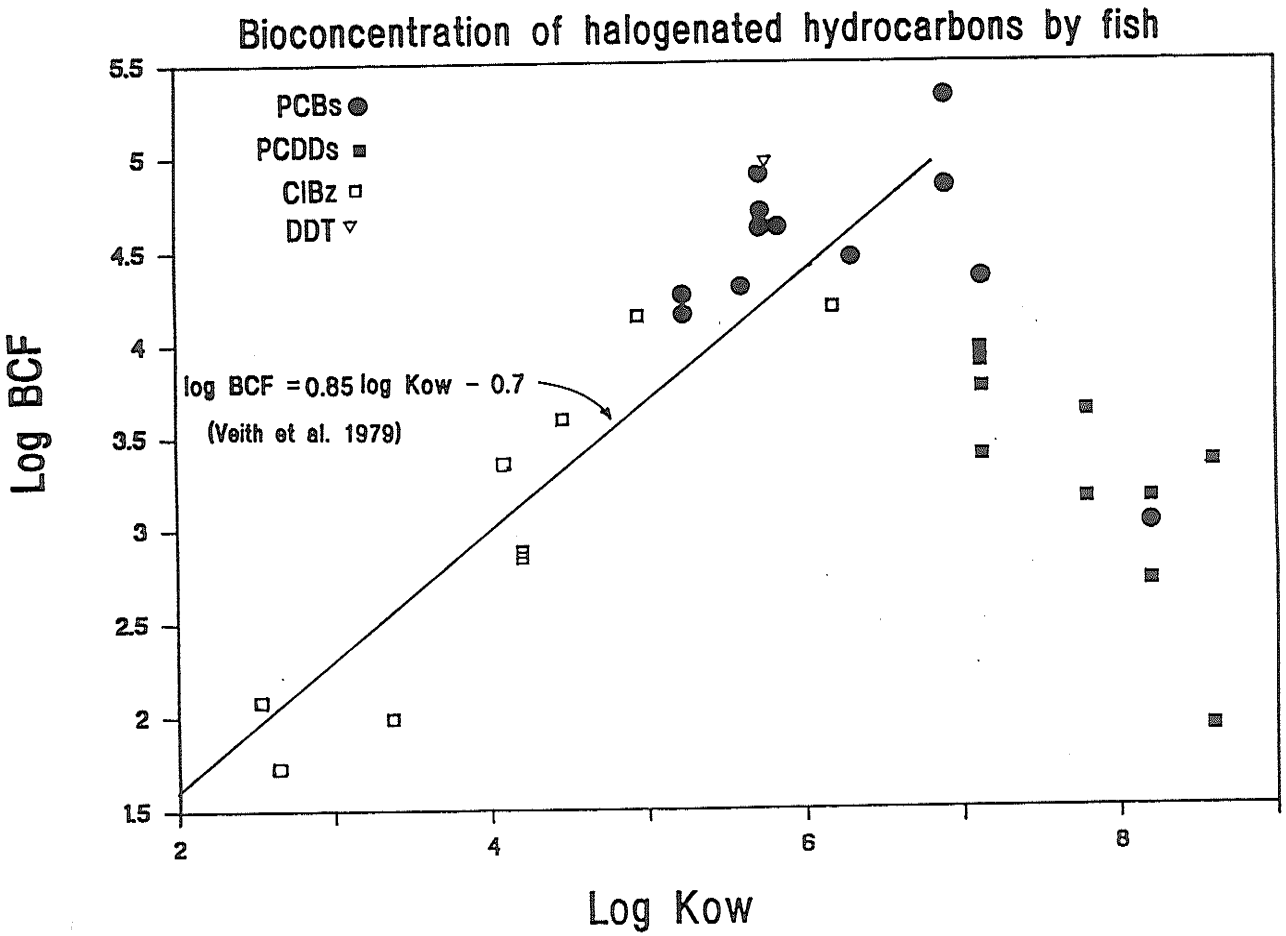

Figure 2. The bioconcentration factors (BCF) of organic compounds relative to their octanol water partition coefficient (K_{OW}). Taken from Muir et al., presented at the Society Environ. Toxicol. Chem., Nov. 2-5 1986, Alexandria, Virginia.

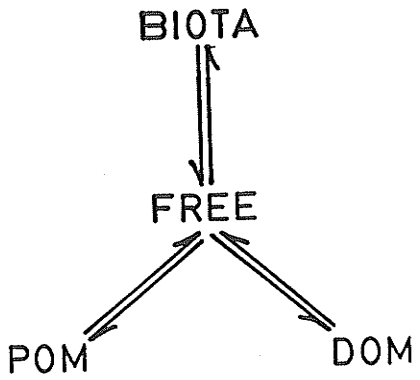

Figure 3. The partitioning of polychlorinated dibenzo-p-dioxins in the water between pariculate organic matter (POM), dissolved organic matter (DOM), truly dissolved (FREE) and biota.

Sorption of PCDDs to organic matter alone cannot explain the pattern of PCDD homologues reported in aquatic biota (Table 5). Higher chlorinated congeners are often found in high concentrations even though their truly dissolved water concentrations are predicted to be extremely low. The high concentration of the higher chlorinated dioxins in the sediments may act as a source of PCDDs to the higher trophic levels (e.g., fish) through a detrital based food chain transfer. A similar mechanism has been suggested for the accumulation of PCBs in the Great Lakes (Thomann and Connolly 1984). Although the assimilation efficiency of PCDDs decreases with the degree of chlorine substitution (Fig. 4), the concentration of the higher chlorinated PCDDs at the bottom of the food chain (i.e., detritus) is so high that there is potential for a significant proportion of the PCDDs found in biota to be derived from food.

Unfortunately there is relatively little information in the literature on the distribution of PCDDs other than 2,3,7,8-T_4CDD in aquatic environments. The majority of this literature is monitoring studies, which rarely report the concentration of PCDDs in more than one environmental compartment. There has been an emphasis placed on measuring trace amounts of PCDDs, especially 2,3,7,8-T_4CDD in fish, and relative little effort has been spent studying the environmental chemistry of these compounds. As a result, the fate, bioavailability and toxicology of PCDDs in aquatic environments remains poorly understood (Baumann and Whittle 1988).

Although the 2,3,7,8-T_4CDD congener is the most toxic and has received the most study, the other congeners are produced and found in large quantities and therefore also represent a hazard to the environment (Table 3-5). 2,3,7,8-T_4CDD toxicity equivalence factors (TEF) in Table 1 can be used to determine the relative toxic potential of the congeners other than

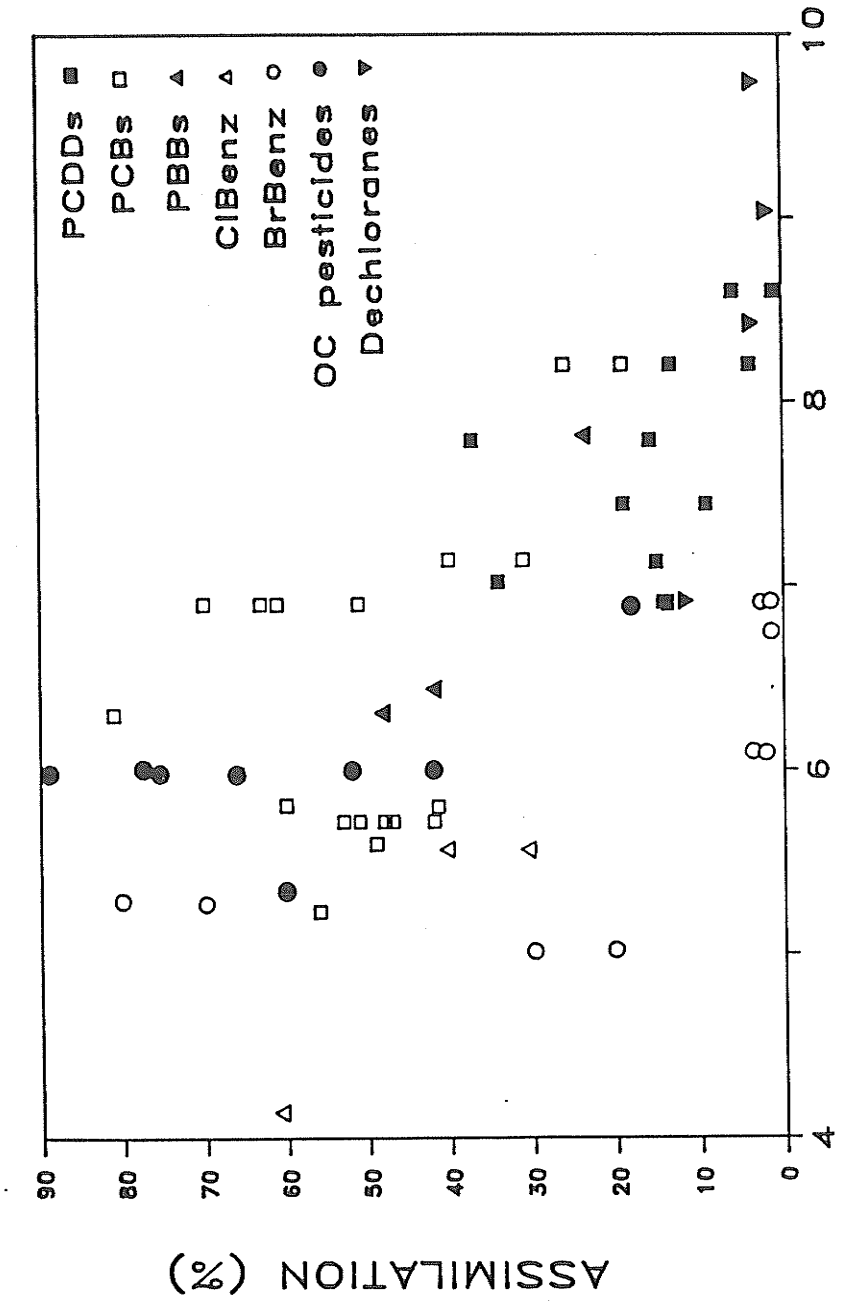

Figure 4. The assimilation efficiencies of organic compounds relative to their octanol water partition coefficients (K_{ow}). Taken from Muir and Yarechewski (1988).

2,3,7,8-T_4CDD in complex mixtures; i.e., toxicity equivalence is equal to the sum of the concentration of each congener multiplied by its TEF. Using this approach the toxicity equivalence of congeners other than 2,3,7,8-T_4CDD in flyash (i.e., Kuelh et al. 1985) is 0.4 to 5.7 times that of 2,3,7,8-T_4CDD in the sample. Similarly the toxicity equivalence in sediments (Stockholm archipelago, Rappe and Kjeller 1987) is 0.6 to 1.5 and in fish (herring and salmon, Rappe et al. 1987) is 0.7 to 2.1 times that of the 2,3,7,8-T_4CDD congener. The toxicity of any matrix may therefore be several times greater than predicted based on consideration of the 2,3,7,8-T_4CDD congener alone. A better understanding of the environmental dynamics of congeners other than 2,3,7,8-T_4CDD is important for the evaluation of the potential hazard of PCDDs in aquatic environments. The study of these less toxic congeners can also give insights into the environmental chemistry of 2,3,7,8-T_4CDD which, because of its extreme toxicity, is difficult and hazardous to work with.

CHAPTER I

The Effect of Suspended Sediment Concentration on the Sediment to Water Partition Coefficient for 1,3,6,8-Tetrachlorodibenzo-p-dioxin.

The extent to which hydrophobic organic pollutants sorb to aquatic sediments will dramatically influence their environmental fate. Accurate determination of K_p is critical for modelling the fate and potential hazard of chemicals in aquatic ecosystems. Sorption of pollutants may affect their availability for biological uptake, volatilization, sedimentation or reactions such as photolysis (Landrum et al. 1985; Macalady and Wolfe 1987; Mudambi and Hassett 1987).

K_p for a pollutant should be constant with increasing sediment concentrations. However, many authors have shown that K_p usually declines when suspended sediment concentrations increase (DiToro 1985; O'Connor and Connolly 1980; Horzempa and DiToro 1983; Voice et al. 1983). This inconsistency has led DiToro (1985) and MacKay and Powers (1987) to hypothesize a particle interaction induced desorption of chemicals from suspended sediments which is intensified as the concentration of suspended sediments increase. An alternative hypothesis put forward by Gschwend and Wu (1985), and Voice and Weber (1985) who explain the decrease in K_p as an artifact of the methodology used. In particular they suggest that sorption of the chemical to a third phase (colloidal) results in overestimation of the concentration in the aqueous solution. These authors showed that as the sediment concentrations increase there is a corresponding increase in the concentration of DOC. It has been shown that DOC can sorb hydrophobic

chemicals and inflate their apparent aqueous solubilities (Chiou et al. 1986; Webster et al. 1986). It would appear that a third phase would explain at least part of the K_p versus suspended sediment effect. To date, measurement of third phase and particle interaction effects have been conducted by centrifugation and there has been no independent assay of the truly dissolved aqueous concentration of hydrophobic chemicals in these sediment-water systems.

Landrum et al. (1984) have described a reverse phase cartridge method to directly measure truly dissolved aqueous concentrations. Yin and Hassett (1986) have also recently described a dynamic headspace method to measure truly dissolved aqueous concentrations. Both of these methods have been employed in this study to determine the K_p of 1,3,6,8-tetrachlorodibenzo-p-dioxin in a sediment-water system and compared directly to determinations of K_p using conventional centrifugation techniques.

MATERIALS AND METHODS

Carbon-14 labelled 1,3,6,8-T_4CDD with a specific activity of 24.16 mCi/mM was purchased from Pathfinder Laboratories (St. Louis, MO). The T_4CDD was purified by thin layer chromatography before use to yield >99.8% radiopurity (determined by HPLC). The T_4CDD is a very hydrophobic compound with a reported log K_{ow} of 7.13 (Burkhard and Kuehl 1986). All water used was distilled deionized water passed through a Milli-Q and 0.2 um filter system (Waters Scientific). Milli-Q water contained <0.24 mg/L dissolved organic carbon (DOC) measured using a high temperature acid persulphate digestion, followed by infrared detection of CO_2 on a OI Corp. Model 700 Carbon Analyser.

Sediments were collected from the sub-littoral zone (2 m) of Lake 304, Experimental Lakes Area in northwestern Ontario (Johnson and Vallentyne 1971). Sediments were freeze-dried, sieved through a 100 mesh screen and stored at 4°C. Sediments were 25.2% total organic carbon and 2.4% total nitrogen. Sediments had a clayey texture with 12% sand, 37% silt and 51% clay.

A preweighed sediment sample was added to one litre of Milli-Q water in a tall-form gas washing bottle. T_4CDD in methanol (10 µL) was spiked directly into the water column and allowed to equilibrate for 24 h with constant mixing using a teflon stir bar. This volume of methanol was not expected to have an effect on the partitioning behaviour of TCDD (Webster et al. 1987; Appendix A).

At the start of each experiment, four 20 mL water samples were collected from a depth of 5 cm below the water surface and placed in 25-mL Corex tubes. A 4 mL water sample was taken directly from each tube immediately after being mixed to prevent settling of the suspended solids. Samples were diluted with scintillation fluor (Atomlight, New England Nuclear) and assayed by liquid scintillation counting (LSC). Samples (4 mL) taken directly from the water column did not differ from the samples taken directly from the tubes. The vertical distribution of radioactivity and suspended sediments was checked by sampling at four depths and varied by less than 10% in all cases. Two samples were then centrifuged at 6000g for 15 min and two samples centrifuged at 20000g for 30 min. Aliquots of the supernatant water (4 mL) were assayed directly by LSC to determine the proportion of radioactivity "in solution". The proportion of radioactivity associated with the dissolved organic matter (DOM) was determined using the method described by Landrum et al. (1984). A 4 mL sample of the supernatant (20000g) was passed through a reverse-phase cartridge (C_{18} Sep-Pak, Waters Scientific), and the eluant assayed by LSC.

The T_4CDD associated with the DOM will pass through the column while the truly dissolved T_4CDD will partition from the C_{18} and remain on the column (Landrum et al. 1984).

The concentration of freely dissolved T_4CDD was also determined using the dynamic headspace method described by Yin and Hassett (1986). The column was then sparged with N_2 ("zero" grade presaturated with H_2O) using a coarse glass frit 25 cm below the water surface (Fig. 5). Flow rates were approximately 300 mL/min and were measured using a bubble flow meter. Immediately, a 3 X 0.6 cm column of 60/80 mesh Tenax-GC was placed in the outflow port of the headspace. After 3 h, the Tenax was removed and placed in a glass test tube for later combustion on Packard 306B sample oxidizer. Recovery efficiency of the T_4CDD from Tenax was determined to be 88.1±2.0%. Ninety-nine percent of the ^{14}C trapped was on the first cm of the Tenax column. The Henry's Law Constant (HLC) was determined in Milli-Q water by determining the removal rate of the T_4CDD by sparging and using the model described by Mackay et al.(1979) where:

$$HLC = - D_r R T V / G \qquad [1]$$

and D_r is the depletion rate constant, R is the ideal gas law constant, T = 283 K (10°C), V = 1 L and G is the flow rate of the gas. The depletion rate constant was estimated using non-linear least squares regression (SAS-NLIN) from plots of cumulative T_4CDD (ng) trapped on Tenax versus time (t). The HLC for T_4CDD was calculated to be 5.96×10^{-6} atm m^3/mol at 10°C. Webster et al. (1985) reported the HLC of T_4CDD to be 6.81×10^{-5} atm m^3/mol at 23°C and Podoll et al. (1986) estimated a value of 1.6×10^{-5} atm m^3/mol for the 2,3,7,8-T_4CDD congener at 25°C.

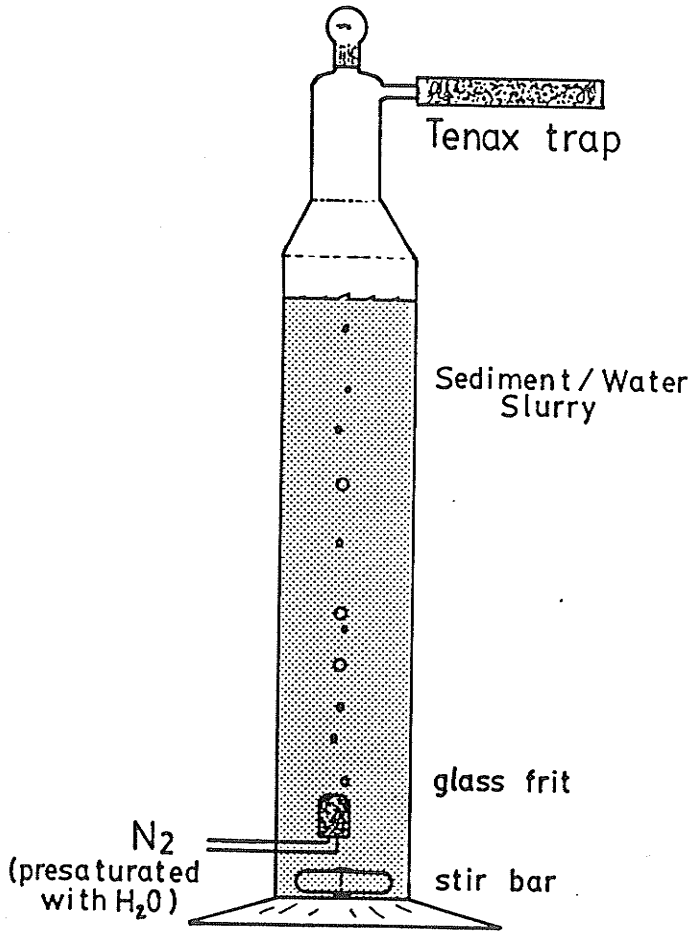

Figure 5. Gas sparging apparatus used to determine truly dissolved aqueous concentrations.

The concentration of the T_4CDD truly dissolved was calculated as

$$\text{free} = (\text{trapped})\ R\ T\ /\ G\ t\ HLC \qquad [2]$$

The amount of T_4CDD trapped (trapped) on the Tenax after 3 h was always less than 2% of the estimated total free in solution.

The sediment water partition coefficient,

$$Kp = \frac{[\text{bound}]\ /\ [\text{suspended sediments}]}{[\text{"in solution"}]} \qquad [3]$$

was determined four times for each experiment using different values for the concentration "in solution" (ng/L): after centrifugation at 6000g or 20000g, using reverse-phase C_{18} cartridge and gas sparging. The log K_p versus log suspended sediment concentration regressions were compared using analysis of covariance (SAS-GLM).

K_p estimated from DiToro's equation (DiToro 1985) was calculated from

$$Kp = f\ Koc^X\ /[1 + (2E)\ m\ f\ Koc^X] \qquad [4]$$

where f is the organic carbon mass fraction, Koc^X is the organic carbon partition coefficient estimated from K_{ow} using the relationship reported by DiToro (1985), 2E=0.7 where E is the collision efficiency term and m is the concentration of suspended sediment. The collision efficiency term (E) was estimated for the Kp calculated in this study using the free water concentrations by fitting equation [4] using nonlinear regression (SAS-NLIN).

Solubility enhancement by DOC was examined independently using a technique similar to that described by Chiou et al. (1986). Twenty-six ng of the T_4CDD was spiked onto the walls of 25 mL Corex glass tubes. This amount is equivalent to 1300 ng/L or 4.1 times the reported water solubility (Friesen et al. 1985). The solvent was evaporated and 20 mL of a Milli-Q-Aldrich humic acid solution added. Aldrich humic acid had been precipitated twice with HCl, dialyzed in distilled water and filtered through a 0.45 µm Millipore filter. The solution was shaken for 24 h and a 4 mL sample taken for determination of ^{14}C by LSC.

RESULTS AND DISCUSSION

The K_p for T_4CDD declined as the suspended sediment concentration increased using conventional centrifugation techniques (Fig. 6). O'Connor and Connolly (1980) and others (DiToro 1985; Horzempa and DiToro 1983; Voice et al. 1983) have previously observed this effect for other pollutants. Centrifugation at 20000g for 30 min resulted in the same relationship for log K_p versus log suspended sediment concentration as centrifugation at 6000g for 15 min (Table 7). Gschwend and Wu (1985) also found that centrifugation at higher speeds and longer times (760g for 20 min vs. 1700g for 60 min) did not affect the value of K_p at low suspended sediment concentrations (i.e., $<10^4$ mg/L). Removing the supernatant and centrifuging a second time also did not change the apparent aqueous concentration; however, the negative slopes of the lines derived from both sparging and C_{18} cartridges were significantly lower (Table 7). Centrifugation therefore does appear to inflate the apparent freely dissolved aqueous concentrations. As the suspended sediment concentration increased the difference between the free concentration

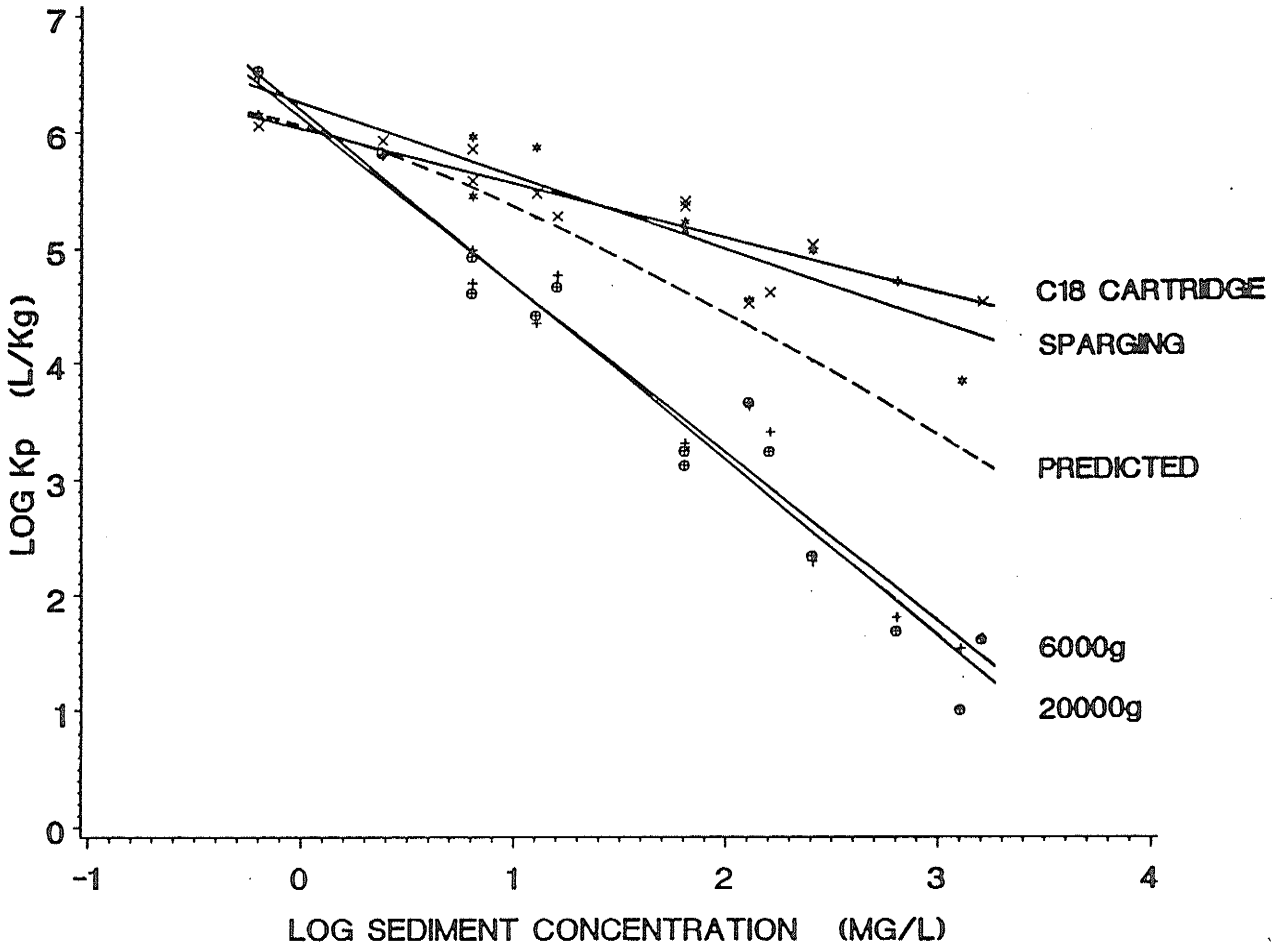

Figure 6. The effect of suspended sediment concentration on K_p. Concentrations of freely dissolved T_4CDD were estimated using centrifugation at 6000g for 15 min, 20000g for 30 min, gas sparging, and C_{18} cartridges. The predicted line was determined using DiToro's equation [4] describing the effect of particle interactions on K_p.

Table 7. Comparison of log K_p versus log suspended sediment concentration regressions. Values followed by similar symbols are not significantly different ($p<0.01$).

method	R^2	intercept	slope
6000g	0.96	6.15 *	-1.46 *
20000g	0.96	6.21 *	-1.54 *
C_{18} cartridge	0.74	5.98 *	-0.46 **
sparging	0.84	6.23 *	-0.65 **

estimated by centrifugation at 6000g and the free concentration estimated using C_{18} cartridges increased (Fig. 7). There was little or no difference between the apparent freely dissolved aqueous concentrations determined by centrifugation at 20000g versus those determined at 6000g. Overestimation of the actual free concentration would result in underestimation of the K_p.

The C_{18} cartridge method indicates that as much as 25% of the T_4CDD in solution was associated with DOM or other material which would pass through the cartridge but which would remain in solution after centrifugation at 20000g (Fig. 8). The DOC concentration increased as the sediment concentration increased (Fig. 9). Gschwend and Wu (1985) also showed a similar relationship between sediment concentration and DOC for Lake Superior sediments. Addition of Aldrich humic acid (DOC) to Milli-Q water caused an increase in the apparent solubility of the T_4CDD (Fig. 10). Above one mg/L DOC, the total amount of T_4CDD in solution approaches the amount of T_4CDD added to the tube and the slope decreases. Keoleian and Curi (1987) have shown that addition of Aldrich humic acid reduces the apparent adsorption of tetrachlorobiphenyl by kaolinite. McCarthy and Black (1987) have also shown that the presence of DOC (Aldrich humic acid) reduces the apparent K_p for binding of benzo[a]pyrene to yeast cells. Although Aldrich humic acid may have a greater binding affinity for T_4CDD than natural DOC (Landrum et al. 1984; 1985; Malcolm and MacCarthy 1986), this solubility enhancement is further evidence that DOC can inflate the apparent freely dissolved concentrations. Centrifugation would have little effect on removing the DOC bound phase from solution. The effect of solubility enhancement by DOC would be to underestimate K_p when centrifugation is used to determine the free water concentrations. Gschwend and Wu (1985) found that prewashing sediments to remove nonsettling particulates (DOC) eliminated the effect of suspended

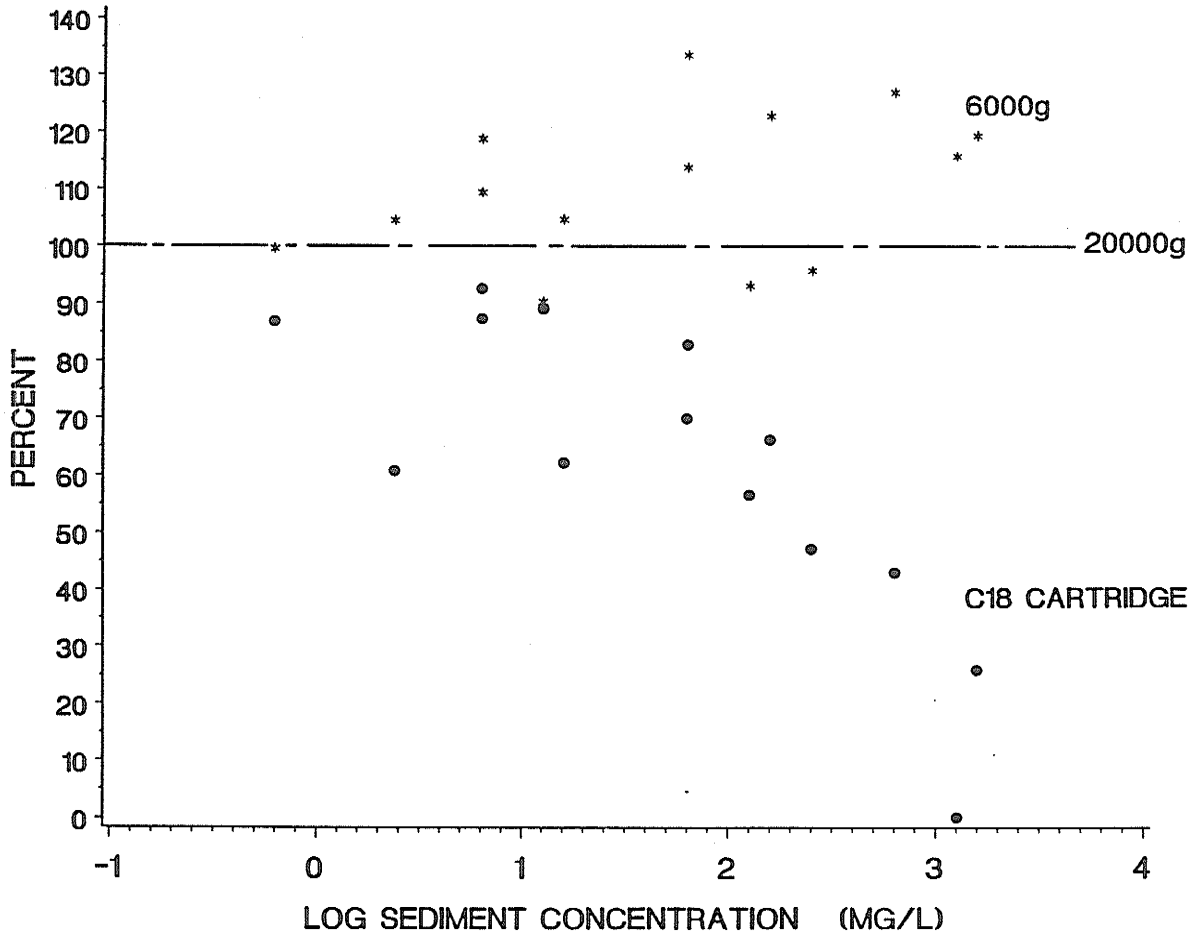

Figure 7. The effect of suspended sediment concentration on the relative estimates of freely dissolved concentrations of T_4CDD. Each estimate is expressed as a percentage of the freely dissolved concentration ("in solution") estimated by centrifugation at 20000g.

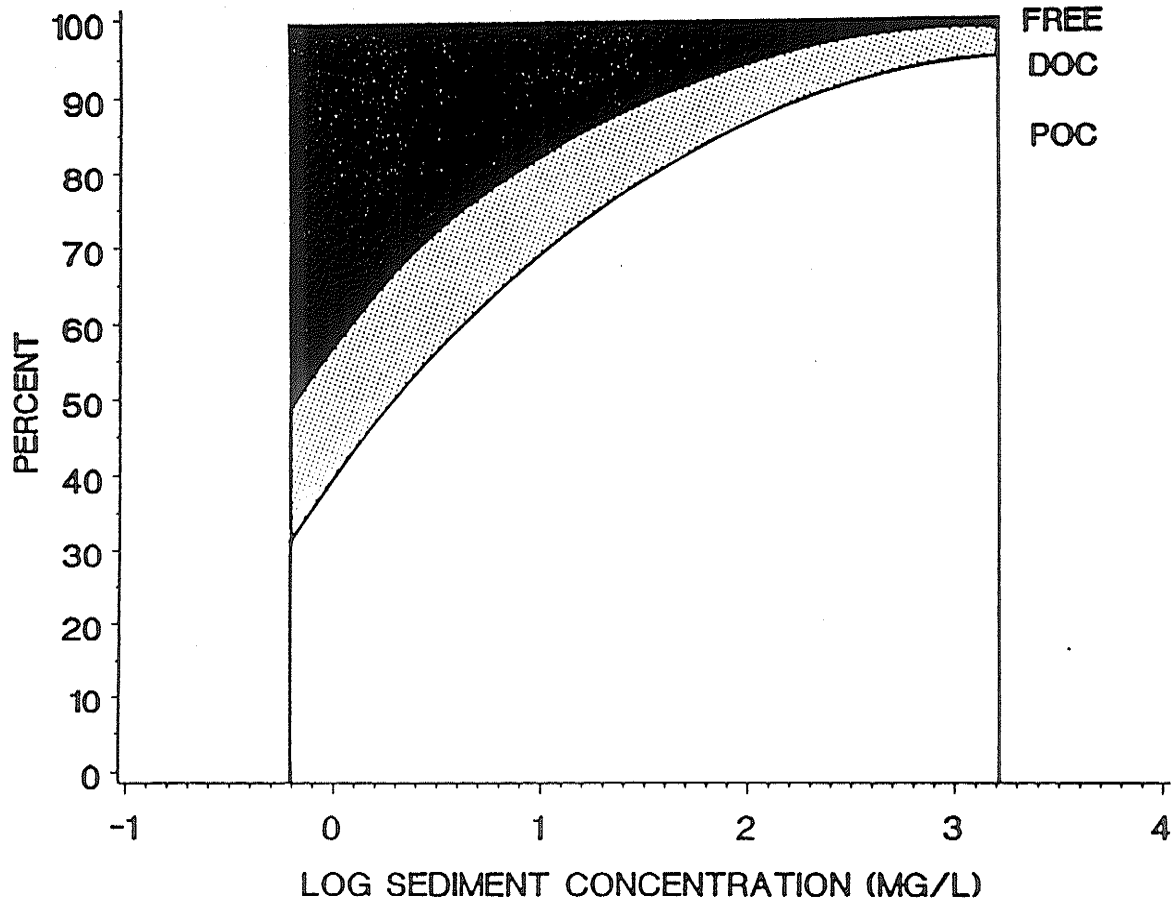

Figure 8. Partitioning between the three phases; particulate organic carbon (POC), dissolved organic carbon (DOC), and freely dissolved (free) T_4CDD in solution, estimated using centrifugation at 20000g and C_{18} cartridges.

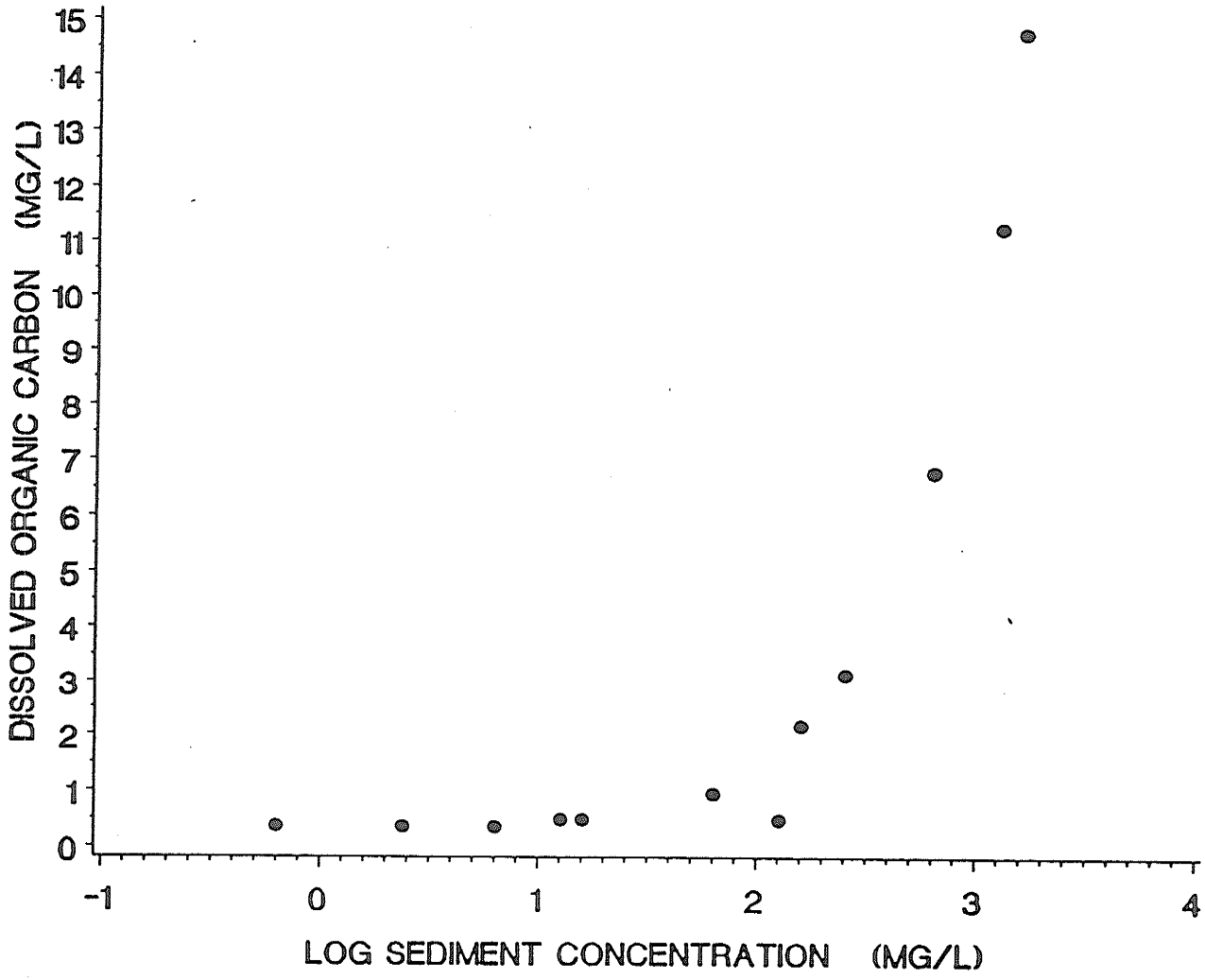

Figure 9. The effect of suspended sediment concentration on dissolved organic carbon concentration.

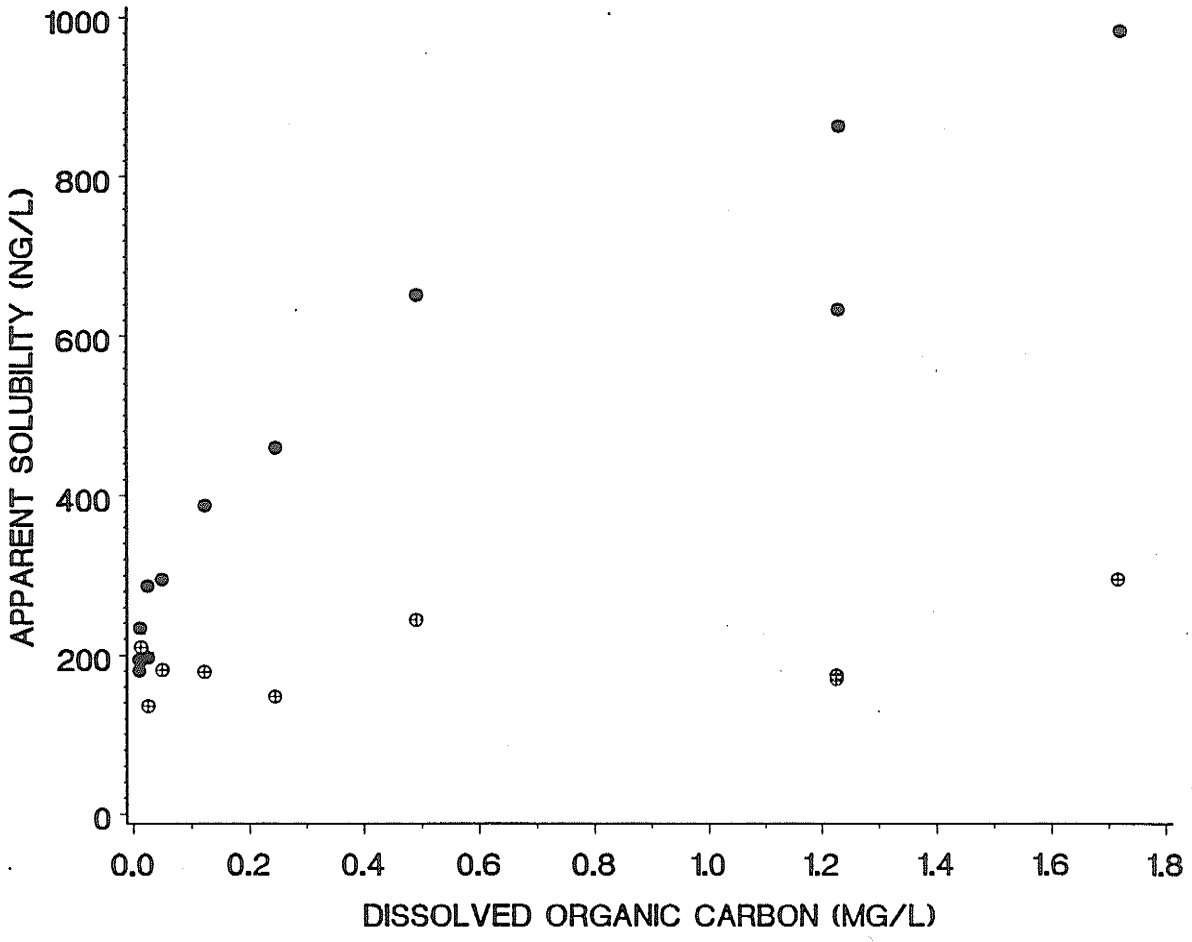

Figure 10. Enhancement of the apparent solubility of 1,3,6,8-tetrachlorodibenzo-p-dioxin in Milli-Q water by Aldrich humic acid. Open circles represent the C_{18} cartidge extractable concentrations and closed circles represent the total concentration in water

sediments on K_p.

Using truly dissolved concentrations estimated using sparging or C_{18} cartridges to calculate K_p did not eliminate the negative relationship between K_p and sediment concentration (Table 7). Both methods predict a slope greater than zero but not significantly different from each other. Another mechanism may be involved in controlling K_p. DiToro (1985) has proposed a particle interaction hypothesis to explain this relationship. In addition to the conventional adsorption/desorption, DiToro (1985) suggests that there is a second desorption reaction which occurs as particles interact via close encounters or collisions. Mackay and Power (1987) have presented a mechanistic explanation for the particle induced desorption and indicates that the apparent variation in K_p is an artifact of the displacement from equilibrium. They hypothesize that the chemical is loosely sorbed to the organic matter in the suspended sediments such that when it collides desorption occurs. The particle then adsorbs the chemical to the equilibrium value or until it encounters another collision. When the concentration of suspended sediments increases, the probability of a collision induced desorption is increased. The K_p values determined by gas sparging or C_{18} cartridge methods are slightly higher than predicted by DiToro (equation 4) while K_p determinations using centrifugation were considerably lower (Fig. 6). It is possible that the collision efficiency term (E) predicted by DiToro is overestimated for this system. When DiToro's equation (DiToro 1985) was fit by nonlinear regression with K_p determined using truly dissolved water concentration from this study, the collision efficiency term (2E) was estimated to be 0.38±0.12; however, estimation of E is very sensitive to the estimate of $K_{OC}{}^{X}$ (and therefore K_{OW}) used in the analysis.

The possibility remains that the negative relationship between K_p and the concentration of suspended sediments for the T_4CDD is due to some consistent bias in the methodology. Failure to reach equilibrium, volatilization, degradation of the chemical and sorption to glass have all been suggested as potential errors influencing K_p (Rao and Davidson 1980; Bowman and Sans 1985a; 1985b). Gas sparging at low free water concentrations may cause some of the bound T_4CDD to desorb, therefore deflating the estimated K_p; however, the amount of T_4CDD trapped on the Tenax never exceeded 2% of the estimated freely dissolved T_4CDD available in the water column. The additional energy supplied to the system by agitation caused by the bubbling of the gas may contribute to enhanced desorption, but the K_p versus suspended sediment relationship was not significantly different from that determined using the C_{18} cartridges (Table 7). Freeze-drying might have an affect on the physical and chemical structure of suspended sediments, but the possible impact on K_p is not clear.

At high sediment concentrations the truly dissolved concentrations approach the detection limits for ^{14}C-T_4CDD using the C_{18} cartridge methodology. Two determinations of the free water concentrations at high sediment concentrations using this methodology were below the detection limits and eliminated from the analysis. Overestimation of the free water concentration could lead to underestimates of K_p. However, the negative slope is seen even in the low sediment concentrations and detection limits are therefore not likely the cause of the K_p suspended sediment relationship. The two methods of determining free water concentrations are independent of each other, yet they give similar results. Sparging gave slightly higher estimates of free water concentrations which is most likely due to an underestimate of the HLC. Changing the HLC would change the estimate of K_p but would not affect

the slope of the K_p suspended sediment relationship.

A third phase (DOC) can explain a major portion of the negative relationship between K_p and suspended sediment concentrations. DOC or nonsettling particulates remaining in the aqueous solution after centrifugation may tend to inflate the apparently free water concentrations, therefore reducing K_p. However, when the free water concentrations determined using sparging or C_{18} cartridges are used to calculate K_p, a negative slope still remains. Although this may be due to errors inherent in measuring free water concentrations, especially at high suspended sediment concentrations, the reduction in K_p does appear to exist. The particle interaction hypothesis may be an explanation for this apparent deviation from conventional thermodynamics.

The importance of accurately determining the K_p of hydrophobic organic contaminants such as PCDDs for predicting their environmental fate can be demonstrated using the National Research Council of Canada (NRC) Persistence model (Roberts et al. 1981). The NRC Persistence model was run using a single input into a system simulating as closely as possible the lake enclosures used in Chapter IV and the chemical parameters were collected or extrapolated from the literature (Table 8). The log K_p values used in the model were 6.53, 5.13 and 1.0 which correspond to the theoretical value of $K_{oc} = K_{ow}$, a measured (centrifugation) K_p at 5 mg/L and 2 g/L suspended solids respectively. Although the fractional degradation is predicted not to dramatically change, the fractional and system retentions are altered considerably by a change in K_p (Table 9). The predicted fractional retention shifts from 98% in sediment to 98% in water and the retentive capacity shifts from 3.2 years to less than 13 h. Even a reduction in the K_p value from 6.53 to 5.13 reduces the retentive capacity of the system to only 57.6 days. Very different behaviour

Table 8. The system and chemical parameters used in the NRC Persistence model for 1,3,6,8-tetrachlorodibenzo-p-dioxin.

System Parameters		Chemical Parameters	
latitude	49°N	molecular weight g/mol	321.9
mean depth	2 m	melting point °C	220
suspended solids	2 mg/L	log Kow[1]	7.13
sediment weight	30 kg	water solubility[2] ng/L	317
attenuation	low	vapour pressure[3] mm Hg	2.7E-8
temperature	20 °C	quantum yield[4]	2.17E-3
volume	67 m^3	bioconcentration factor[5]	2299
organic carbon	25.2 %	depuration rate constant[5]	0.08
fish weight	300 g	proportion biodegradable[5]	0.66

1. Burkhard and Kuehl (1986)
2. Friesen et al. (1985)
3. Rordorf et al. (1986)
4. Choudhry and Webster (1988)
5. Chapter II

Table 9. The effect of K_p on the predicted behaviour of 1,3,6,8-tetrachlorodibenzo-p-dioxin in mesocosms using the NRC Persistence model[1].

	Log K_p		
	6.53	5.13	1.00
Fractional retention			
water	<0.01	0.02	0.99
suspended sediment	0.01	<0.01	<0.01
sediment	0.99	0.98	<0.01
biota	<0.01	<0.01	0.01
Fractional degradation			
volatilization	0.00	0.47	0.00
photolysis	1.00	1.00	1.00
hydrolysis	0.00	0.00	0.00
System retention (days)			
retentive capacity	2295.3	89.5	1.5
half-life	1591.0	62.0	1.1

1. Roberts et al. 1981

of the T_4CDD in aquatic systems is predicted depending on the K_p value used in the model. Accurate determination of the K_p of hydrophobic contaminants such as T_4CDD is required to adequately model their behaviour in the environment.

Although this study is limited to the examination of only one chemical and one sediment type the importance of accurately measuring the free water concentration when determining K_p has been demonstrated. Future work on this question using this approach should include a larger number of chemicals and sediment types. Research should focus on measuring the truly dissolved water concentrations which are important in determining the fate and bioavailability of chemicals in the environment.

CHAPTER II

The Effect of Particulate and Dissolved Organic Matter on the Bioavailability of Polychlorinated Dibenzo-p-dioxins

Hydrophobic organic pollutants have a high affinity for both POM and DOM in aquatic systems (McCarthy 1983; Carter and Suffet 1982; Landrum et al. 1984; Morehead et al. 1986). Partitioning of hydrophobic contaminants to POM or DOM may strongly influence their fate and bioavailability in natural ecosystems (Landrum et al. 1985; McCarthy et al. 1985; Carlberg et al. 1986). An equilibrium will exist between the pollutant sorbed to the POM or DOM and that "free" or in true solution (Fig. 2). Only the fraction which is truly dissolved in solution is believed to be bioavailable to aquatic biota (Landrum et al. 1985; McCarthy et al. 1985). Any increase in the concentration of POM or DOM should cause the equilibrium to shift away from the truly dissolved (free) compartment resulting in a lower concentration of pollutant available to the biota. Similarly, any increase in the affinity (i.e., increased K_{ow}) of the pollutant for organic matter should result in reduced bioavailability of the pollutant.

PCDDs are extremely hydrophobic compounds (Table 10) which are ubiquitous anthropogenic trace contaminants in aquatic environments (Czuczwa and Hites 1986). A simple extrapolation of the relationship between K_{ow} and BCF established by Veith et al. (1979) would predict extremely high BCFs for PCDDs. However, Muir et al. (1985a) and others (Bruggeman et al. 1984; Opperhuizen et al. 1985) have shown that the BCFs for PCDDs and other superlipophilic compounds are actually 2 to 5 orders of magnitude lower than

Table 10. The physical/chemical properties and predicted bioconcentration factors (BCF) for polychlorinated dioxins.

congener	water solubility[1] ng/L (20°C)	log Kow[2]	Predicted log BCF[3]	molar volume[4] cm^3/mol	MIC[5] Å
1,3,6,8-T_4CDD	317	7.13	5.36	275.6	8.7
1,2,3,4,7,8-H_6CDD	56	7.79	5.92	338.3	9.8
1,2,3,4,6,7,8-H_7CDD	2.4	8.20	6.27	359.2	9.8
O_8CDD	0.4	8.60	6.61	359.2	9.8
2,3,7,8-T_4CDD	19.3(22°C)	7.02	5.27	275.6	7.6

1. Friesen et al. (1985); Marple et al. (1986).
2. Burkhard and Keuhl (1986); Marple et al. (1986).
3. Estimated from Veith et al. (1979); log BCF = 0.85 log Kow - 0.70.
4. Estimated using LaBas method (Reid et al. 1977).
5. Minimum internal cross section, from Opperhuizen et al. (1986).

would be predicted on the basis of K_{ow} alone. At least part of the lower than predicted BCFs may be due to the sorption of PCDDs to DOM in the exposure system. Although most recent studies on hydrophobic organics have recognized the importance of removing particulates, very few studies have attempted to measure the actual free concentration of the contaminant in water and include it in the determination of BCFs. Failure to measure the concentration of truly dissolved pollutant will lead to an underestimate of its BCF. The objective of this study was to determine the extent to which DOM influenced the bioavailability of PCDDs to fish. The BCFs for rainbow trout fry (Salmo gairdneri) of four PCDD congeners were determined in the presence of Aldrich humic acid or filtered lake water as DOM sources. Although the effect of DOM on bioavailability has been examined for several other compounds, PCDDs represent a more lipophilic (log K_{ow} = 7.12-8.60; Burkhard and Kuehl 1986) group of compounds than have been previously studied.

MATERIALS AND METHODS

Carbon-14 labelled 1,3,6,8-T_4CDD (T_4CDD), 1,2,3,4,7,8-H_6CDD (H_6CDD), 1,2,3,4,6,7,8-H_7CDD (H_7CDD) and O_8CDD purchased from Pathfinder Laboratories, St. Louis, MO, had specific activities of 102.1, 137.2, 126.1 and 98.5 DPM/ng respectively. All compounds were purified by thin layer chromatography to yield products >99.8% radiochemically pure. Aldrich humic acid obtained from Aldrich Chemical Co., Milwaukee, WI (lot no. 1204PE) was prepared by acid precipitation (twice), followed by dialysis in distilled water, centrifugation at 4000g for 1 h and filtration through a Whatman GFA filter (Whatman, Maidstone, England).

Rainbow trout fry (Salmo gairdneri) were obtained from the Rockwood Hatchery, Canada Dept. of Fisheries and Oceans, Winnipeg, and acclimated to $10^{0}C$ in declorinated tap water for several weeks prior to use. Fish were not fed for 24 h prior to use in the experiments.

DOM partitioning

The percentage of TCDD bound to DOM (0-18 mg Aldrich humic acid / L) was estimated using three independent methods: reverse-phase cartridges (Landrum et al. 1984), dynamic headspace analysis (Yin and Hassett 1986), and equilibrium dialysis (Carter and Suffet 1982).

Aldrich humic acid from a stock solution was added to one litre of distilled deionized water which was passed through a Milli-Q and 0.2 µm filte system (Waters Scientific, Milford, MA) in a tall-form gas washing bottle. Milli-Q water contained <0.24 mg/L DOC measured using a high temperature acid persulphate digestion, followed by infrared detection of CO_2 on a Model 700 Carbon Analyser (OI Corp., Austin, TX). The T_4CDD (200-400 ng) in <10 µL of methanol was added directly into the water column and allowed to equilibrate for 24 h at $10^{o}C$ with constant mixing using a Teflon stir bar. Webster et al. (1987) have shown that this amount of methanol does not affect the partitioning behaviour of T_4CDD to DOC (Appendix A). At the start of each experiment, two 20 mL water samples were collected at a depth of 5 cm below the water surface and placed in 25-mL Corex tubes. A 4 mL water sample was taken directly from each tube, diluted with scintillation fluor (Atomlight, New England Nuclear, Boston, MA) and assayed by LSC. Four mL samples taken directly from the water column did not differ from the samples taken from the tubes indicating that sorption to glass was minimal. The vertical distribution of radioactivity checked by sampling at four depths varied by

less than 10%. The two 20 mL water samples were then centrifuged at 20000g for 30 min to remove particulates. Duplicate 4 mL samples of the supernatant were assayed directly by LSC. The proportion of radioactivity associated with DOM was determined using the method described by Landrum et al. (1984). A 4 mL sample of the supernatant was passed through a reverse-phase cartridge (C_{18} Sep-Pak, Waters Scientific) and the eluant assayed by LSC. The PCDD associated with DOM should pass through the column, while truly dissolved PCDD should partition to the C_{18} and remain on the column (Landrum et al. 1984). Greater than 90% of the DOC passed through the reverse-phase cartridges, similar to that reported previously (Landrum et al. 1984).

The dynamic headspace analysis described by Yin and Hassett (1986) was also used to determine the truly dissolved concentrations. After the initial water samples had been taken, the water column was sparged with zero grade N_2 presaturated with H_2O using a coarse glass frit 25 cm below the water surface (Fig. 5). Flow rates were approximately 100 mL/min and were measured using a bubble flow meter. A 3.0 x 0.6 cm column of 60/80 mesh Tenax-GC was placed in the outflow port of the headspace. After 2 h the Tenax was removed, placed in a glass test tube and later combusted on a Packard 306 Sample Oxidizer (Packard Instument Co., Downers Grove, IL.) and the $^{14}CO_2$ trapped in Carbosorb was assayed by LSC. Recovery efficiency of the T_4CDD from the Tenax was 88.1±2.0% (N=6). Ninety-nine percent of the ^{14}C was trapped on the first cm of the Tenax column. The truly dissolved concentration of T_4CDD was then calculated as

$$\text{free} = [(\text{trapped})\ R\ T] \ /\ [G\ t\ HLC] \qquad [5]$$

where R is the ideal gas law constant, T = 283 K, G is the flow rate of the

gas, t is time (2 h) and HLC is the Henry's Law Constant. A value of 5.96 x 10^{-6} atm m^3/mol at 10°C for the HLC has been reported in Chapter I was used in the calculation. The amount of the T_4CDD trapped on the Tenax after 2 h was always less than 1.4% of the estimated total in true solution.

The freely dissolved concentrations were also estimated using equilibrium dialysis (Carter and Suffet 1982). One litre of the Aldrich humic acid-Milli-Q water solution was added to a 1 L flask. Fifteen mL of Milli-Q water was placed inside 20 cm of dialysis tubing (6,000-8,000 MW cut off) and placed in the flask. The solution was gently shaken at 10°C for 24 h. Duplicate 4 mL water samples were taken from both outside and inside the dialysis tubing and assayed by LSC. The T_4CDD associated with the DOM was not expected to cross the membrane because the complex was assumed to be too large; thus when equilibrium was established the concentration of freely dissolved T_4CDD inside and outside the dialysis tubing was expected to be equal. The concentration of DOC measured inside the dialysis tubing after 24 h was 4% of that outside, indicating that only a small amount of the DOC leaked across the dialysis membrane.

The DOC partition coefficient (K_{doc}) was determined as the ratio of the compound bound per gram of DOC divided by the concentration of truly dissolved compound.

Exposure system

Aqueous solutions of each PCDD were obtained by a procedure similar to that described by Veith and Comstock (1975) and modified by Muir et al.(1985d) (Fig. 11). Acetone-washed 60/80 mesh glass beads (50 g) were coated with each PCDD (15 to 25 x 10^6 DPM) by slowly evaporating off the tetrahydrofuran in a rotary evaporator. The beads were air dried then packed into 3 x 15 cm glass

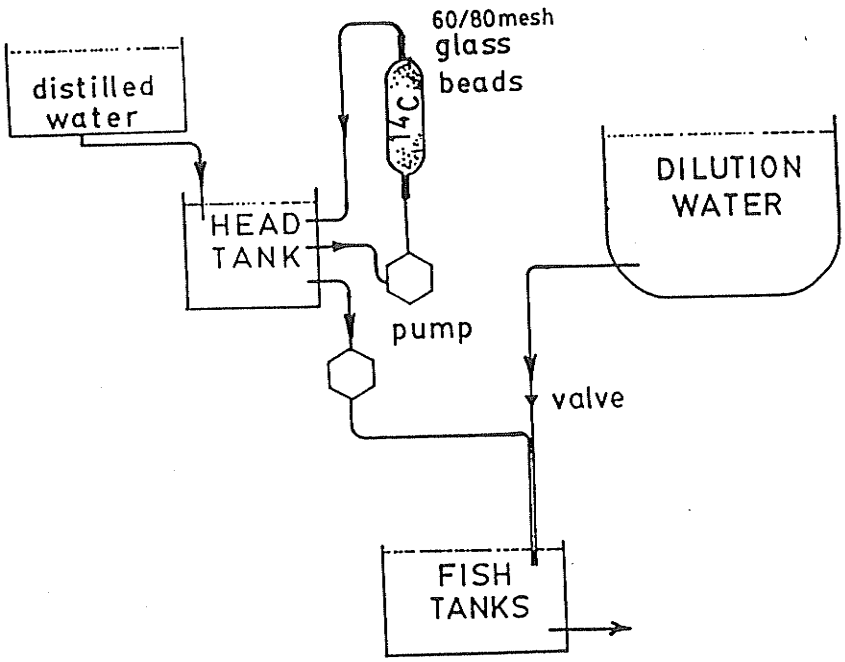

Figure 11. Apparatus used to create exposure solutions.

columns stoppered at each end with glass wool plugs. Distilled water from a head tank was circulated through the glass bead column and returned to the head tank (Fig. 11).

Water from the head tank was mixed with dilution water in Teflon tubing prior to flowing into 4 L glass exposure aquaria. Exposure concentrations ranged from 13 to 167 ng/L. Dilution water was osmotically balanced distilled water (48 mg/L $NaHCO_3$, 30.0 mg/L $CaSO_4.2H_2O$, 30.0 mg/L $MgSO_4$, and 2.0 mg/L KCl) with 0 to 8.6 mg/L Aldrich humic acid added or filtered natural lake water. Lake water was collected at a depth of 15 m from Lake 239 (L239) in the Experimental Lakes Area in Northwestern Ontario (Johnson and Vallentyne 1971). Water was filtered through a 0.45 µm Pellicon Cassette System (Millipore Filter, Millipore Corp., Bedford, MA.) and held at $4^{o}C$ prior to use. Water flows were maintained at a minimum of 12 L/d. Exposure concentrations were maintained by a combination of adjusting the rate of flow through the glass bead column and the rate of flow of the dilution water. For safety the overflow from each aquarium was passed through a column of activated charcoal. All exposures were done in a controlled environment room at $10^{o}C$.

Rainbow trout fry were exposed for 4 to 10 d to each PCDD congener in simulated lake water, simulated lake water plus Aldrich humic acid or filtered L239 water. Fish ranged in weight from 0.4 to 1.1 g and loading densities were less than 4 g/L. At each sampling time, 2-4 fish were collected and frozen at $-20^{o}C$ until analysis. At the end of each exposure the remaining fish were transferred to clean flowing dechlorinated tap water (2 L/min) to study the elimination of radioactivity for up to 48 d. Fish were fed 1.5% body weight/d during the elimination phase. Three dead and 3 living fish were placed into three of the T_4CDD treatments at the end of the uptake experiment

for 24 h. Dead fish accumulated less than 5% of the amount accumulated by living fish indicating that uptake via adsorption to the outside of the fish was of minor importance.

Water and fish analyses

Duplicate 4 mL water samples were collected at the start of each exposure, and at each fish sampling time, and assayed by LSC. Additional 25 mL water samples were collected 2-4 times during each experiment and centrifuged at 20000g for 30 min to remove POM. Four mL of the supernatant was assayed directly by LSC and a second 4 mL of the supernatant was passed through a reverse-phase cartridge (C_{18} Sep-Pak) and the eluant assayed by LSC to determine the proportion of radioactivity sorbed to DOM as described above. The pH ranged from 7.0 to 7.8 and total suspended solids (TSS) ranged from <1 to 8 mg/L.

Fish were rinsed with distilled water, blotted dry, weighed and freeze-dried. Freeze-dried fish were homogenized with a Polytron (Kinematica, Luzern, Switzerland) in 4 mL of toluene. The slurry was centrifuged and 2 mL of the extract assayed by LSC. The tissue residue was air dried and combusted on a Packard 306 Sample Oxidizer. Combustion efficiency was 94±4% (N=6). In two T_4CDD treatments, the whole fish were weighed and oxidized directly.

Extractable and total radioactivity were calculated as ng equivalents of each congener/g fish (wet weight). Elimination rate constants (K_d) were determined by fitting the data from the elimination phase to a first order decay curve. Uptake rate constants (K_u) were estimated using nonlinear regression (SAS-NLIN) to fit the two-compartment kinetic rate model,

$$C(t) = [(K_u * C_w)/K_d] * [1-e^{(-Kd*t)}] \qquad [6]$$

where C_w is the concentration of the PCDD in the water (ng/L), and C(t) is the concentration in the fish (ng/kg) at time t. Concentrations of PCDDs in the water used in the model were time weighted means of the concentration in solution after centrifugation (apparent) or reverse-phase cartridge extractable (truly dissolved). K_ds were means of all treatments except for the K_d for H_7CDD (K_d = 0.04) which was taken from Muir et al. (1985d). Equilibrium BCFs were calculated as the ratio K_u/K_d. The slopes of K_u against 1/DOC for T_4CDD were compared using analysis of covariance (SAS-GLM).

RESULTS

All three methods (reverse-phase cartridges, dynamic headspace analysis and equilibrium dialysis) gave a similar relationship between the percentage of the T_4CDD bound and the concentration of DOC (Fig. 12). However, reverse-phase cartridges gave consistently higher percent bound estimates above DOC concentrations of 3 mg/L. In Milli-Q water, 14-46% (mean of all treatments = 28±12%) of the T_4CDD was bound. Addition of 18 mg/L Aldrich humic acid increased the amount bound to 87±9% which results in an estimated log K_{doc} of 5.4.

In the fish exposure studies, a large proportion of the PCDDs were associated with POM. POM sorbed 19.6-74.5% of the total T_4CDD in solution and 84.2-91.4% of O_8CDD (Table 11). In simulated lake water treatments, 23.7-39.2% of the T_4CDD, 13.0% of the H_6CDD, 4.2% of the H_7CDD and 4.6% of the O_8CDD were in true solution as measured using reverse-phase cartridges. Addition of Aldrich humic acid reduced the percentage of all PCDDs in true solution. However L239 treatments with 2.6-3.3 mg/L DOC had free water concentrations similar to simulated lake water.

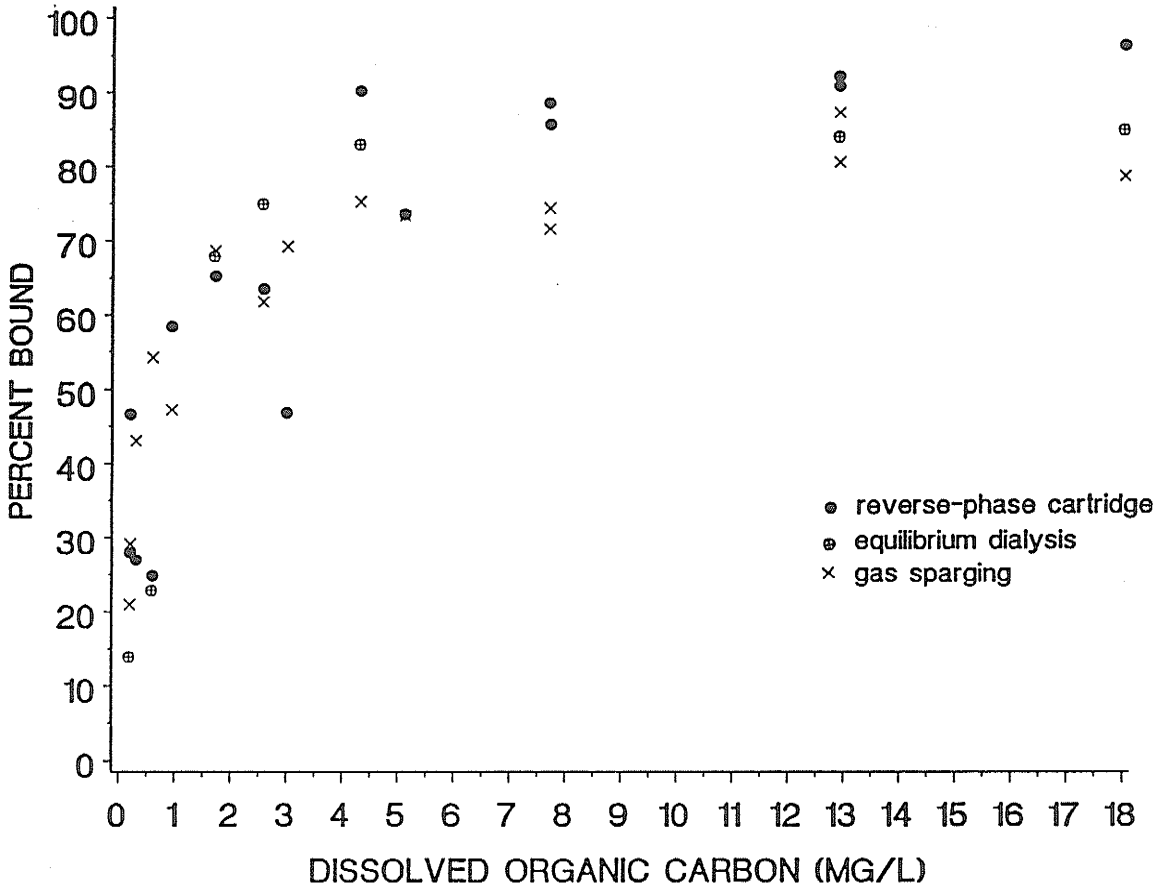

Figure 12. Comparison of the reverse phase cartridge, dynamic headspace, and equilibrium dialysis, methods to determine the partitioning of 1,3,6,8-tetrachlorodibenzo-p-dioxin to Aldrich humic acid.

Table 11. Exposure concentrations and dissolved organic carbon partition coefficients (K_{doc}) for fish uptake experiments.

Treatment	exposure duration (days)	DOC (mg/L)	total conc. (ng/L)	percent in solution after centrifugation	percent C_{18} cartridge extractable	log K_{doc}
T_4CDD-S1[2]	5	0.6(0.2)[1]	39.9	35.9(26.3)	26.7(31.0)	5.74
T_4CDD-S2	5	0.7(0.2)	31.8	67.6(26.1)	38.7(29.0)	5.99
T_4CDD-S3	10	1.1(0.2)	39.7	37.0(17.1)	23.4(19.3)	5.58
T_4CDD-S4	10	1.7(0.5)	41.9	80.4(23.0)	39.2(27.3)	5.79
T_4CDD-L239[3]	10	3.3(0.5)	41.7	25.5(14.2)	17.7(14.7)	5.12
T_4CDD-AHA[4]	4	1.4(0.6)	106.0	37.1(5.0)	17.2(2.9)	5.92
T_4CDD-AHA	4	2.3(0.4)	167.2	37.6(5.7)	19.8(3.8)	5.59
T_4CDD-AHA	10	2.5(1.4)	50.7	38.2(12.9)	15.9(17.2)	5.75
T_4CDD-AHA	10	4.1(0.5)	36.6	69.6(28.0)	14.1(15.2)	5.98
T_4CDD-AHA	10	8.6(1.3)	41.1	60.1(17.1)	11.1(10.0)	5.71
H_6CDD-S1	10	0.3(0.1)	13.9	16.6(17.3)	13.0(17.3)	5.76
H_6CDD-L239	10	2.9(0.2)	13.1	0.8(1.3)	nd[5]	
H_6CDD-AHA	10	1.6(1.4)	21.6	54.7(16.9)	9.3(13.8)	5.72
H_7CDD-S1	10	0.2(0.1)	15.2	49.6(18.5)	4.2(6.2)	7.39
H_7CDD-L239	10	2.6(0.3)	14.3	30.0(12.4)	1.6(3.2)	6.85
H_7CDD-AHA	10	1.2(1.6)	23.5	26.3(18.8)	1.1(8.8)	7.28

Table 11 continued

O_8CDD-S1	10	0.7(0.3)	57.5	15.8(14.5)	4.6(11.0)	6.50
O_8CDD-L239	10	3.3(0.6)	69.6	10.1(8.1)	3.4(10.5)	5.78
O_8CDD-AHA	10	2.0(1.8)	65.9	8.6(10.7)	2.3(4.6)	6.16

1. numbers in parentheses represent one S.D.
2. simulated lake water
3. Lake 239 water
4. Aldrich-humic acid treatment water
5. not detectable

The mean depuration rate constants (K_d) were 0.12±0.01, 0.04±0.01, 0.08±0.01 day^{-1} for the T_4CDD, H_6CDD and O_8CDD respectively. In simulated lake water, the apparent uptake rate constants ($K_{u(a)}$ L Kg^{-1} $days^{-1}$, based on total ^{14}C) were 285±110 (mean ± S.D. of all exposures) for the T_4CDD and declined with the degree of chlorination to 30 for the O_8CDD (Fig. 13). The log K_{doc} generally increased in simulated lake water with the degree of chlorination from 5.58-5.99 for the T_4CDD, 5.76 for the H_6CDD, 7.39 for the H_7CDD and 6.50 for the O_8CDD (Table 12). $K_{u(a)}$ for L239 exposures followed a similar pattern to the simulated lake water exposures (Fig. 13). For all congeners, the log K_{doc} was lower for L239 water than for either simulated lake water or Aldrich humic acid solution exposures (Table 11). Addition of Aldrich humic acid reduced $K_{u(a)}$ of the T_4CDD by 80%, and H_6CDD by 86% (Fig. 13, 14; Table 12). A similar pattern exists for the K_u estimated using the extractable ^{14}C concentrations in fish (Table 12). The $K_{u(a)}$ for both the H_7CDD and O_8CDD increased slightly with the addition of Aldrich humic acid.

DISCUSSION

A comparison of reverse-phase cartridge, dynamic headspace and equilibrium dialysis methods in this study gave similar trends for the partitioning behaviour of the T_4CDD. Addition of less than 1 mg/L Aldrich humic acid decreased the percentage of the truly dissolved T_4CDD to 53% of the total in solution, while addition of 18 mg/L reduced it to 13%. The reverse-phase cartridge method first described by Landrum et al. (1984) was the easiest and quickest of the three methods to use. The similarity of the results using these three independent methods lends confidence to the results obtained using the simple reverse-phase cartridge method. However, above 3

Table 12. The uptake rate constants (K_u) and bioconcentration factors (BCF) for polychlorinated dioxins.

treatment	uptake rate constant				bioconcentration factor			
	apparent[1]		true[2]		apparent		true	
	ext.[3]	total	ext.	total	ext.	total	ext.	total
T_4CDD-S1[5]	146(5)[4]	446(18)	197(9)	602(25)	1704	3717	2299	5017
T_4CDD-S2	81(3)	252(19)	146(6)	441(33)	210	2100	1700	3675
T_4CDD-S3	53(3)	253(18)	85(5)	402(29)	402	2109	992	3348
T_4CDD-S4	37(3)	191(11)	77(7)	390(23)	438	1592	895	3255
T_4CDD-L239[6]	70(9)	292(3)	101(13)	419(51)	825	2437	1183	3493
T_4CDD-1.4A[7]	-	168(18)	-	363(40)	-	1400	-	3025
T_4CDD-2.3A	-	134(10)	-	255(19)	-	1116	-	3025
T_4CDD-2.5A	14(2)	126(9)	35(5)	305(21)	170	1054	410	2545
T_4CDD-4.1A	15(1)	80(3)	75(5)	398(14)	177	672	873	3315
T_4CDD-8.6A	10(1)	57(3)	56(3)	307(14)	121	474	654	2565
H_6CDD-S1	63(3)	76(3)	78(4)	94(4)	1370	1935	1690	2387
H_6CDD-1.6A	8(11)	12(11)	14(20)	22(20)	168	310	308	568
H_7CDD-S1	6(1)	39(5)	131(21)	864(104)	142	938	999	6594
H_7CDD-L239	5(1)	60(10)	94(22)	1082(118)	125	1441	2440	28027
H_7CDD-1.2A	13(3)	56(8)	277(8)	1225(171)	300	1335	7150	31789

Table 12 continued

O_8CDD-S1	18(2)	30(4)	59(7)	94(12)	299	387	543	705
O_8CDD-L239	21(4)	36(6)	62(12)	100(17)	360	465	493	637
O_8CDD-2.0A	31(4)	52(6)	124(16)	202(23)	510	676	1140	1489

1. Based on concentration in solution after centrifugation
2. Based on reverse-phase cartridge extractable
3. extractable ^{14}C.
4. numbers in parentheses represent S.E.
5. simulated lake water
6. Lake 239 water
7. Aldrich humic acid treatment water

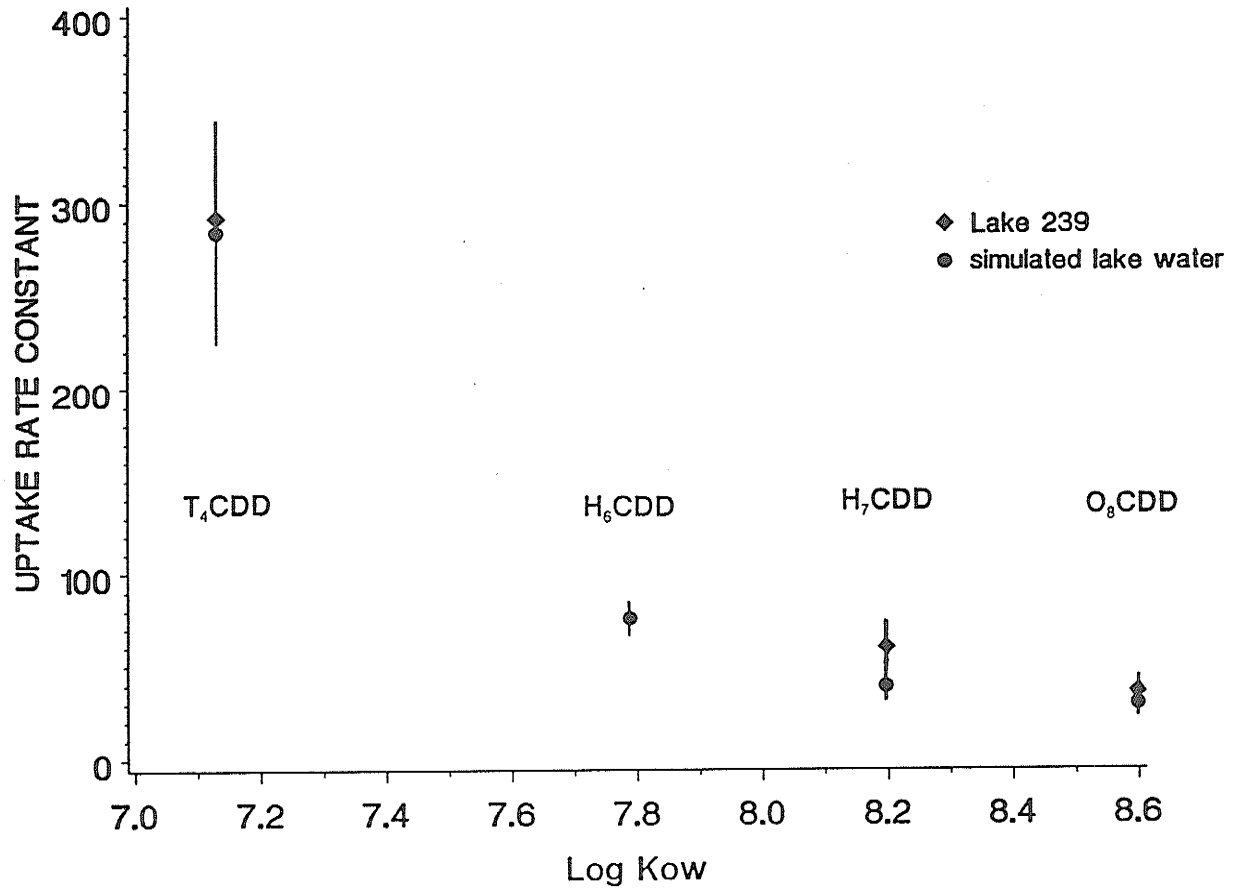

Figure 13. The uptake rate constants (L Kg^{-1} day^{-1}) for polychlorinated dioxins in simulated lake water and natural lake water (Lake 239).

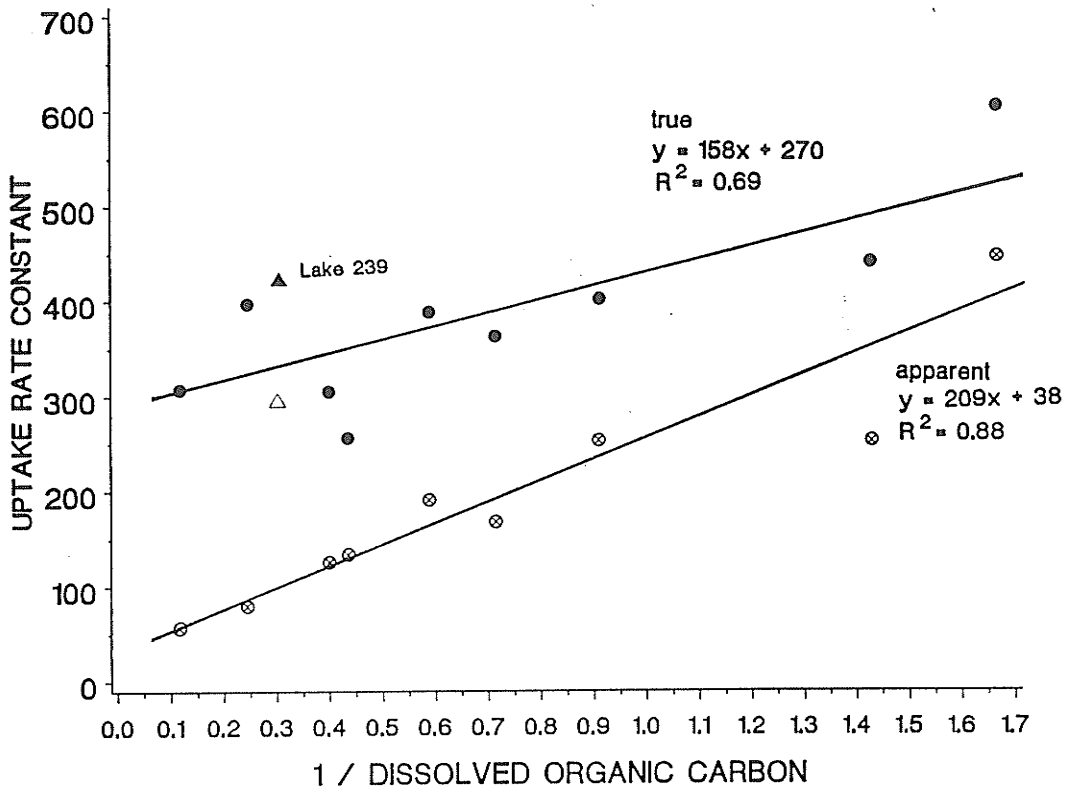

Figure 14. The effect of dissolved organic carbon (Aldrich humic acid) on the uptake rate constants (L kg^{-1} day^{-1}) for 1,3,6,8-tetrachloro-dibenzo-p-dioxin. The slopes were determined to be significantly ($p<0.01$) different using analysis of covariance. Closed and open circles represent the true and apparent uptake rate constants, respectively. Triangles represent the results for L239.

mg/L DOC, the reverse-phase cartridge method gave higher estimates of the percent bound than the other two methods. The reverse-phase method may therefore underestimate the concentrations of truly dissolved PCDD at high DOC concentrations. This may be due to the failure of up to 10% of the DOC to pass through the reverse-phase cartridge. For the higher chlorinated dioxins, this may lead to a significant overestimation of the concentration in true solution.

Substituting log K_{doc} = 5.4 into the relationship developed by Landrum et al. (1985) between K_{doc} and the DOC concentration required to reduce the $K_{u(a)}$ by 50% (I_{50}) predicts that only 1.9 mg/L of Aldrich humic acid would be required to reduce $K_{u(a)}$ of the T_4CDD by 50%. Addition of Aldrich humic acid to simulated lake water reduced $K_{u(a)}$ for the T_4CDD. The I_{50} calculated for the T_4CDD in this study was 2.0 mg/L Aldrich humic acid. This is an excellent agreement considering that Landrum et al. (1985) used an amphipod as the test species, short term exposures, higher temperature, and a different group of compounds (PAHs) with lower log K_{ow}s, to determine the relationship.

The reduction in $K_{u(a)}$ appears to result from overestimating the concentration of the available T_4CDD (truly dissolved). In comparison, $K_{u(t)}$ calculated using estimated free water concentrations declined only slightly with the addition of Aldrich humic acid. Because K_d is not strongly affected by DOM (McCarthy and Jimenez 1985), using $K_{u(a)}$ rather than $K_{u(t)}$ in the calculation of BCF will lead to an underestimate of the true BCF. In most studies, little attention is given to "quality" of the exposure water. Because such a small amount of DOC (Aldrich humic acid) will significantly reduce $K_{u(a)}$ this appears to have led to serious underestimation of the BCF of many hydrophobic compounds including PCDDs.

The natural DOC in L239 water appears to have a much lower affinity for the PCDDs than Aldrich humic acid. Lake 239 water treatments had lower K_{doc} than Aldrich humic acid treatments and the $K_{u(a)}$ was similar to the simulated lake water treatments. Other studies have shown a similar result (Morehead et al. 1986; Landrum et al. 1987) for less hydrophobic compounds. Aldrich humic acids may strongly overestimate the impact of DOM on the uptake of PCDDs. Chiou et al. (1986) have shown that the partitioning (solubility enhancement) of several hydrophobic pesticides and PCBs to DOM is related to the molecular size and polarity of the DOM. They found that the DOC partition coefficient for soil-derived humic acids was 5 to 7 times higher than for aquatic humic and fulvic acids (Chiou et al. 1986). DOM from soils, sediments and the water column differ in their chemical and physical characteristics (Melcer and Hassett 1986; Malcolm and MacCarthy 1986). Commercially available humic acids are likely more similar to the more polar, high molecular weight soil derived humics than to aquatic humics. Landrum et al. (1985) reported that the DOC partition coefficients for several hydrophobic compounds were approximately one order of magnitude greater for Aldrich humic acid than for natural waters. Chiou et al. (1987) showed that the solubility enhancement of DDT and PCB is consistently greater for commercially available humic acids than natural river waters. Morehead et al. (1986) also showed that the binding affinity of Aldrich humic acid for benzo[a]pyrene was greater than for two natural lake waters. However, DOM from sediment interstitial waters showed similar binding behaviour to Aldrich humic acid. Morehead et al. (1986) also concluded that the quality or site of collection of DOM was a more important factor determining K_{doc} than the hydrophobicity of the compound. Although there was a correlation between K_{doc} and hydrophobicity of the compound for different water samples, this correlation was greater for sediment interstitial waters

and Aldrich humic acid than for lake waters (Morehead et al. 1986). A similar result has been presented in Chapter III for Canadian Shield waters using the T_4CDD where sediment interstitial waters had generally greater K_{doc} than epilimnetic waters from the same lake. DOM from any given source does affect the uptake of hydrophobic compounds, and this effect appears to be greater for more hydrophobic compounds, but the quality of the DOM may be the dominant factor. Although Aldrich humic acid appears to be similar to sediment interstitial water in some respects, caution should be used if data obtained using Aldrich humic acid are to be extrapolated to the environment. The ability of aquatic DOM to decrease the apparent BCF may be minimal unless the DOM concentration is relatively high.

The addition of Aldrich humic acid slightly increased $K_{u(a)}$ for both H_7CDD and O_8CDD. It is possible that the ^{14}C is loosely associated with the the Aldrich humic acid and is bioavailable even though it is not extracted by the C_{18} cartridge. However, increased $K_{u(a)}$ in the presence of Aldrich humic acid could also be due to some form of experimental error. The exposure concentrations in both of these treatments were considerably higher than their true water solubilities. DOM has been shown to enhance the apparent solubility of the T_4CDD (Chapter I, III) and other compounds (Chiou et al. 1986; 1987). Aldrich humic acid had a greater affect on solubility enhancement than natural lake waters but had a similar effect on K_u (Chapter III). This suggests that a fraction of the T_4CDD was loosely associated with the DOM when it was present at concentrations above the water solubility. Aldrich humic acid may increase the fraction of this loosely associated PCDD making it more bioavailable. The K_{doc} of O_8CDD may be lower than expected (i.e., lower than the H_7CDD) because exposure concentrations were much higher than its reported water solubility. Morehead et al. (1986) showed that the

sorption isotherms for benzo[a]pyrene were not linear above its water solubility such that there was more compound in true solution than would be predicted. Only a small proportion of the H_7CDD and O_8CDD remained in solution after centrifugation. Most of the ^{14}C was associated with the POM excreted by the fish during the exposures or with microparticulates (>0.45 μm present in the dilution water which were not separated prior to the exposures. This led to extremely low water concentrations after centrifugation which were near or at the detection limits (1 ng/L). Although POM has been shown to bind hydrophobic organic compounds, McCarthy and Black (1987) have demonstrated that the binding affinity of benzo[a]pyrene for Aldrich humic acid is unaffected by the presence of particulate material over a wide range of particulate concentrations. The presence of POM in the exposure system will reduce the concentration of the compound in solution (similar to sorption to glass) but it should not influence the general conclusions about the affects of DOM.

Even when the free water concentrations are used to predict K_u for PCDDs, they are considerably lower than would be predicted by their log K_{ow} (Table 10, 12), i.e., by extrapolating the relationship developed by Veith et al. (1979). Steric hindrances, water solubility, and metabolic transformation have been suggested as other possible explanations for the low BCFs for PCDDs.

Metabolic transformation of the lower chlorinated PCDDs by the fish may result in low BCFs by inflating the K_d. Metabolic transformation might explain part of the difference between the BCFs of 1,3,6,8-T_4CDD and 2,3,7,8-T_4CDD which is metabolized to a much less extent (Muir et al. 1986; Kleeman et al. 1986a; 1986b; Branson et al. 1985). Very little metabolic transformation of the higher chlorinated PCDDs has been reported (Muir et al. 1985d; Muir et al. 1986); thus, it is unlikely that such reactions are

responsible for low BCFs observed.

Molecular volume and shape affect the activity coefficients of solutes in membranes differently from those seen in 1-octanol and n-hexane and these differences become larger with larger solute size (Gobas and Mackay 1987). Chemicals with molar volumes from 230 to 400 cm^3/mol showed a difference in solubility between membrane vesicles (dimyristoyl-phosphatidyl choline) and 1-octanol probably resulting from an increase in the free energy of cavity formation in the membranes (Gobas et al. 1988). The PCDDs do follow this pattern such that PCDDs with larger molecular volume (Table 10) have lower K_u. Opperhuizen et al. (1985) suggested a lack of membrane permeation for hydrophobic molecules with minimum internal cross sections over 9.5 Å. H_6CDD, H_7CDD and O_8CDD all have minimum internal cross sections of 9.8 Å which indicates that these compounds would not permeate the polar holes of the epithelial membranes. However, all three of these compounds are accumulated in the internal tissues of fish (Muir et al. 1985d; Muir et al. 1986). Previous studies which have reported a complete lack of accumulation of O_8CDD may have suffered from detection limit or other experimental limitations (Opperhuizen et al. 1985; Bruggeman et al. 1984). However, the higher chlorinated dioxins might have been taken up via another route such as micellar transport which would not be restricted by steric factors (Opperhuizen et al. 1985).

Because BCFs are independent of kinetic factors, they should be unaffected by reduced membrane permeation if the exposure period is long enough. However, the time required to reach equilibrium may be greater than the life time of the fish for such superlipophilic compounds (Hawker and Connell 1985; Connell 1988). Gobas and MacKay (1987) have suggested that for hydrophobic chemicals such as PCDDs, uptake rates are controlled by solute

transport in the aqueous phase rather than in the lipid phase. Because transport in the aqueous phases of the fish is a basic property of the fish uptake kinetics should be independent of the compounds hydrophobicity (i.e., K_u is constant at high K_{ow}).

Measured apparent BCFs of PCDDs in this study were below those predicted from their K_{ow}s alone (i.e., Veith et al. 1979; Gobas and Mackay 1987) but only part of this reduction can be explained by calculating the true BCFs using the estimated free water concentrations. This may be due to experimental errors leading to an overestimation of the truly dissolved concentration and therefore an underestimation of the BCF. Steric hindrances, solubility factors, or metabolic transformation may also be important factors controlling the BCFs of PCDDs.

CHAPTER III

The 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*

Hydrophobic organic chemicals have been shown to sorb to POM and more recently to DOM (McCarthy 1983; Carter and Suffet 1982; Landrum et al. 1984; Chapters I, II). Partitioning of the hydrophobic compound to DOM may result in an increase in the apparent solubility (Chiou et al. 1986; Chapter I). The relatively water insoluble hydrophobic compound is rendered apparently more soluble by partitioning into the more water soluble DOM. Although the apparent solubility may increase in the presence of DOM, the concentration of truly dissolved (free) chemical in the water may be reduced (i.e., if the truly dissolved concentrations are below the true water solubility). Because biota appear to be able to accumulate only the compound truly dissolved, sorption of hydrophobic compounds to DOM may lead to a reduction in bioavailability (Landrum et al. 1985; 1987; McCarthy et al. 1985; McCarthy and Jimenez 1985; Carlberg et al. 1986; Chapter II).

The source or "quality" of the DOM can be as important or more important than the concentration of DOM (Carter and Suffet 1982; Landrum et al. 1987; Carlberg et al. 1986; Morehead et al. 1986). Landrum et al. (1984; 1985; 1987) found that sorption of polycyclic aromatic hydrocarbons (PAH) to aquatic DOM from the Great Lakes varied both temporally and spacially. Although the DOM concentration at any one site affects the bioavailability, this variable is less important than changes in the DOM source (Landrum et al. 1984; 1987).

The ability of DOM to sorb hydrophobic compounds appears to be related to the molecular size and polarity (Choiu et al. 1986;1987). Chiou et al. (1986) found that the DOM partition coefficient of PCBs and several pesticides for soil derived humic acids was 5 to 7 times higher than for aquatic humic and fulvic acids.

Many of the studies on the interaction between DOM and hydrophobic compounds have been done with commercially available humic acids, particularly Aldrich humic acid. Although these commercially available humic acids are similar in some respects to soil humic material, they are not representative of soil or water humic and fulvic acids (Malcolm and MacCarthy 1986; Choiu et al. 1987). Studies using Aldrich humic acid may overestimate the sorption of hydrophobic compounds to natural DOM from lake or sediment interstitial waters (Landrum et al. 1985; Choiu et al. 1987; Chapter II). Other studies have avoided the use of commercial humic acids by extracting the DOM from sediments or soils; however, it is not clear if there is an effect on the sorption characteristics of the DOM due to the extraction procedures.

T_4CDD is a hydrophobic (S_w = 317 ng/L), superlipophilic (log K_{ow} = 7.13) compound (Friesen et al. 1985; Burkhard and Kuehl 1986) which is readily accumulated by invertebrates and fish (Muir et al. 1985a; 1985b). Both the apparent solubility and bioavailability of dioxins, including the T_4CDD have been shown to be affected by DOM (Muir et al. 1985a; Webster et al. 1986; Chapter I, II). The present study evaluates the effect of natural DOM from the epilimnion and sediment interstitial waters of Canadian Shield lakes on both the apparent solubility and bioavailability of the T_4CDD. This study extends the examination of DOM-contaminant interactions to a more hydrophobic compound and to a wider variety of natural DOM than have been previously examined. Understanding the effects of DOM on the partitioning and

bioavailability of T_4CDD will provide further insight into the environmental behaviour of the more toxic dioxin congeners, especially 2,3,7,8-T_4CDD which has similar chemical properties (Friesen et al. 1985; Adams and Blaine 1986; Burkhard and Kuehl 1986; Marple et al. 1986; Table 10).

MATERIALS AND METHODS

The ^{14}C-1,3,6,8-tetrachlorodibenzo-p-dioxin, with a specific activity of 24.16 mCi/mM (Pathfinder Laboratories, St. Louis, MO.) was purified prior to use by thin layer chromatography to give a radiopurity of >99.8%. Aldrich humic acid (Aldrich Chemical Co., Milwakee, WI., lot no. 1204PE) was precipitated twice with HCl, dialysed in distilled water and filtered through a 0.45 µm type HA Millipore filter (Millipore Corp., Bedford, MA). Benthic amphipods, Crangonyx laurentianus, were collected on August 15, 1986, from the littoral zone (0-0.5 m) of Lake 470 in the Experimental Lakes Area, Northwestern Ontario (Johnson and Vallentyne 1971). Animals were held in Lake 470 water at 24°C with 12 h light 12 h dark photoperiod for 7-20 d.

Samples of natural lake water, sediment interstitial water, or simulated lake water were used in these studies. Water was collected from the epilimnion of 12 Canadian Shield lakes in the Experimental Lakes Area (Table 13). Sediment interstitial water was collected from seven of these lakes at the deepest point in the lake using an Ekman dredge. An additional sample was taken at a depth of 2 m from Lake 304. Interstitial water was separated by centrifugation at 5000g for 30 min, followed by filtration through a Whatman GFC filter and 0.45 µm type HA Millipore filter. Water was stored at 4°C and used within two weeks. Simulated lake water was Milli-Q water (Millipore Corp.) reconstituted with inorganic ions (48 mg/L $NaHCO_3$, 30.0 mg/L $CaSO_4.H_2O$,

Table 13. Physical and chemical characteristics of the Experimental Lakes sampled. Water samples collected from the epilimnion at the deepest point in the lake during August 1986 (unpublished data provided by J. Shearer, G. Linsey and D. Malley[1])

	max depth (m)	area (ha)	DOC (µg/L)	Susp. C (µg/L)	Susp. N (µg/L)	pH	alkalinity (meq/L)
Lake 224	27.4	25.9	2.9	480	34	7.1	78
Lake 373	21.5	28.0	4.3	630	9	7.3	166
Lake 305	32.7	52.0	4.4	550	41	7.3	132
Lake 377	19.0	26.7	1.2	225	68	7.0	109
Lake 303	2.5	9.5	6.2	2050	205	6.7	56
Lake 623	21.3	36.0	6.8	450	38	7.2	113
Lake 239	30.4	56.1	6.0	530	48	7.1	141
Lake 382	13.1	37.1	7.4	830	76	6.7	88
Lake 225	2.0	4.0	8.8	1420	81	5.1	-5
Lake 222	5.8	16.4	10.7	770	80	7.0	196
Lake 470	1.7	5.7	-	-	-	7.2	154
Lake 304	6.7	3.6	8.8	1620	186	7.1	93

1. Freshwater Institute, Dept. of Fisheries and Oceans.

30.0 mg/L $MgSO_4$ and 2.0 mg/L KCl) with 0 to 9.5 mg/L Aldrich humic acid added.

Solubility enhancement

The ^{14}C-T_4CDD (28.3 ng) was spiked onto the walls of 25-mL Corex glass tubes and the carrier solvent allowed to evaporate off. Twenty-five mL of each treatment water (Table 14) was added to each of six tubes to give a nominal concentration of 1131 ng/L or 3.5 times the reported water solubility. Each tube was capped and gently shaken at 23°C for 24 h. Replicate 4 mL water samples were taken from each tube, diluted with scintillation fluor (Atomlight, New England Nuclear, Boston, MA) and assayed by LSC. The tubes were then centrifuged at 20000g for 30 min and 4 mL of the supernatant was passed through a reverse-phase cartridge (C_{18} Sep-Pak, Waters Scientific, Milford, MA) and the eluant assayed by LSC. Previous studies have shown that the ^{14}C sorbed to the DOM passed through the cartridge while the ^{14}C free in solution or weakly bound remained on the cartridge (Landrum et al. 1984; Chapters I, II). More than 95% of the DOC from Lake 304 passed through the cartridge, consistent with results reported by Landrum et al. (1984). DOC concentrations were determined using a high temperature acid persulphate digestion followed by infrared detection of CO_2 on a Model 700 Carbon Analyser (OI Corp., Houston, TX).

Bioavailability

A solution of the T_4CDD was introduced directly into 100 mL of each water solution (125 mL flask) in less than 10 µL of a methanol carrier. This amount of methanol does not have an effect on the partitioning of the T_4CDD to DOM (Webster et al. 1987; Appendix A). Solutions were allowed to equilibrate for 3 h at 23°C. Allowing solutions to equilibrate for 24 h instead of only 3

h did not affect the uptake rate constants. Total water concentrations were generally less than the reported water solubility of 317 ng/L (Table 14).

Each treatment consisted of 5 or 6 replicate flasks with two amphipods in each spiked with 147±24 ng/L T_4CDD as described above. After 6 h both animals were removed, blotted dry with a paper towel, weighed and oxidized on a Packard 306 Sample Oxidizer (Packard Instrument Co., Downers Grove, IL). The $^{14}CO_2$ was trapped in 2-methoxyethylamine, diluted with scintillation fluor and assayed by LSC.

Depuration experiments (4 replicates) were conducted by exposing animals in 250 mL of Lake 304 epilimnetic water. Two animals were removed at 0.25, 0.5, 1, 2, 3, 6 h, and the remainder were transferred to uncontaminated Lake 304 water and two animals collected at 0.5, 1, 4 and 18 h.

Replicate 4 mL water samples were taken at 0, 3 and 6 h and assayed by LSC. Additional duplicate 25 mL water samples were collected at 3 h and the DOM-bound and truly dissolved water concentrations were determined using reverse-phase cartridges as described above. The DOC partition coefficient (K_{rp}) was determined as

$$K_{rp} = (C_b \; / \; DOC) \; / \; C_f \qquad [7]$$

where C_b is the concentration bound (ng/L), DOC is the concentration of dissolved organic carbon (Kg/L) and C_f is the concentration truly dissolved (ng/L) (Landrum et al. 1985).

The net flux of T_4CDD between water and biota can be described by a two-compartment model,

$$dC/dt = K_u \; C_w - K_d \; C(t) \qquad [8]$$

where K_u is the uptake rate constant (L Kg^{-1} h^{-1}), C_w is the concentration of T_4CDD in the water (ng/L), K_d is the first-order depuration rate constant (h^{-1}) and A(t) is the amount of T_4CDD in the animal (ng/kg) dependent on time (t). If the water concentration is assumed to be constant, integrating the differential equation results in

$$C(t) = [(K_u\ C_w)/K_d]\ [1-e(-K_d\ t)]. \qquad [9]$$

If the depuration rate is considered to be negligible then equation [9] can be writen as

$$K_u = C(t)\ /\ (C_w\ t). \qquad [10]$$

The first order depuration rate constant determined from depuration experiments was determined to be not significantly different from zero during the first 18 h (Fig. 15) so that the above assumption appears to be valid.

The DOC partition coefficient (K_{doc}) can also be estimated from the biological uptake data (Landrum et al. 1985). The truly dissolved water concentration was estimated from the kinetics according to

$$C_f = C(t)\ /\ (K_{u(0)}\ \ t) \qquad [11]$$

where $K_{u(0)}$ is the uptake rate constant determined in the absence of DOC (i.e., in simulated lake water). The bound concentration can be determined by the difference between the measured total water concentration and the estimated freely dissolved water concentration. The partition coefficient K_b can then be determined as described by equation [7] for K_{rp}.

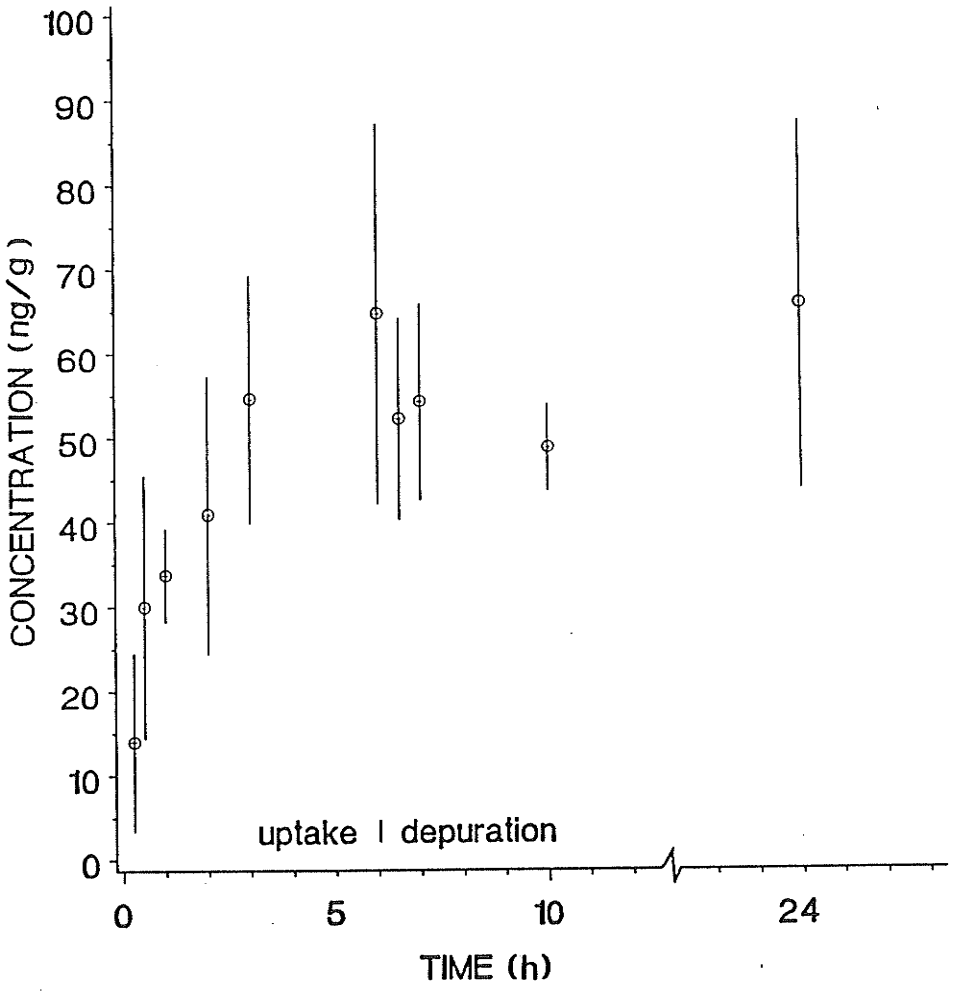

Figure 15. Uptake and depuration curves for 1,3,6,8-tetrachloro-dibenzo-p-dioxin for Crangonyx laurentianus in Lake 304 epilimnetic water.

RESULTS

Solubility enhancement

The apparent solubility of the T_4CDD in the Milli-Q treatment was 283±2 ng/L (N=6). The apparent solubility of the T_4CDD was increased in the lake waters relative to Milli-Q water (Fig. 16). The lake waters resulted in a significant relationship ($p<0.01$) between apparent solubility and DOC concentration with a slope of 37.2±10.1 ng/mg DOC and an intercept of 326±53 ng/L. The T_4CDD had a higher apparent solubility in simulated lake water than in Milli-Q water (328±24; 366±52 ng/L). Aldrich humic acid had a greater effect on apparent solubility than lake water DOM. The relationship between apparent solubility and DOC concentration for Aldrich humic acid had a slope of 47.8±6.5 ng/mg DOC, but had a similar intercept, 296±41 ng/L. The amount of the T_4CDD in the high DOC treatments approached the total amount of the T_4CDD added to the tubes and may have limited the enhancement of the apparent solubility. This explanation is supported by the fact that the truly dissolved concentrations measured using reverse-phase cartridges declined as the DOC concentration increased. The solubility enhancement effect for several of the sediment interstitial waters and the Aldrich humic acid treatments may have thus been underestimated.

Bioavailability

The uptake rate constant for the T_4CDD in C. laurentianus declined as the DOC concentration increased (Fig. 17). The percent of the T_4CDD in the bound phase increased as the DOC concentration increased (Fig. 18). Approximately 30% of the T_4CDD was bound in the simulated lake water treatment while more than 75% and as much as 99% was bound in the sediment interstitial

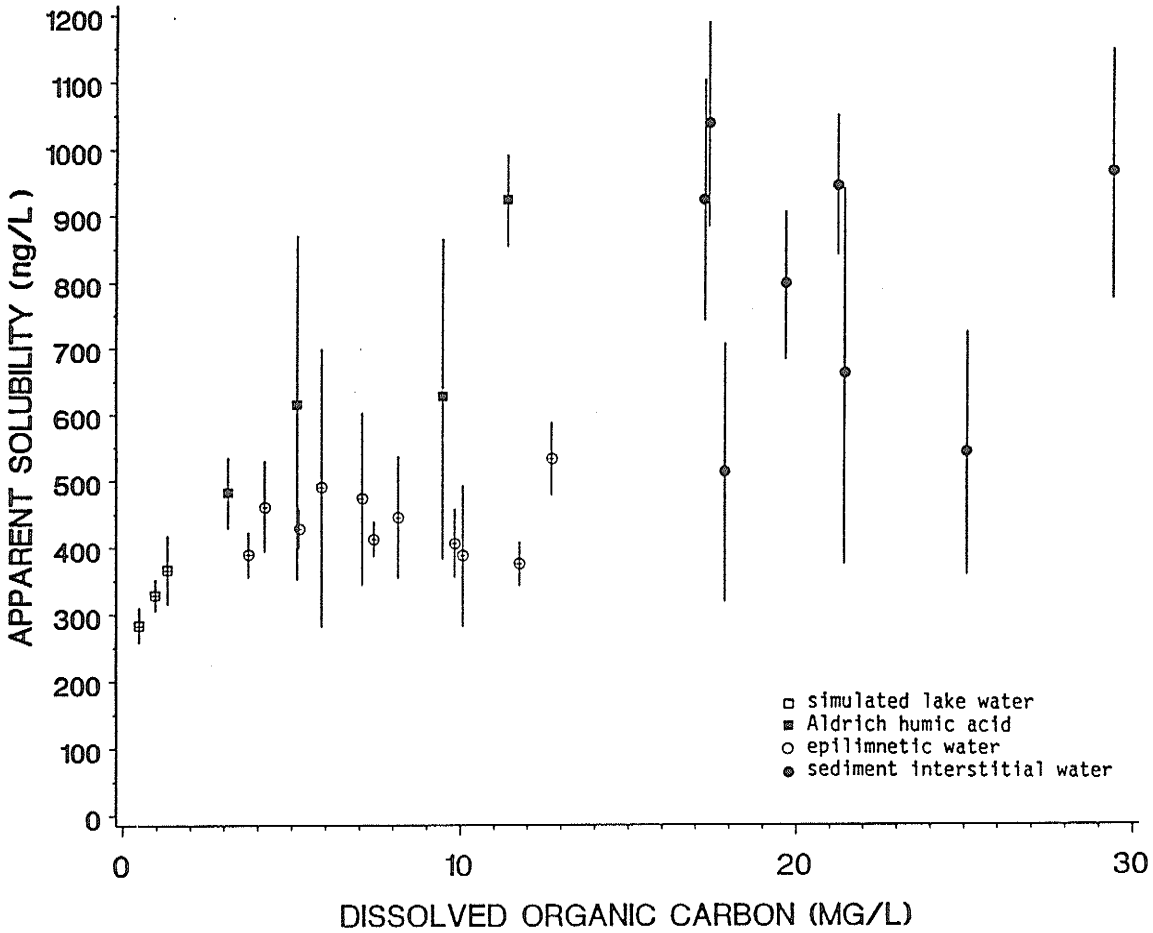

Figure 16. Solubility enhancement of 1,3,6,8-tetrachlorodibenzo-p-dioxin due to dissolved organic carbon.

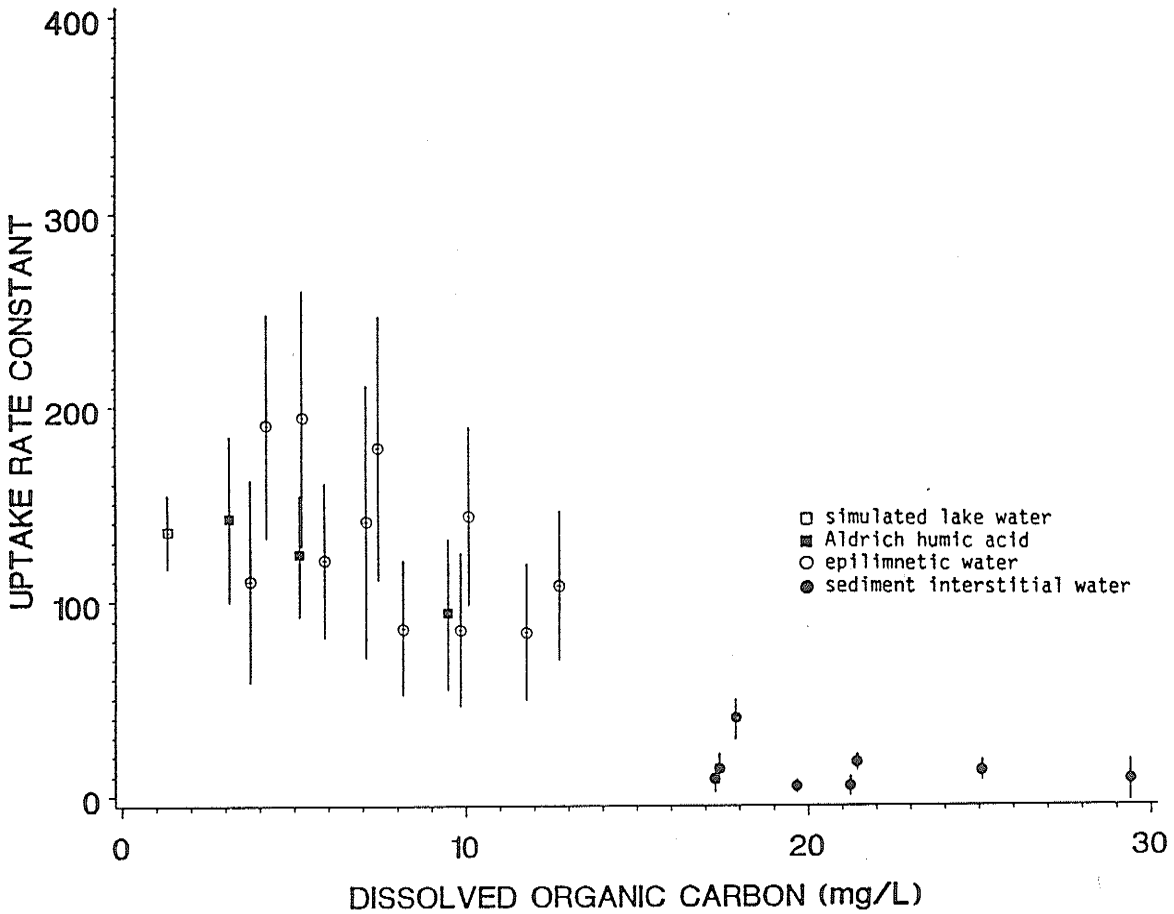

Figure 17. The effect of dissolved organic carbon on the uptake rate constant ($L\ kg^{-1}h^{-1}$) of 1,3,6,8-tetrachlorodibenzo-p-dioxin for Crangonyx laurentianus

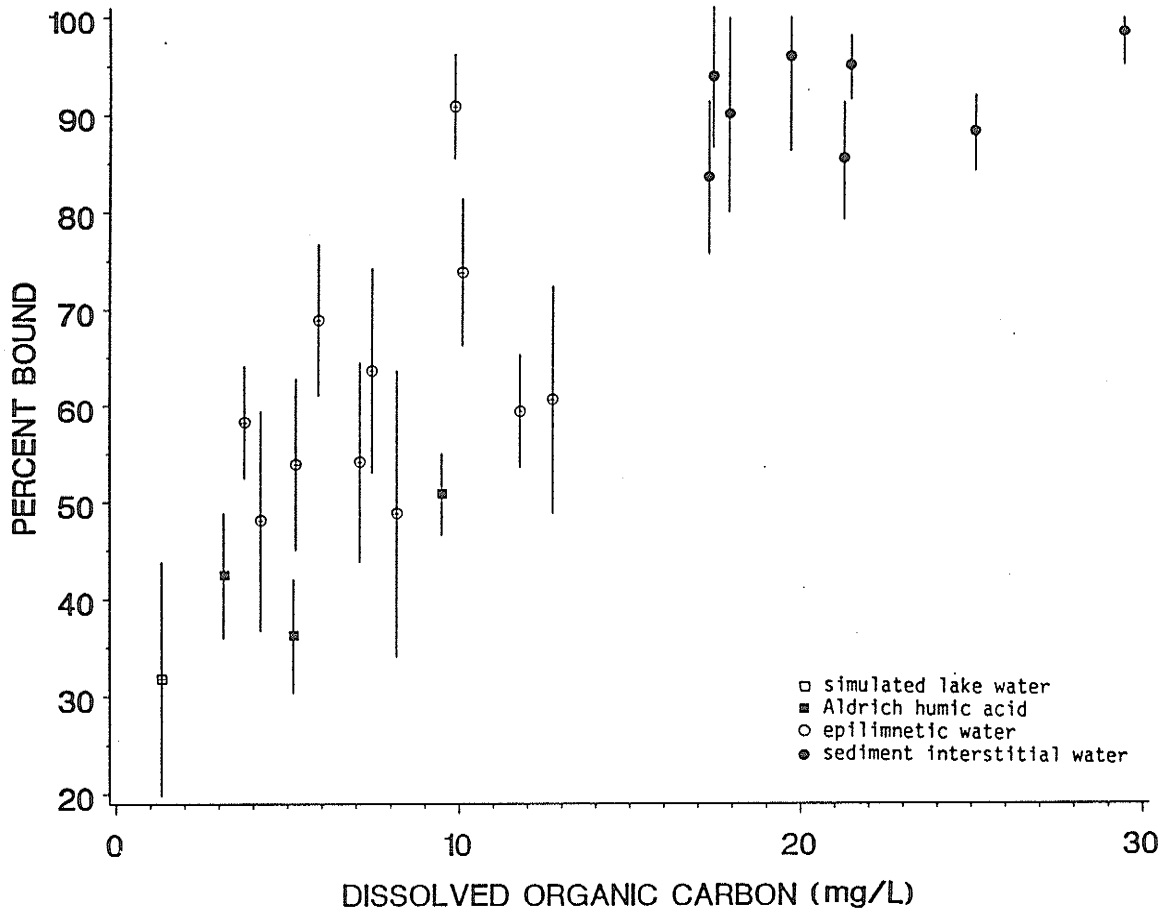

Figure 18. The percentage of 1,3,6,8-tetrachlorodibenzo-p-dioxin bound to dissolved organic carbon in bioavailability tests determined using reverse-phase cartridges.

waters. There was a significant relationship (R^2=0.67) between the truly dissolved concentration predicted by reverse-phase methodology and the truly dissolved concentration predicted by the biota using equation [11] (Fig. 19). The truly dissolved concentrations predicted by biota were higher than the truly dissolved concentrations predicted by the the reverse-phase method. K_{rp} was weakly correlated to the DOC concentration such that the use of sediment interstitial waters generally led to higher K_{rp} values than use of the epilimnetic waters (Table 14).

DISCUSSION

An increase in the apparent solubility of the T_4CDD through the action of DOM may have an impact on its movement and ultimate fate in the environment. The apparent solubility of the T_4CDD was weakly correlated with the DOC concentration, such that it was increased slightly in epilimnetic lake water and more than doubled in most interstitial waters compared to Milli-Q water. DOM may mobilize the T_4CDD from soils or sediments which would otherwise be permanent sinks for the compound. Apparent solubility enhancement by DOM in the water column may therefore result in a wider dispersion than expected for such a hydrophobic compound. Because sorption of nonionic hydrophobic compounds such as T_4CDD is believed to be completely reversible (McCarthy and Jimenez 1985b), gradual desorption of the T_4CDD from the DOM may provide a continuous source of truly dissolved T_4CDD to the water column. This could lead to low level exposure of aquatic organisms to T_4CDD which would otherwise be spatially isolated from the source.

Binding of the T_4CDD to DOC in the exposure systems reduced the truly dissolved concentrations leading to a decrease in the uptake rate constants

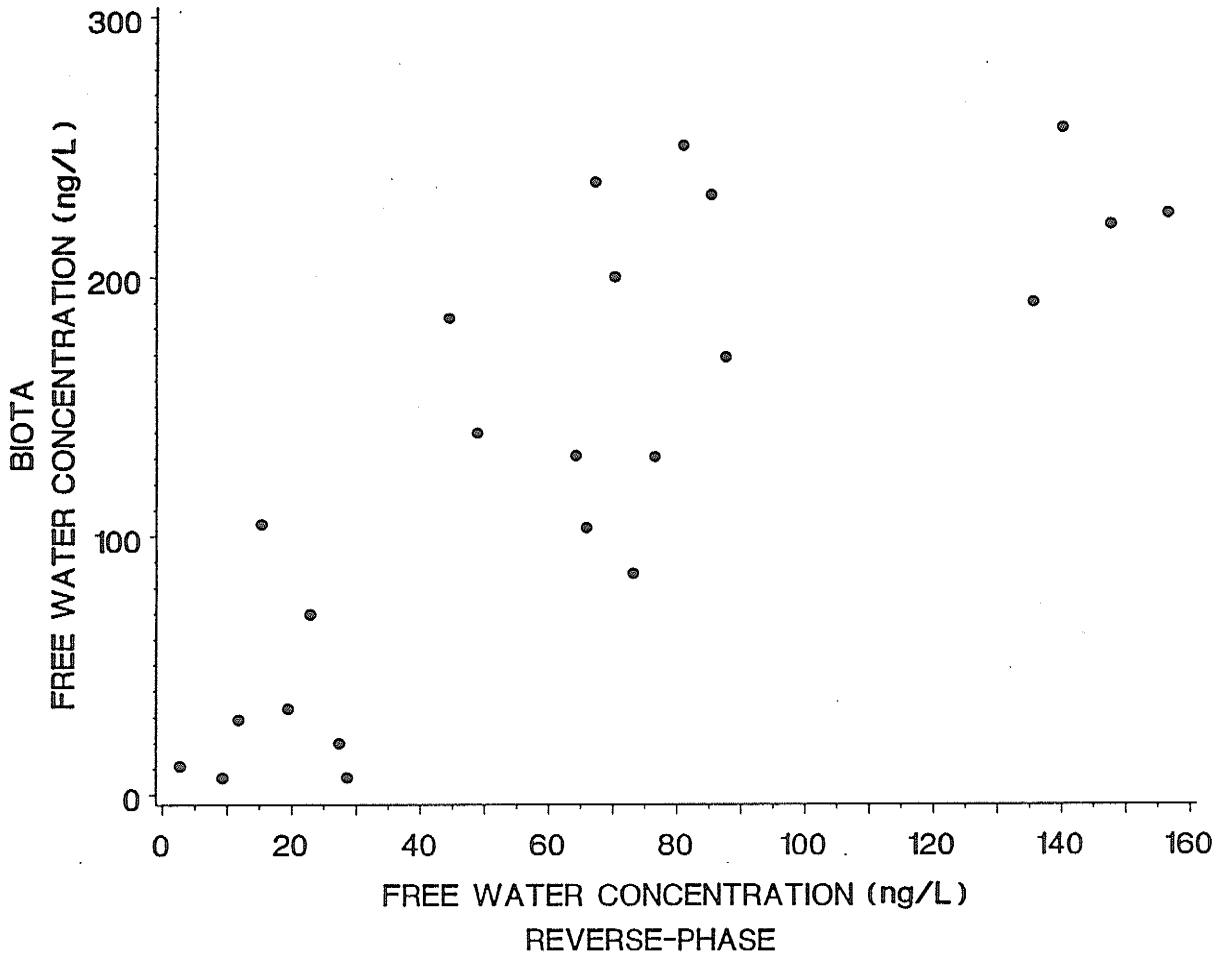

Figure 19. Comparison of free water concentrations determined using reverse-phase cartridges and uptake kinetics.

Table 14 The dissolved organic carbon (DOC) and partition coefficients (K) for the bioavailability tests; rp = reverse-phase cartridges, b = biota.

Treatment	DOC (mg/L)	Total water conc. (ng/L)	K_{rp} (L/g)	K_b (L/g)
Lake 224 epi[1]	3.7	209 (19)[3]	385 (79)	193 (154)
Lake 373 epi	4.2	164 (12)	249 (148)	12 (15)
Lake 305 epi	5.2	201 (60)	248 (140)	25 (21)
Lake 377 epi	5.9	154 (16)	404 (124)	59 (57)
Lake 303 epi	7.1	180 (8)	231 (189)	74 (63)
Lake 623 epi	7.1	139 (10)	146 (46)	27 (26)
Lake 239 epi	7.4	180 (28)	269 (149)	84 (57)
Lake 382 epi	8.2	142 (27)	160 (183)	109 (83)
Lake 225 epi	9.8	164 (28)	1092 (431)	113 (87)
Lake 222 epi	10.1	172 (10)	328 (187)	55 (57)
Lake 470 epi	11.8	163 (12)	130 (42)	86 (49)
Lake 304 epi	12.7	168 (32)	142 (79)	56 (75)
Lake 303 sed[2]	17.3	217 (35)	332 (224)	1389 (951)
Lake 224 sed	17.4	326 (23)	5675(4977)	819 (404)
Lake 239 sed	17.9	238 (13)	1057 (711)	150 (58)
Lake 304 sed-2m	19.7	188 (23)	350 (217)	4058 (5072)
Lake 470 sed	21.2	196 (12)	350 (238)	5589 (8416)
Lake 222 sed	21.4	232 (15)	636 (92)	816 (404)

Table 14 continued

Lake 225 sed	25.1	224(30)	361 (240)	444 (185)
Lake 304 sed-6m	29.4	203 (22)	462 (295)	4459 (6592)
Aldrich H.A.-(0)	1.4	215 (18)	246 (147)	137 (168)
Aldrich H.A.	3.1	243 (17)	241 (54)	82 (92)
Aldrich H.A.	5.6	245 (6)	109 (24)	58 (65)
Aldrich H.A.	9.5	279 (31)	111 (16)	85 (80)

1. epilimnetic water
2. sediment interstitial water
3. Numbers in parentheses represent one S.D.

(K_u). The percentage of the T_4CDD bound to DOC as determined by reverse-phase methodology increased to almost 100% above a DOC concentration of 17 mg/L while only 30% was bound to DOC in the simulated lake water. The free water concentration estimated using the uptake rate constants ($C_{f(b)}$) was correlated (R^2=0.67) to the free water concentration estimated using reverse-phase methodology ($C_{f\ (rp)}$) although $C_{f(b)}$ was approximately twice as high as $C_{f(rp)}$. This is possibly due to the use of simulated lake water as a reference when it is not completely free of DOC. Milli-Q water (<0.24 mg/L DOC) had considerably lower DOC than the simulated lake water. Respiration and excretion by the animals or contamination of inorganic ions might have added trace amounts of organic carbon to the treatment water. This would therefore underestimate the $K_{u(0)}$ and lead to an overestimate of $C_{f(b)}$.

The DOC-TCDD complex is apparently too large or too polar to penetrate the respiratory membranes or the TCDD does not have sufficient time to diffuse out of the DOM complex and interact with the biological membranes (Landrum et al. 1987). The amount of the hydrophobic compound such as PAHs or PCBs bound to DOM has been shown to be directly correlated to the DOM concentration using several independent methodologies, including equilibrium dialysis (Carter and Suffet 1982, MaCarthy and Jimenez 1985), dynamic headspace analysis (Yin and Hassett 1986), solubility enhancement (Choiu et al. 1986) and reverse-phase cartridges (Landrum et al. 1984; Morehead et al. 1986). Although only a few studies have simultaneously measured the free water concentration and the biological uptake rates there is considerable evidence that the uptake rates are inversely correlated to the DOM concentrations in the exposure systems (Landrum et al. 1985; McCarthy et al.1985; McCarthy and Jimenez 1985; Carlberg et al. 1986). McCarthy et al. (1985) found that the uptake of PAHs in Daphnia magna was reduced as the concentration of Aldrich humic acids was increased.

The percentage of PAHs bound to DOM, measured using equilibrium dialysis, was directly related to the decrease in BCF and had a slope not significantly different from one (McCarthy et al. 1985). Landrum et al. (1985; 1987) determined that for PAHs there was a good correlation ($r = 0.86$) between the log DOM partition coefficient determined using the reverse-phase cartridges (K_{rp}) and using biota (K_b). These results support the conclusion that little or none of the contaminant associated with DOM is bioavailable.

Although the binding of T_4CDD is related to the concentration of DOC the composition of the DOC can be an important factor. Morehead et al. (1986) found that the DOC partition coefficient (K_{rp}) for PAHs ranged over several orders of magnitude in different natural waters and appeared to be most affected by the source of the DOM. Landrum et al. (1987) found that the site of collection of sediment interstitial waters was a more important factor than the DOM concentration for the uptake of PAHs by amphipods. At least part of the variability of binding and uptake rates of T_4CDD with DOC concentration may be due to variability in the composition of the DOM. Only a small fraction of the DOM may be responsible for the binding of T_4CDD to DOM. Binding of nonionic hydrophobic organics can be viewed as a partition-like interaction between the solute and an intra-molecular nonpolar environment of the organic matter (Chiou et al. 1986). The cosolute (DOM) molecule must be large enough that it will allow a nonpolar environment to exist within it. Chiou et al. (1986) found that the solute partition coefficient of soil derived humics acids was 4 times greater than with soil fulvic acids and 5 to 7 times greater than aquatic humic and fulvic acids and concluded that the ability of DOM to enhance the solute solubility appears to be controlled by both the DOM molecular weight and polarity. Melcer and Hassett (1986) have shown that humic substances from different origins, (i.e., soils, sediments

and water column) differ considerably in their physical and chemical characteristics. In the present study, the T_4CDD generally had a greater affinity for the DOM in the intersitial waters relative to that in the epilimnetic waters as indicated by an increase in K_{rp} (Table 14). This may be due to the DOM in epilimnetic waters being composed of more recently created organic matter while the sediment interstitial waters are likely composed of more reduced, larger molecular weight organic material which has gradually accumulated from the water column.

Aldrich humic acid had a greater effect on the solubility enhancement than DOM from natural waters. Other workers have also found that Aldrich humic acid has a greater affinity for hydrophobic organics than other aquatic humics (Carter and Suffet 1982; Landrum et al. 1985; Choiu et al. 1987). Commercially available humic acids are generally not characteristic of aquatic humics. Aldrich humic acid is more characteristic of the less polar, high molecular weight soil derived humic acids (Malcolm and MacCarthy 1986). Although Aldrich humic acid caused a greater solubility enhancement of the T_4CDD, it had a similar effect on the T_4CDD to DOM from natural waters in terms of the fraction bound and the effect on the uptake rate constant in biota. This may indicate that at least a fraction of the T_4CDD is loosely associated with the DOM and is available to the reverse-phase column and to biota. However, Landrum et al. (1985) have shown that Aldrich humic acid has a greater influence on decreasing the uptake rates of PAHs than natural waters. Caution should be used when extrapolating partitioning and uptake data obtained using commercially available humic acids to natural environments.

In general the results obtained for the T_4CDD should be applicable to other tetrachlorinated dioxins including the more toxic 2,3,7,8-T_4CDD isomer.

The physical/chemical properties of 1,3,6,8-T_4CDD are similar to 2,3,7,8-T_4CDD (Table 10). Although the BCF for 2,3,7,8-T_4CDD is higher than for 1,3,6,8-T_4CDD, this may be due primarily to biotransformation of the the latter congener, especially in the presence of 2,3,7,8-T_4CDD (Muir et al. 1985d; 1986; Adams et al. 1986; Kuehl et al. 1985; 1987b). Metabolism of 1,3,6,8-T_4CDD in this study was assumed to be minimal (based on the elimination rates), partly due to the short exposure times (Fig. 15). Steric factors may also be involved in reducing the BCF of 1,3,6,8-T_4CDD relative to the more planar 2,3,7,8-T_4CDD structure. 2,3,7,8-T_4CDD may vary from the results obtained for 1,3,6,8-T_4CDD in this study but the general trends are expected to be similar. The BCF for 2,3,7,8-T_4CDD has been reported to be less than predicted by its water solubility but these studies have not considered the effect of DOM on reducing the bioavailability of 2,3,7,8-T_4CDD to fish or invertebrates (Mehrle et al. 1988; Kleeman et al. 1986a; Adams et al. 1986; Kuehl et al. 1987b). Based on the results of this study the true BCF for 2,3,7,8-T_4CDD may be considerably higher than reported. The environmental mobility of 2,3,7,8-T_4CDD may also be influenced by DOM enhancing its apparent water solubility. Future studies should attempt to measure the truly dissolved concentrations and report the quality of the water used including the DOM concentration.

CHAPTER IV

The Chemical Limnology of Polychlorinated Dibenzo-p-dioxins in Lake Enclosures: 1. Environmental Fate

Polychlorinated dioxins (PCDDs) are a large group (75 congeners) of hazardous environmental contaminants. Sources of PCDDs include incineration of municipal and industrial waste, chlorophenols, pesticides derived from chlorophenols (e.g., 2,4-D, 2,4,5-T) and automobile exhaust (Czuczwa and Hites 1986; Yasuhara et al. 1987; Cochrane et al. 1980; Ballschmiter et al. 1986a). PCDDs are found in remote lake sediments indicating they are ubiquitious environmental contaminants (Czuczwa and Hites 1986; Czuczwa et al. 1984). PCDDs are also extremely toxic and have a tendency to bioaccumulate in the environment. Twenty-eight day LC_{50}s for 2,3,7,8-T_4CDD in fathead minnows are less than 63 ppt and BCFs are as high as 39,000 (Adams et al. 1986; Mehrle et al. 1988). Although chlorinated dioxins other than 2,3,7,8-T_4CDD are less toxic (Esposito et al. 1980; Barnes et al. 1986) and have lower BCFs (Muir et al. 1985a) they are produced and found in large quantities in the environment (Czuczwa and Hites 1986). The concentration of PCDDs found in sediments from Great Lakes and other aquatic environments generally increases with the degree of chlorine substitution (Czuczwa and Hites 1986; Ballschmiter et al. 1986a; Hagenmaier et al. 1986). The concentrations of PCDDs in surficial sediments (0-1 cm) in Lake Huron range from <1 pg/g for total tetrachloro congeners to as high as 1300 pg/g for octachlorodibenzo-p-dioxin (O_8CDD) (Czuczwa and Hites 1986). PCDDs other than 2,3,7,8-T_4CDD therefore also present a potential hazard in the environment. However, very little effort has been spent on

understanding the environmental dynamics of these less toxic PCDDs. In this study the environmental fate of a 1,3,6,8-tetrachloro- (T_4CDD) and the octachlorodibenzo-p-dioxin (O_8CDD) was investigated in aquatic mesocosms.

T_4CDD was selected for study because it is a dominant tetrachloro isomer found in incinerator fly ash (Yasuhara et al. 1987; Kuehl et al. 1985) and is also a contaminant of various 2,4-D ester formulations, and diphenyl ether herbicides (Cochrane et al. 1980; Tamagishi et al. 1981). T_4CDD has similar chemical/physical characteristic to the other tetrachlorinated isomers, including the more toxic 2,3,7,8-T_4CDD (see Tables 1 and 6). 1,3,6,8-T_4CDD should therefore be representative of the tetrachlorinated congeners for environmental fate purposes. The study of this less toxic congener can give insights into the environmental chemistry of 2,3,7,8-T_4CDD which because of its extreme toxicity is difficult and hazardous to study. O_8CDD is the most lipophilic PCDD congener and it is one of the most abundant congeners in both fly ash and pentachlorophenol (Czuczwa and Hites 1986). O_8CDD is also the dominant PCDD congener detected in air particulates and lake sediments (Czuczwa and Hites 1986). The physical and chemical properties of these two PCDDs have been reported in the literature (Friesen et al. 1985; Burkhard and Kuehl 1986; Rordorf et al. 1986; Choudhry and Webster 1988; Table 5) and both congeners have been previously studied in small ponds (Corbet et al. 1988; Marcheterre 1985). The objective of this study was to examine and compare the environmental behaviour of these two PCDDs which represent the extremes in the physical/chemical properties of environmentally significant PCDDs under natural field conditions simulating lakes. Examination of the environmental dynamics of PCDDs in aquatic mesocosms may lead to a better understanding of the environmental distribution of PCDDs in the Great Lakes and other contaminated environments.

MATERIALS AND METHODS

Chemical Analyses

Uniformly ring labelled ^{14}C-T_4CDD and ^{14}C-O_8CDD were purchased from Pathfinder Laboratories (St. Louis, MO) and had specific activites of 24.16 and 20.58 mCi/mM respectively. Both congeners were determined to be >99.8% radiochemically pure by HPLC using conditions described in detail below. Stock solutions of 3.92 mg were made up in 50 mL of tetrahydrofuran. Tritiated water was supplied by New England Nuclear (Boston, MA).

^{14}C and ^{3}H activities were determined by diluting environmental samples or extracts with scintillation fluor (Atomlight, New England Nuclear) and assaying by LSC. ^{14}C and ^{3}H activities were determined simultaneously using a dual-label calculation routine on a Beckman LC 7500 with $H^{\#}$ automatic quench compensation (Beckman Instruments, Irvine, CA). Selected samples were combusted using a Packard 306 Sample Oxidizer (Packard Instruments Co., Downers Grove, IL). The $^{14}CO_2$ was trapped in 2-methoxyethylamine, diluted with scintillation fluor and assayed by LSC.

HPLC was performed on a Waters model 6000A solvent delivery system using a µBondapak C_{18} column (Waters Scientific., Milford, MA). The mobile phase wa methanol and 0.5 min fractions were collected and assayed by LSC. The T_4CDD and O_8CDD had retention times of 4.3 and 6.2 min respectively. All solvents were distilled in glass grade supplied by Caledon Laboratories (Georgetown, ONT).

Mesocosms

Five 5 m diameter x 2 m deep enclosures were constructed in the littoral zone of Lake 304 at the Experimental Lakes Area in Northwestern Ontario (Fig.

20; Johnston and Vallentyne 1971). Sediments collected from Lake 304 at 2 m had a clayey texture with 12% sand, 37% silt and 51% clay. Sediments were 25.2% total organic carbon and 2.4% total nitrogen. DOC in the sediment interstitial water was 28.8±3.6 mg/L. The sediment porosity (0-6 cm) was estimated to be 0.85-0.92 mL of H_2O/mL of sediment, assuming 1 g H_2O = 1 mL, inorganic sediment has a specific gravity of 2.6 g/cm^3 and organic sediment has a specific gravity of 1.2 g/cm^3. The water and surface sediment chemistry of Lake 304 has been described in more detail previously (Armstrong and Schindler 1971; Brunskill and Schindler 1971; Brunskill et al. 1971). The chemical and physical characteristics of the enclosures are summarized in Table 15 and Appendix B.

Enclosures were constructed of nylon-reinforced polyethylene supported by a tubular frame floated on the surface using styrofoam blocks inside a pocket sewn into the top edge. The tube was sunk into the soft bottom sediments using heavy chain and angle iron held inside a fold sewn into the bottom edge. A SCUBA diver then pushed the bottom of the corral into the sediments as far as possible, i.e., at least 30 cm. The enclosures were constructed and put into place May 8-11 and allowed to equilibrate for approximately 4 weeks.

On June 12, 1985 PCDDs were added to the mesocosms as a sediment slurry to simulate inputs occurring from runoff into the littoral areas of the lake. The stock solutions (3.92 mg in tetrahydrofuran) were transferred into 4 L glass bottles which contained approximately 0.5 kg wet weight sediment collected from the littoral zone of Lake 304 (2 m). The bottles were shaken repeatedly for 3-4 h prior to addition to the lake enclosures. Sediment (^{14}C) was mixed into the enclosures by slowly (over 15-20 min) pouring the sediment from the bottle into the prop wash of an electric outboard motor. Two

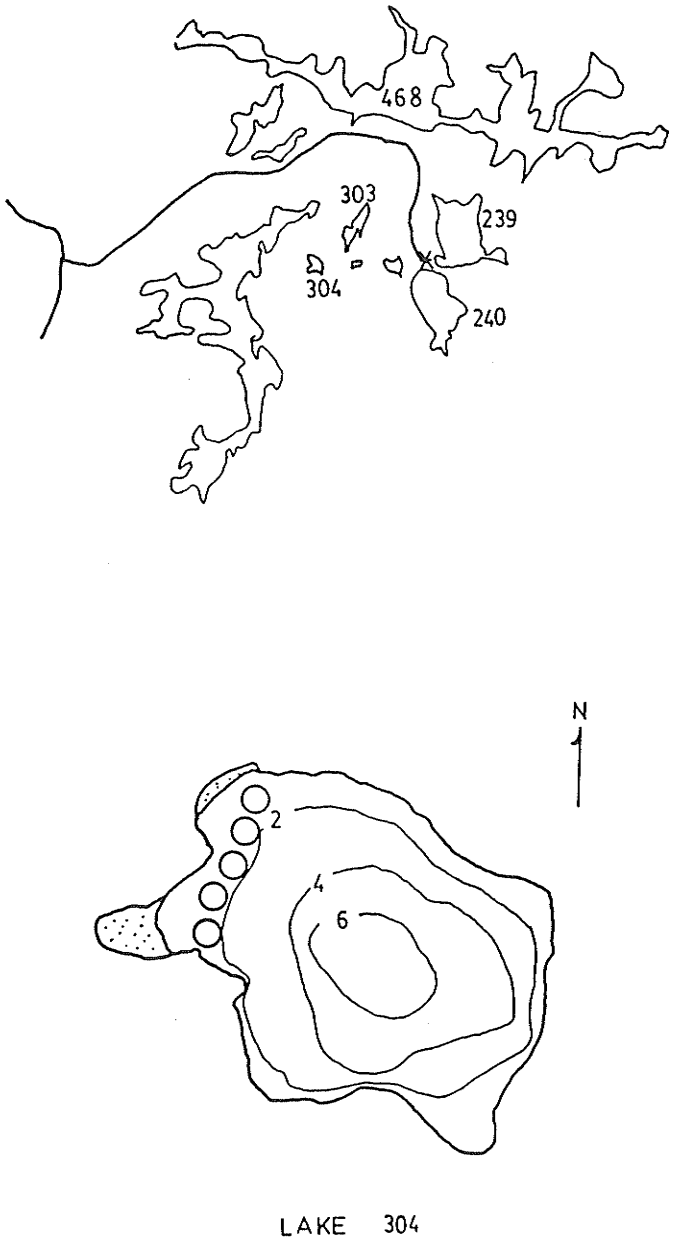

Figure 20. The location of enclosures in Lake 304, Experimental Lakes Area.

Table 15. The chemical characteristics of enclosure and lake water prior to the addition of polychlorinated dioxins (May 15 - June 11, 1985).

	enclosure		lake	
	mean±S.D.	N	mean±S.D.	N
Suspended P µg/L	11± 2	20	10± 2	6
Suspended N µg/L	125± 15	20	106± 8	6
Suspended C µg/L	1110±128	20	997± 67	6
DOC mg/L	7.8±0.6	20	8.9±0.3	6
Conductivity µmhos	19± 1	20	20± 1	6
temperature ^{o}C	16± 1	20	16± 1	6
alkalinity µeq/L	95± 4	4	85	1

enclosures were treated with the T_4CDD, two with the O_8CDD and one served as a control. A site approximately 10 m away from the enclosures served as a lake reference. Two mCi of tritiated water were added to each enclosure to determine leakage and to estimate the total volume at the beginning of the experiments. Four mL water samples (N = 8) were taken from each enclosure on day 1, assayed by LSC and the volume of each enclosure estimated as:

$$\text{Volume } (m^3) = \text{mCi added} / \text{mCi}/m^3 \qquad [12]$$

Water

A 4 L water sample was collected from the centre of each corral at 1 m depth at each sampling date. One L (days 0 - 9) or 4 L of unfiltered water was extracted twice with 200 mL of dichloromethane (DCM) in either a 2 L shake flask or a 4 L bottle with constant stirring for 30 min. Extracts were dried on approximately 50 g of anhydrous Na_2SO_4, rotoevaporated to 1 mL and subsamples counted by LSC. Selected extracts were also assayed by HPLC as described above. The extraction efficiency was determined (on day 1) to be 78±8 and 77±5 % for the T_4CDD and O_8CDD, respectively.

During the first 72 h additional water samples were also taken to determine the partitioning of PCDDs among POM, DOM and that truly dissolved in solution (free). The term "free" refers to the solute in true solution and not associated with other components in the aqueous phase such as DOM (Landrum et al. 1984). Replicate 4 mL samples of whole water were counted directly by LSC. Replicate 25 mL whole water samples were centrifuged for 30 min at 20000g in Corex glass centrifuge tubes. A 4 mL aliquot of the supernatant was assayed directly by LSC to determine the proportion of radioactivity "in solution". The proportion of radioactivity associated with the DOM was

determined using a method described by Landrum et al. (1984). A 4 mL aliquot of the supernatant was passed through a reverse-phase cartridge (C_{18} Sep-Pak, Waters Scientific), and the eluant assayed by LSC. The PCDD associated with the DOM will partition to the C_{18} and remain on the column (Landrum et al. 1984). Half-lives of PCDDs in water and other compartments were estimated by linear regression (SAS-GLM) assuming a pseudo-first-order kinetic rate model.

Surface films

The surface film was collected using a technique similar to that described Harvey and Burzell (1972). A 20 x 20 cm glass plate was lowered until it touched the surface of the water, then the plate was lifted and washed with DCM. DCM was reduced to 0.5 mL and aliquots of both the water and DCM were assayed by LSC. Glass plate samplers collected a 0.56±0.04 mm layer of water.

Particulate organic matter >100 µm (zooplankton)

A 30 cm diam. Wisconsin net (100 µm) was pulled vertically through the top 1.5 m of each enclosure, washed in the adjacent lake, transferred to glass jars and frozen at -50°C for later analysis. Samples were filtered through #40 ashless Whatman filter (Whatman, Maidstone, England) and combusted on a sample oxidizer.

Periphyton/polyethylene

Strips of the polyethylene wall material 0.0025 x 2 m were covered with packing tape on one side. The strips were hung from the north side of each enclosure 3 weeks prior to the "spike" date. On each sampling date two strips were collected by rolling the strip under the surface of the water while

removing the tape backing. Each strip was separated into 0.5 m sections. Samples were transferred into separate glass jars and frozen until analyzed. The strips were scraped with a glass slide and the periphyton collected on #40 ashless Whatman filters and combusted on a sample oxidizer. After day 14, periphyton was collected directly from the walls by scraping the wall with glass slides in the field.

On the "spike" date, additional polyethylene strips were added to each enclosure. On each sampling date these strips were collected as described above. The strips served as a comparison for the effect of colonization on the sorption of PCDDs. Duplicate 2 cm pieces of polyethylene were cut from the centre of each section of the strips collected (i.e., 0.25, 0.75, 1.25 m depth). The polyethylene pieces were placed into scintillation vials diluted with scintillation fluor and assayed directly by LSC. Because there was no correlation of ^{14}C concentration with depth, all samples on each date were pooled for comparisons.

Sediment

Jars: Three days prior to the spike date, sediment collected from Lake 304 at a depth of 2 m, using an Ekman dredge, was placed into 7 cm diam. x 8 cm deep glass jars such that each jar was approximately 2/3 full. Jars were lowered into each enclosure attached to strings so that they could be easily retrieved without disturbing the sediments. On each sampling date (up to 24 d) jars were collected and immediately capped and frozen until analyzed. Sediments were freeze-dried, then throughly mixed and a 3 g aliquot refluxed in 1:1 hexane:acetone for 24 h. The slurry was filtered through a Whatman GFC filter and the eluant rotoevaporated to approximately 10 mL, further reduced to 2 mL

under a stream of N_2 and then an aliquot assayed by LSC. Aliquots of the extracts were also analyzed by HPLC to determine the percentage of each congener remaining as the parent compound. Triplicate samples (approximately 0.10 g) of both the extracted and unextracted sediments were combusted on a sample oxidizer.

Recovery of the T_4CDD and O_8CDD spiked onto dried sediments was 77.6±5. and 78.8±5.2% respectively. To determine if there were significant losses of PCDDs during freeze-drying, 10 wet sediment samples (0.5 g of sediment filtered through a Whatman GFC filter) were spiked with each congener. Half of the samples were freeze-dried then combusted and the other half were combusted directly. Mean recoveries for TCDD and OCDD after freeze-drying were 103±3 and 109±11% respectively.

Cores: Duplicate core samples of the sediment were taken with a 5 cm diam. KB Corer. Water was siphoned off, then the sediment pushed up the core tube and the 0-6 cm fraction collected. Sediments were treated and analysed as described for sediment jars. Fewer sediment-core samples were collected during the first 24 d than sediment-jar samples, and only a single core was taken from each enclosure on day 3 to reduce the probability of disturbing the water column.

Profiles: Sediment core samples were collected as described above but the sediments were cut into 2 cm slices and transferred into glass jars. To avoid contamination from the layer above, a 2.5 cm core was taken of the inside of each core slice. Samples were transferred into 25 mL Corex glass centrifuge tubes and centrifuged for 15 min at 20000g and the supernatant taken off and recentrifuged for 30 min at 20000g. A 4 mL sample of the supernatant was

assayed for both ^{14}C and ^{3}H. The pellet was transferred back to the sample jars and frozen until analyzed. The sediment was freeze-dried and 2 subsamples (0.10 g) combusted on a sample oxidizer.

RESULTS

Total water concentrations (total ^{14}C) of the T_4CDD were higher than that of O_8CDD until day 2 but declined rapidly with a half-life of 2.6±0.2 days compared to a half-life for O_8CDD of 4.0±0.3 days (Fig. 21; Table 16). The concentration of O_8CDD remained higher than that of the T_4CDD after day 3 and until the termination of the experiment. During the first 48 h 10 to 15% of the T_4CDD and <1% of the O_8CDD were extractable by C_{18} Sep-Paks (Fig. 21). Approximately 10-30% of both PCDDs were associated with the DOC (unextractable with C_{18} Sep-Pak) while the remaining 75 to 90% was associated with POC (centrifugable).

The surface films contained both T_4CDD and O_8CDD with concentrations (total ^{14}C) that were 4 to 18 times higher than in the water column. The concentrations of the O_8CDD were 2-20 times higher than the concentrations of T_4CDD (Fig. 22). The concentrations of O_8CDD remained high (125-220 ng/L) for the first 4 d then declined rapidly with a half-life of 1.3±0.2 days.

The initial concentration (day 1) of the T_4CDD (total ^{14}C) in POM >100 µm (zooplankton) was 3.8 times higher than the concentration of O_8CDD (Fig. 23). However, the concentration of T_4CDD in POM >100µm declined rapidly with a half-life of 3.2±0.9 d. Concentrations remained between 20 to 67 ng/g dry weight after day 9 with the exception of day 54 when concentrations rose to 108-248 ng/g.

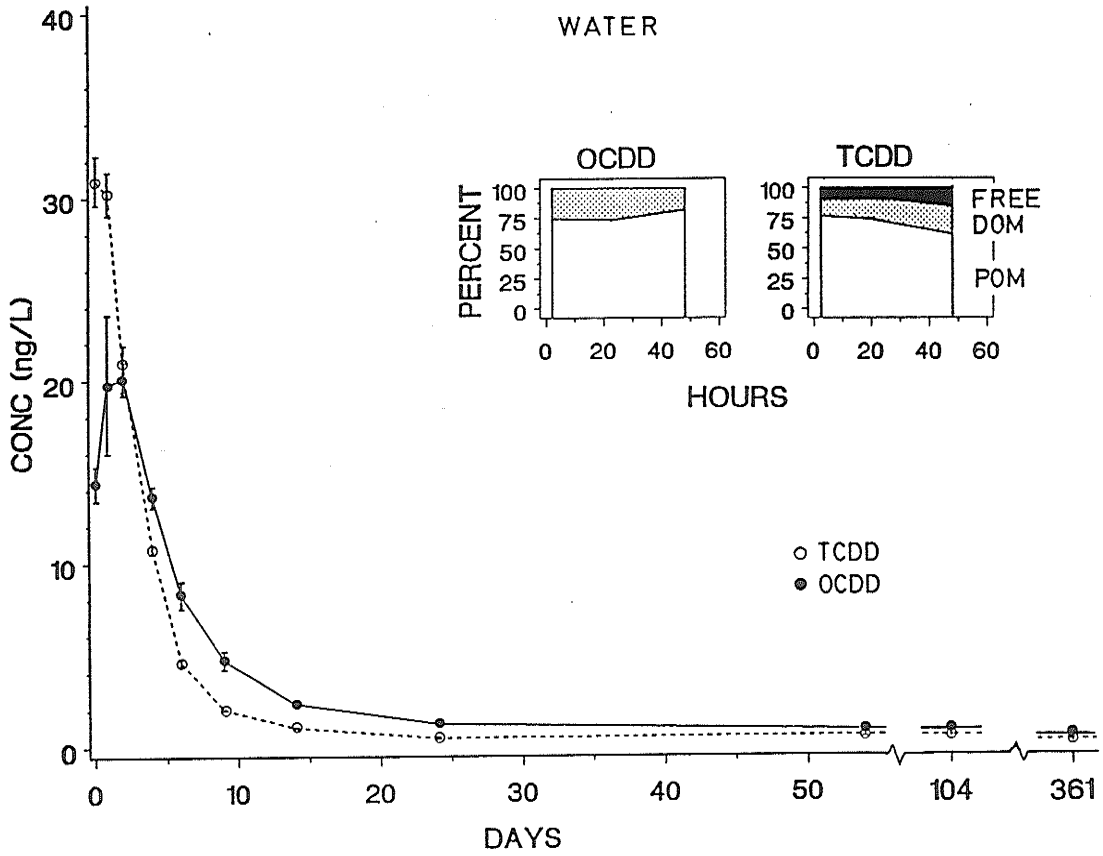

Figure 21. The total water concentration of PCDDs (extractable ^{14}C) in lake enclosures. Bars represent the range in the enclosure means. Nominal water concentrations were 59.0 and 58.4 ng/L for T_4CDD and O_8CDD respectively. Insert represents the percentage of the ^{14}C associated with particulate (POM) or dissolved (DOM) organic matter or in true solution (free).

Table 16. First order half-lives of total ^{14}C disappearance (days) in lake enclosures.

	O_8CDD		T_4CDD	
compartment	$t_{1/2}$	R^2	$t_{1/2}$	R^2
water	4.0±0.3	0.97	2.6±0.2	0.96
surface film	1.3±0.2	0.74	NF[1]	
POM>100µm	NF		3.2±0.9	0.96
periphyton	193±32	0.62	NF	
plastic/peri.[2]	NF		NF	
sediment/cores	NF		NF	
sediment/jars	NF		NF	

1. does not fit the model, i.e. slope is not significantly different from zero.
2. polyethylene strips

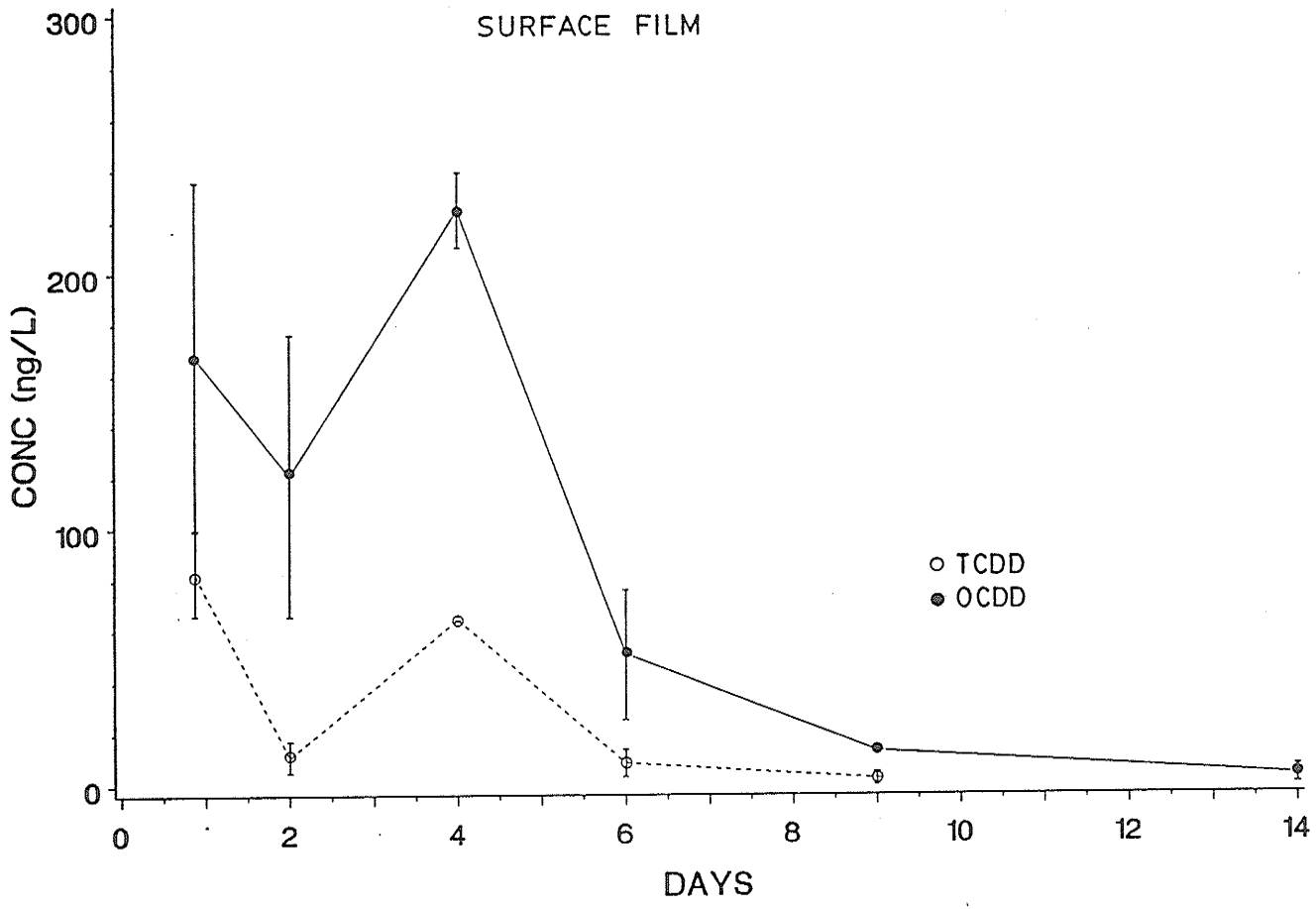

Figure 22. The concentration of PCDDs (total ^{14}C) in surface films in lake enclosures. Bars represent the range in the enclosure means.

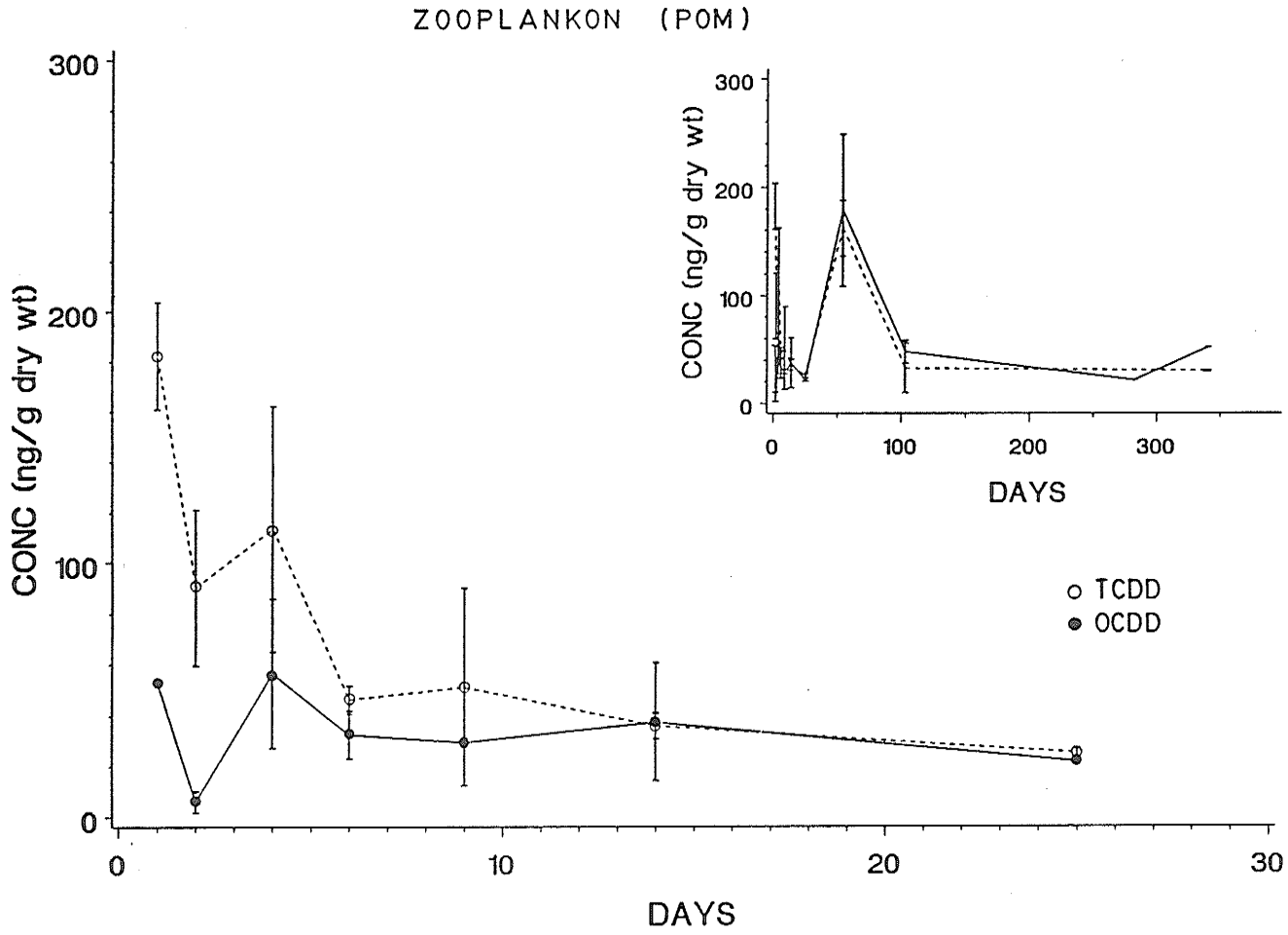

Figure 23. The concentration of PCDDs (total ^{14}C) in particulate organic matter >100 µm (zooplankton) in lake enclosures. Bars represent range in the enclosure means.

Unlike the POM, the concentrations of the T_4CDD and O_8CDD (total ^{14}C) in periphyton increased gradually with time until 14-103 d (Fig. 24). The concentrations of O_8CDD were as much as 25 times higher than that of T_4CDD. After day 24 the O_8CDD concentrations in periphyton declined slowly with a half-life of 193±32 d. The concentrations of T_4CDD and O_8CDD in the colonize polyethylene were up to 6400 and 65 times, respectively, the concentrations in the periphyton (Fig. 25). The concentration (ng/cm^2) of the T_4CDD in the uncolonized polyethylene (i.e., placed in enclosures on day 0) was considerably higher than that from the colonized polyethyene, but this trend was less obvious for O_8CDD.

Although the sediment concentrations (total ^{14}C) measured in jars were highly variable, the mean concentrations were relatively constant over the 25 d sampling period (Fig. 26). However, the concentrations measured in sediments from jars were approximately 4 times higher than those measured using cores (Fig. 27). The sediment concentrations appeared to increase slightly over the first few days then remain relatively constant over two years. Analysis of sediment extracts by HPLC indicates that all (>99%) of the T_4CDD remained as the parent compound even after two years. Interpretation of the HPLC chromatograms for O_8CDD were difficult. All O_8CDD chromatograms showed a broad peak of activity between 3 to 5 min. This peak was eliminated if the sample was cleaned up using a silica Sep-Pak and eluted with 5 mL hexane but recovery from the Sep-Pak was only 40%. Clean up of the extracts using a C_{18} Sep-Pak and eluting with 5 mL methanol resulted in the same HPLC chromatogram. When this broad peak was collected, concentrated, and reinjected into the HPLC, it had the same retention time, i.e., 3-5 min. Shaking the extracts with concentrated sulfuric acid reduced the percentage of ^{14}C in the broad peak but did not eliminate it. When this peak was collected

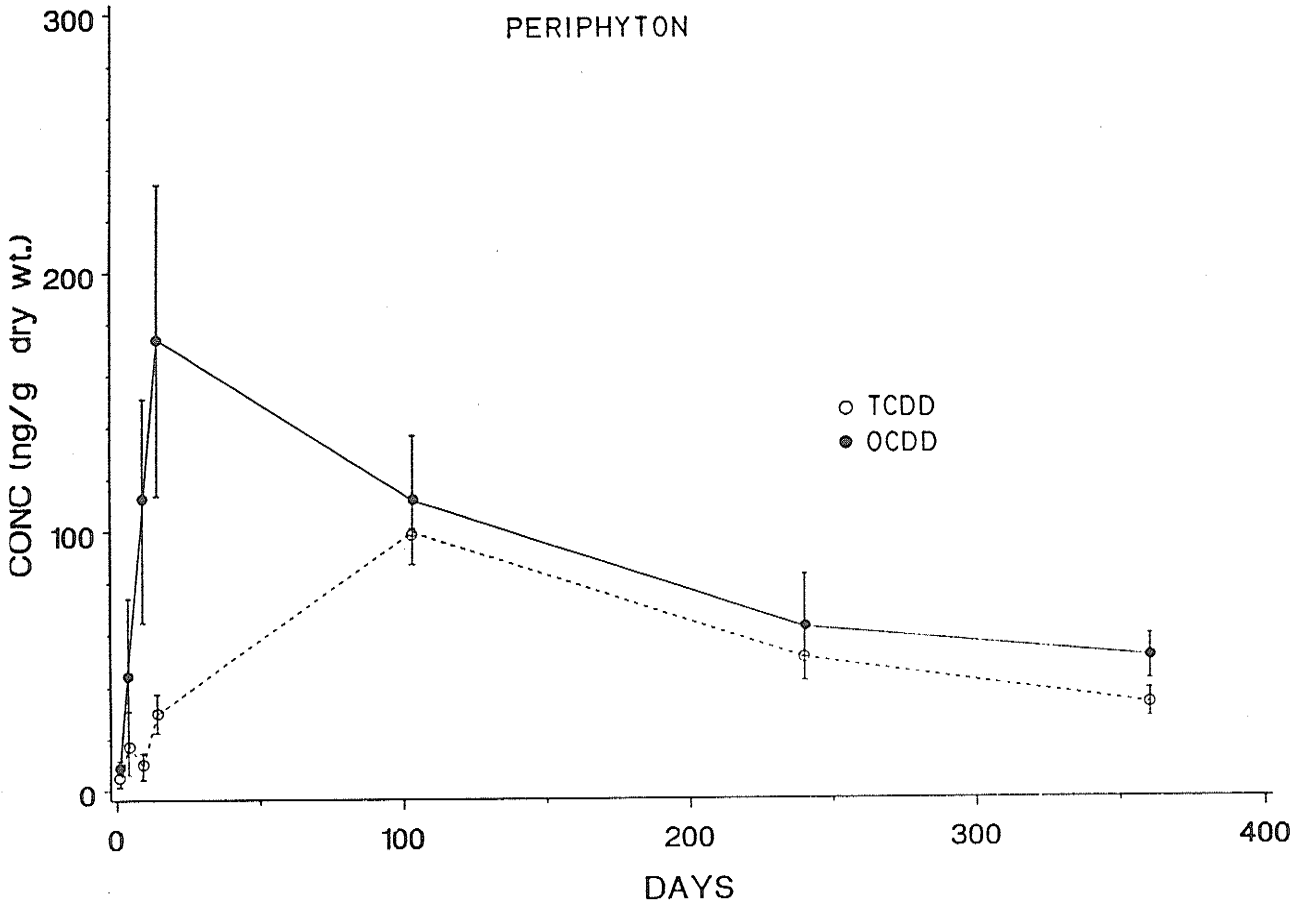

Figure 24. The concentration of PCDDs (total ^{14}C) in periphyton in lake enclosures. Bars represent the range in the enclosure means.

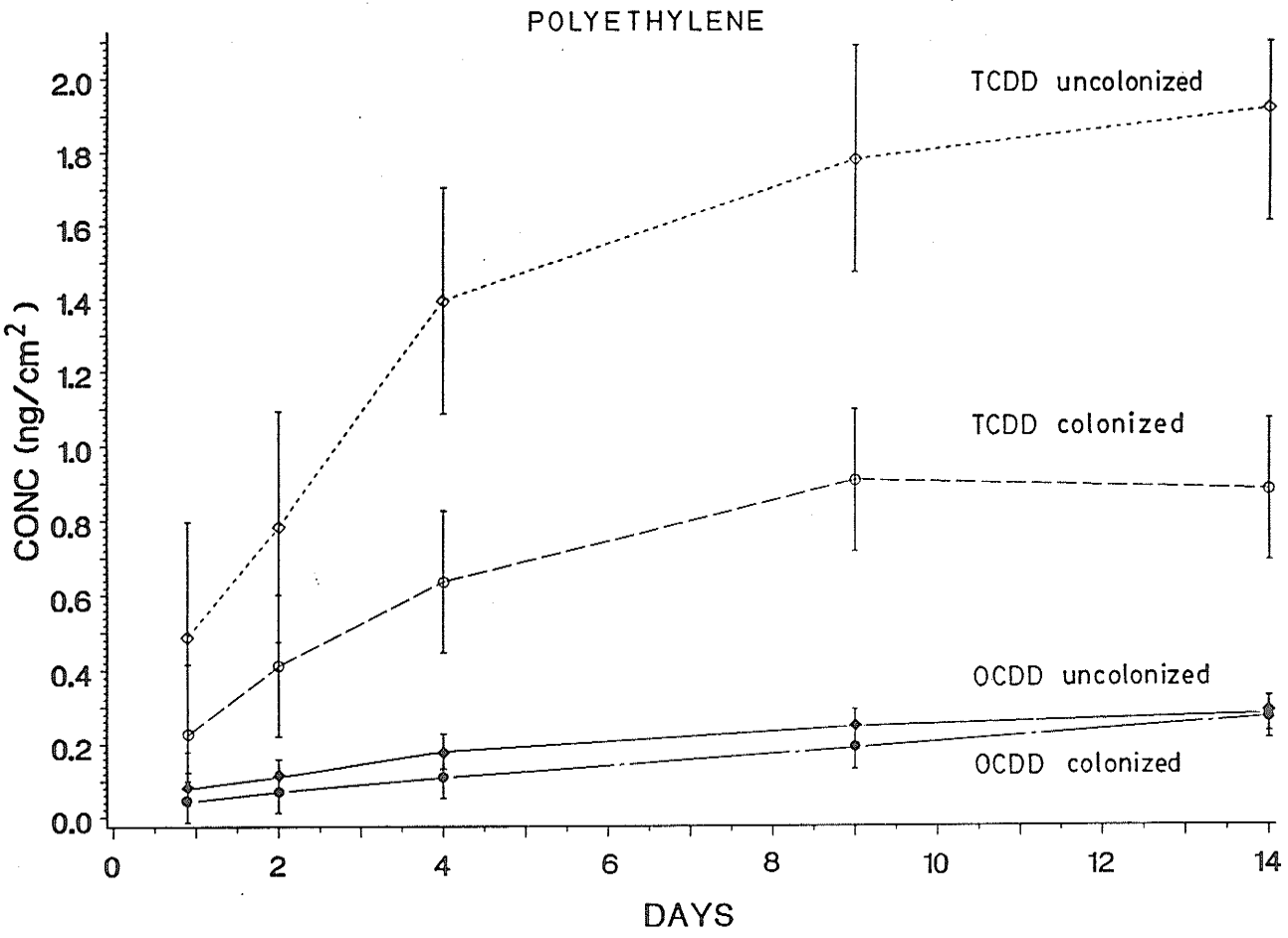

Figure 25. The concentration of PCDDs (total ^{14}C) in polethylene wall material in lake enclosures. Bars represent the range in the enclosure mean

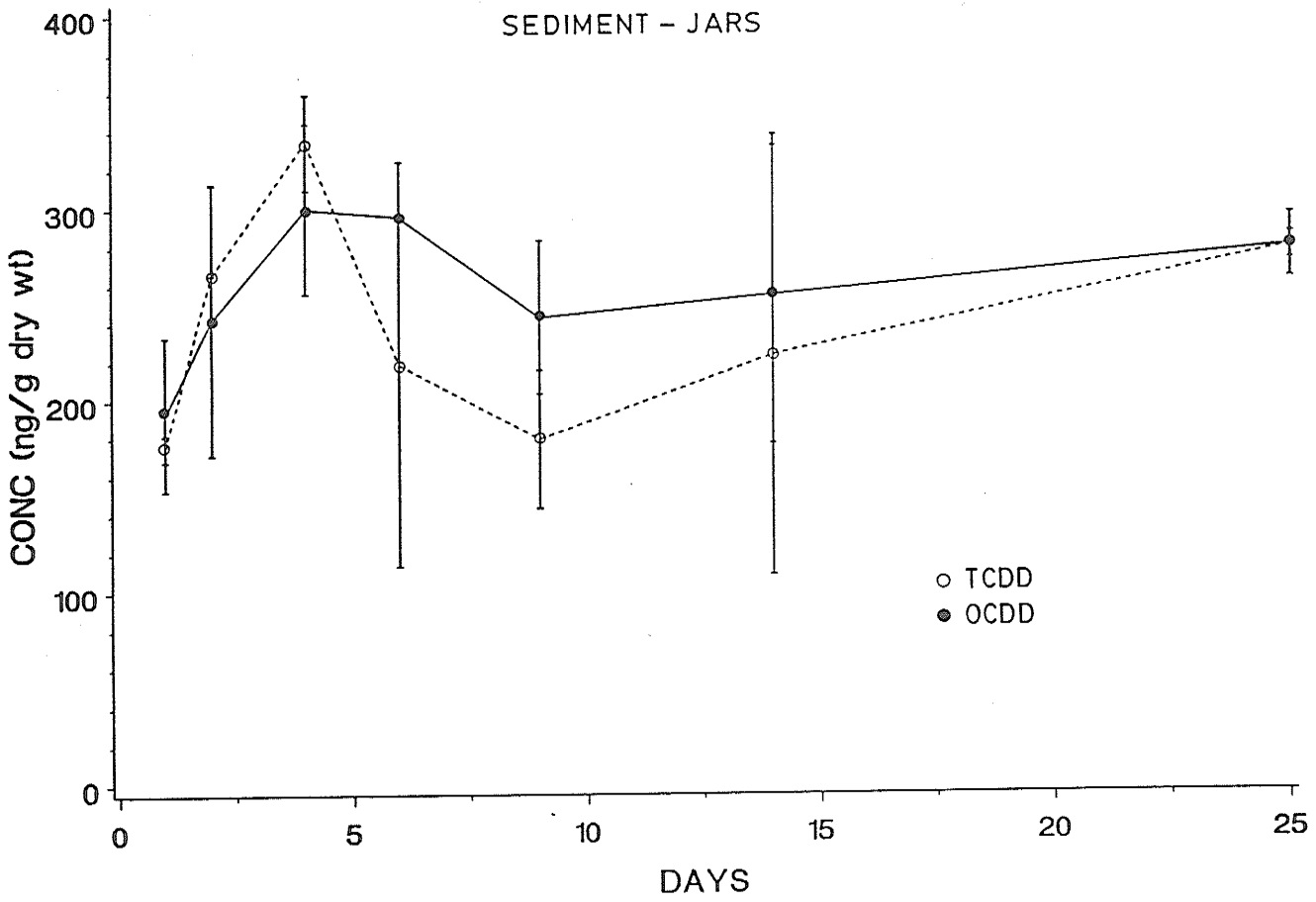

Figure 26. The concentration of PCDDs (total ^{14}C) in sediment collected using jars in lake enclosures. Bars represent the range in the enclosure means.

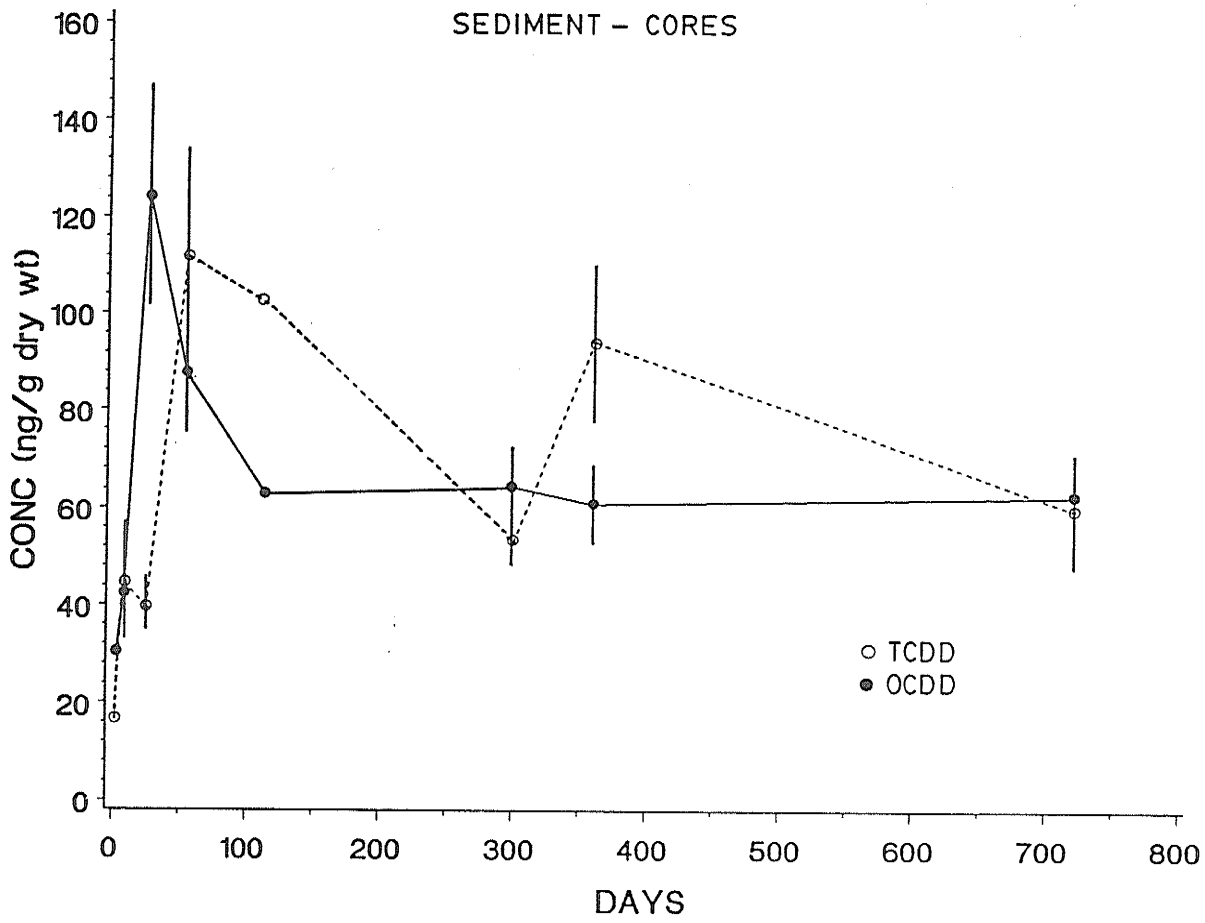

Figure 27. The concentration of PCDDs (total ^{14}C) in sediment collected using cores (0-6 cm) in lake enclosures. Bars represent the range in the enclosure means.

and analyzed by capillary gas chromatography (GC-ECD) an O_8CDD peak was not detected. However, if this broad peak was collected, shaken with concentrated sulfuric acid, then passed through a normal phase Sep-Pak and eluted with hexane, an O_8CDD peak was detected by GC-ECD. When a sediment extract from the reference enclosure was spiked with O_8CDD, then chromatographed by HPLC, it gave a similar broad peak. This indicates that the broad peak of activity may be due to the O_8CDD interacting with other components in the sample to change its chromatographic characteristics. The ^{14}C activity corresponds to the appearance of colour in the fractions collected. Using HPLC it was not possible to determine if O_8CDD remained intact as the parent compound during the duration of this study although there is strong evidence that it did.

The ^3H_2O moved quickly into the interstitial waters reaching at least 10 cm within 3 d (Fig. 28). The ^3H activity was only slightly lower in the first 2 cm of sediment than in the overlying water column. The ^3H_2O continued to penetrate into the sediments reaching at least 24 cm by day 54. The ^{14}C moved into the sediments much slower than the ^3H_2O. By day 3, the ^{14}C had not moved below 2 cm in any of the enclosures, and by day 8, only a small amount was detected in the 2-4 cm slice. By day 25, the ^{14}C had penetrated down to a depth of 8 cm.

DISCUSSION

Both PCDD congeners quickly partitioned/settled from the water column to the surficial sediments (Fig. 21, 27). Although the T_4CDD is less hydrophobic than O_8CDD (log K_{OW} = 7.13 and 8.60 respectively; Burkhard and Kuehl 1986) it was removed from the water column faster with a half-life of 2.6±0.2 d

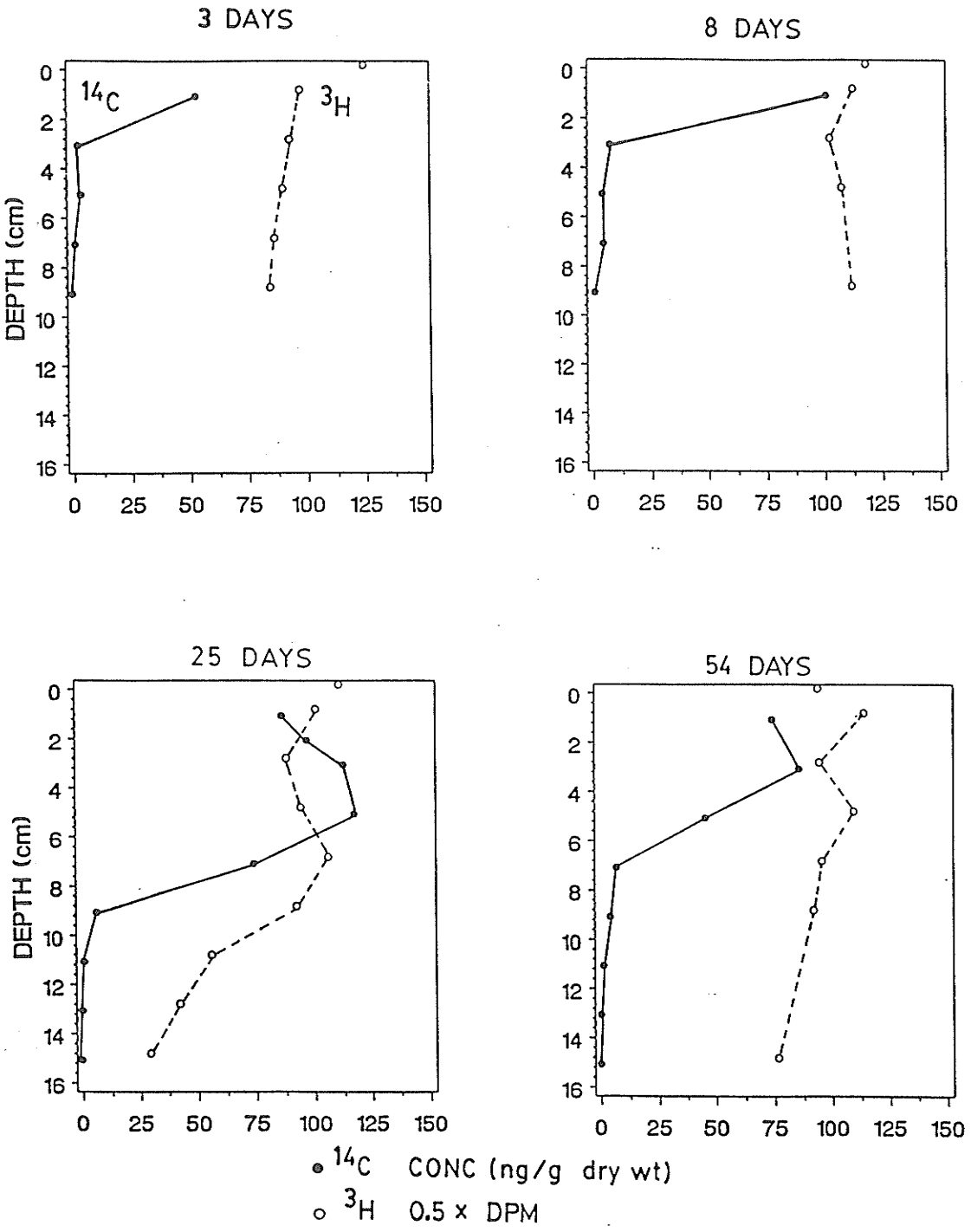

Figure 28. The profile of ^{14}C-O_8CDD and $^{3}H_2O$ in sediment cores from lake enclosures.

compared to 4.0±0.3 d for O_8CDD (Table 16). A similar trend was seen in pond studies where T_4CDD and O_8CDD had half-lives in the the water column of 0.6-1.2 and 1.6-1.9 d, respectively (Corbet et al. 1988; Marcheterre et al. 1985). The initial concentrations of the T_4CDD in unfiltered water were higher than that for O_8CDD as would be predicted by its higher water solubility (Friesen et al. 1985). However, the total concentrations of the O_8CDD in the water column after three days remained higher than that of T_4CDD even though the water solubility of O_8CDD is more than 3 orders of magnitude lower. This inconsistency may be explained by the sorption of PCDDs to DOM or fine POM which may have a longer residence time in the water column or be resuspended into the water column. DOM has been previously shown to enhance the apparent water solubility of PCDDs (Webster et al. 1986; Chapter I, III). In this study O_8CDD was almost totally bound to DOM or POM in the water column while 10-15% of T_4CDD was truly dissolved. This difference in the partitioning of PCDDs between POM, DOM and that in true solution is important in interpreting the bioavailability and fate in the various compartments of the environment (Fig. 29, 30).

Possible transformation of ^{14}C-PCDDs to more polar degradation products in the environment makes the interpretation of total ^{14}C results difficult. Although, no degradation products of ^{14}C-TCDD were detected in the water column (1-5 d) or the sediments (1-720 d) and there is evidence to indicate that ^{14}C-O_8CDD remained intact throughout the duration of the experiment, caution should be used when interpreting total ^{14}C values. For example, even small amounts of more polar degradation products in the water could lead to overestimates of the actual amount of PCDDs passing through the reverse-phase cartridges (therefore overestimating the truly dissolved concentrations). This problem is most acute for the O_8CDD congener. Although this does not

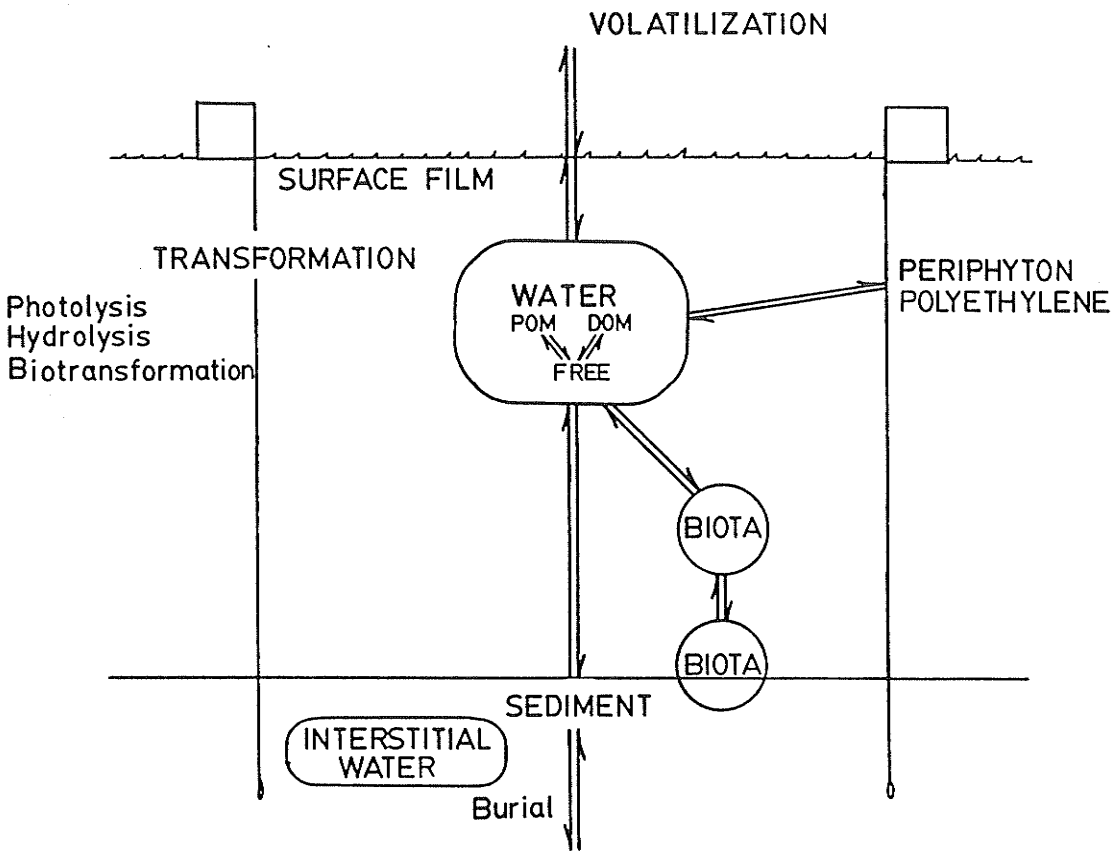

Figure 29. A schematic representation of the environmental compartments and reactions in lake enclosures.

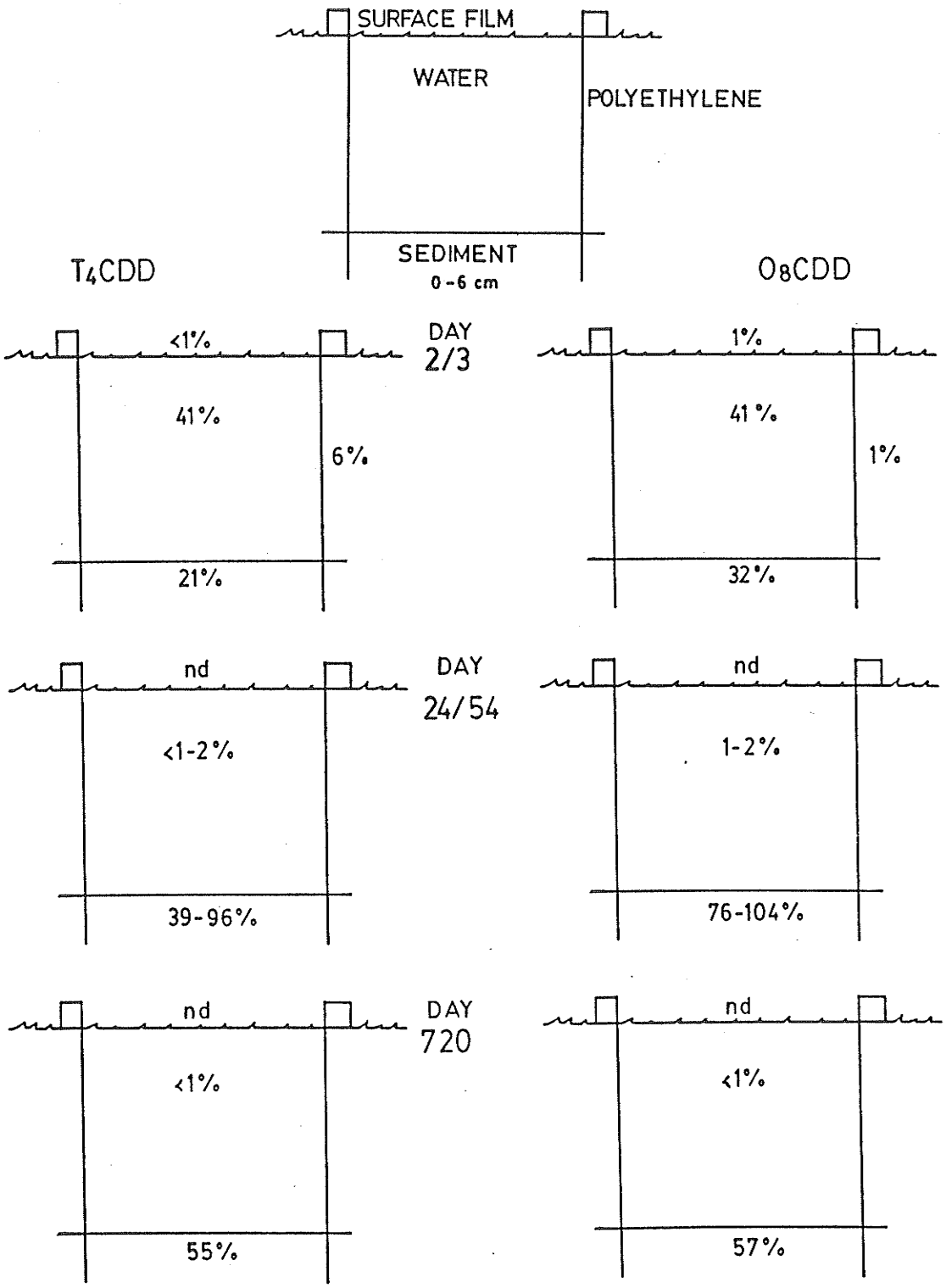

Figure 30. The distribution of total ^{14}C in lake enclosures

appear to be the case it is an alternative which can not be ignored.

Both PCDD congeners had elevated concentrations (extractable ^{14}C) in the thin organic films on the water surface (Fig. 21). The concentrations of the more hydrophobic O_8CDD were 2-20 times higher than those for the T_4CDD. The O_8CDD may preferentially partition into the hydrophobic environment of the surface films (Meyers and Kawka 1982) or be incorporated into these films as the hydrophobic organic matter spiked into the enclosures is incorporated into the surface films. Enrichment of surface films with hydrophobic organic contaminants relative to the water column has been reported in both marine and freshwater systems (Rice et al. 1982; Hardy and Kiesser 1988). The high concentrations of PCDDs found at the air-water interface in this study may have implications for prediction of the volatilization and photolysis of these compounds in lake environments. This effect of the surface films may depend on the extent to which PCDDs are bound to the organic matter within this compartment. The PCDDs in true solution may be more available for volatilization or direct photolysis, although PCDDs may also undergo photosensitized transformations in the sorbed state (Zafiriou et al. 1984; Zepp et al. 1985; Miller et al. 1987).

The higher concentrations of the T_4CDD (total ^{14}C) in POM relative to O_8CDD during the first 6 d might have been due to the higher concentrations of PCDDs truly dissolved in the water column which resulted in more rapid uptake of the T_4CDD by zooplankton (Fig. 23). Muir et al. (1985a) have shown that the apparent uptake rate constants for PCDDs, especially those for the more hydrophobic congeners (e.g., O_8CDD) are considerably lower than those predicted from their K_{OW} alone. In laboratory studies, sorption of PCDDs to POM and DOM reduced the apparent uptake rate constants (and therefore BCFs) in fish and invertebrates by reducing the amount of PCDD in true solution and

therefore bioavailable (Muir et al. 1985a; Chapter II, III). Although sorption to organic matter may reduce bioavailability, other factors such as steric hindrance may also be important (Opperhuizen et al. 1985; Gobas and Mackay 1987; Gobas et al. 1988). The concentration of the T_4CDD in POM (zooplankton) declined rapidly from 160-205 ng/g with a half-life of 3.2±0.9 but remained above the detection limits at about the same concentration as O_8CDD (20-67 ng/g). The low levels of PCDDs in net hauls may be due to suspended sediments in the water column resulting from the addition of the "spike" as a sediment slurry or resuspension of bottom sediments during sampling of sediments or biota. On day 54, all of the enclosures had a similar large increase in concentration to 108-248 ng/g. On this date there was a three-fold increase in the suspended carbon concentration detected in the water column of the enclosures relative to earlier in the experiment and the lake site (Appendix B). There was a 6 fold increase in the sedimentation rate in Lake 304 open water during this same period (unpublished data, J. Curtis, Freshwater Institute). This large increase may therefore be the result of a resuspension of sediments from wind action which affected the whole lake rather than the result of disturbing the bottom sediments during sampling.

Although the O_8CDD is more hydrophobic than the T_4CDD it was present at lower concentrations (total ^{14}C) in the polyethylene wall material (Fig. 25). The sorption of O_8CDD to POM and DOM makes it less available to the polyethylene even though the total water concentrations were consistently higher than T_4CDD after day 3. The explanation for this may be that when the polyethylene is colonized by periphyton (3 weeks), a more hydrophilic environment is created through which the PCDDs must diffuse before partitioning into the polyethylene. The concentration of the T_4CDD in the

colonized wall material was less than half of that in the uncolonized material on day 14. However, colonization of the wall material did not affect the concentration of O_8CDD in this compartment. The periphyton that could be scraped off of the polyethylene contributed less than 6% of the O_8CDD activity on the colonized wall material.

The high concentrations of O_8CDD relative to those of the T_4CDD in periphyton seem inconsistent with other results (i.e., polyethylene). It is possible that the concentrations of PCDDs in periphyton were due primarily to a physical phenomenon rather than to direct partitioning. The concentration of O_8CDD is not increased above that of the T_4CDD until after day 4 when water concentrations had declined below 15 ng/L. After day 2, the total water concentrations of O_8CDD were higher than those for T_4CDD and were primarily associated with particulate organic matter. The perphyton may have filtered out and trapped particles from the water column. Since the concentration of O_8CDD associated with particles was greater than that of T_4CDD, this might have led to the higher concentrations of O_8CDD in periphyton. As total water concentrations declined with time, the concentration of the two congeners in the periphyton became more similar but that for O_8CDD remained higher, as it did in the water column.

Both congeners moved rapidly into the sediment compartment (Fig. 26, 27). Sediment jar samples indicated that the sediment concentrations of the T_4CDD and O_8CDD were similar over the first 24 d with both congeners reaching a maximum concentration of approximately 300 ng/g dry weight by day 4 (Fig. 26). However, sediment core samples indicated that the sediment concentrations of both congeners peaked at approximately 120 ng/g dry weight by 24-54 d (Fig. 27). If sediment concentrations derived from jars are used to determine a mass balance, then sediments would contain as much as 4 times the amount of ^{14}C

actually added to the enclosures. The use of jars therefore appears to inflate the values for the sediment concentrations. The shape of the jars may result in over trapping or focusing of the suspended material (^{14}C) added to the enclosures. Sediment traps have been shown to collect an excess of material, especially under turbulent conditions (Hargrave and Burns 1979). This problem may have been exaggerated in this study because the PCDDs were added to the enclosures as a sediment slurry and mixed into the water column. Great care should therefore be used when interpreting any sediment data derived in this way (i.e., with jars).

Burial or movement of PCDDs into the sediments (>6 cm) was only minor during the first year of the experiment. $^{3}H_2O$ moved very rapidly into sediments below 10 cm by day 3 while the ^{14}C (most likely the intact congener) remained in the surface sediments (Fig. 28). Even after 54 d, very little of the ^{14}C had moved >6 cm into the sediment even though the $^{3}H_2O$ had penetrated down to at least 24 cm. The PCDDs apparently partitioned strongly to the highly organic (25.2% total carbon) sediments and remained at the surface. With time, the PCDDs (total ^{14}C) slowly penetrated into the sediments either by moving with the interstitial waters (free or sorbed to the dissolved organic matter) or by mixing of contaminated sediment particles (Anderson et al. 1987). The "apparent" K_p of T_4CDD was experimentally determined using Lake 304 sediments to be <10 L/kg at very high suspended sediment concentrations (i.e., $>10^4$ mg/L; Chapter I). Assuming a concentration in sediment of 120 ng/g dry weight and a K_p of 10 the estimated total concentration of T_4CDD in sediment interstitial water would be approximately 0.5 ng/L. Although no ^{14}C could be detected (<0.5 ng/L) in the interstitial waters (28.8±3.6 mg/L DOC) this does not preclude DOM as a means of transport of PCDDs into the sediments. DOM from Lake 304 interstitial waters has been

shown to inflate the apparent solubility of the T_4CDD (Chapter III). Sorption of PCDDs to DOM may increase their mobility in sediments.

Sediment core samples indicate little or no decrease in the sediment concentrations (i.e., total ^{14}C) of either congener over two years (Fig. 28). The sediment core data did not fit the first order kinetic rate model ($t>25$ d), i.e., the slope was not significantly different from zero. This may be due in part to the large variability among samples within each enclosure. No degradation products of ^{14}C-T_4CDD were detected using HPLC over the duration of the study. ^{14}C-O_8CDD likely also remained intact in the sediment although this could not be confirmed using HPLC.

Corbet et al. (1988) showed that T_4CDD in sterile and non-sterile pond water degraded at a similar rate ($t_{1/2}$ = 6.3 to 8.0 days) in sunlight but did not degrade under darkened conditions. Photolysis may have been an important pathway for removal of the T_4CDD from the enclosures even though no degradation products were detected in the water column during the first 5 d or in the sediments during the duration of the experiment. Corbet et al. (1988) reported two polar degradation products of the T_4CDD in sediment of small ponds by day 54 at 6.3 and 16% of the total ^{14}C. This difference may be due to several factors including the smaller depth (<1 m) and size (5 m^3), and lower organic carbon content of the sediment (3.5%) of the ponds relative to the lake enclosures. The low organic content of the pond sediments might have resulted in a slower movement from the water to the sediment compartment, therefore increasing the availability of the T_4CDD for biological and chemical interactions. The T_4CDD in these pond studies was added with acetone as a cosolvent at much higher nominal concentrations (viz., 980, 240, 98 ng/L). Because the T_4CDD in the present study was adsorbed to the sediments prior to being added to the enclosures, the amount free in solution and therefore

available to undergo direct photolysis or biotransformation was reduced. Quensen and Matsumura (1983) found that 2,3,7,8-T_4CDD in laboratory experiments was transformed only during the initial phases of the experiments. They suggested that this was due to micoorganisms which utilized the initally high concentration of truly dissolved 2,3,7,8-T_4CDD. This same trend was observed for TCDD in sediment/water incubations (Muir et al. 1985c). Muir et al. (1985c) reported a maximum of 7% of the 1,3,6,8-T_4CDD in these laboratory systems as polar metabolites over 675 days. This low rate of transformation in water/sediment systems observed in the laboratory is supported by these enclosure studies. A large proportion (94-104%) of both PCDDs could be accounted for as untransformed compound in the sediment on days 24-54 (Table 19). This suggests low rates of transformation and removal from the system.

A large proportion of both congeners (as total ^{14}C) remained in the sediments (55-81% T_4CDD and 57-58 O_8CDD) after one year. Corbet et al. (1988) reported less than 14.2% of the T_4CDD remaining in small ponds after 426 d and Tsushimoto et al. (1982) reported only 29.4% of 2,3,7,8-TCDD remaining in slightly larger ponds after 25 mo. A larger fraction (approximately 7.2 times given log K_p = 6.53) of the T_4CDD in the less organic pond sediments would be expected to be in true solution and therefore available for volatilization, photolysis, etc.. Although the truly dissolved concentrations would be very low, over two years this slight difference may be significant. In contrast to the results for T_4CDD, Marcheterre (1985) reported 61.8-88.2% of the O_8CDD remaining in small ponds (same site as Corbet et al. 1988) after 652 d. The higher retention of O_8CDD is expected because of its reduced availability in the water column. The fact that similar amounts of T_4CDD and O_8CDD could be accounted for in the enclosures may be due again to the high organic content of the sediments.

The fixed solution (equilibrium) of the National Research Council (NRC) Persistence model (Roberts et al. 1981) was run using an aquatic system simulating as closely as possible an enclosure used in this study (Table 17; Appendix E). The chemical parameters required to be entered into the model for each congener were collected or extrapolated from the literature (Table 17). The model integrates the many processes occuring in the environment and provides a framework from which to examine and interpret laboratory and field results.

The model predicts that both T_4CDD and O_8CDD will be predominately found in the sediments (>98%) at equilibrium (Table 18). The remainder is predicted to be found in the suspended sediments, while only trace amounts are predicted to remain truly dissolved in the water column. These predictions are supported by the field results. By day 24, >98% of the total ^{14}C detected in all enclosures was found in the sediments. Although 2 to 5% of the ^{14}C was found in the water column only a small fraction of the T_4CDD (10%) and the O_8CDD (<1%) in the water column was determined to be truly dissolved. The majority of the ^{14}C in the water column (2-48 h) was associated with POM (60-80%) and DOM (10-25%).

The predicted retentive capacities of T_4CDD and O_8CDD in the enclosures are 4 and 6000 years, respectively. Sorption of PCDDs to organic matter in the water column and sediments limits their availability, resulting in low removal rates (volatilization and photolysis) and long retentive capacities. Because the O_8CDD is less available in the water column, the predicted rates of removal are extremely low, resulting in a very long retentive capacity. The NRC Persistence model predicts that volatilization from the lake enclosures would be minimal. Although both Corbet et al. (1988) and Marcheterre et al. (1985) reported detecting intact PCDDs in the air above ponds, volatilization

Table 17. The system and chemical parameters used in the NRC Persistence model.

System Parameters		Chemical Parameters		
			O_8CDD	T_4CDD
latitude	49°N	molecular weight g/mol	459.8	321.9
mean depth	2 m	melting point °C	325.5	220
suspended solids	2 mg/L	log Kow[1]	8.60	7.13
sediment weight	30 kg	water solubility[2] ng/L	0.4	317
attenuation	low	vapour pressure[3] mm Hg	6.3E-12	2.7E-8
temperature	20 °C	quantum yield[4]	2.26E-5	2.17E-3
volume	67 m^3	bioconcentration factor[5]	387	2299
organic carbon	25.2 %	depuration[5] d^{-1}	0.02	0.08
fish weight	300 g	proportion biodegradable[5]	0.25	0.66
		hydrolysis[6] d^{-1}	0.0	0.0
		microbial trans.[7] d^{-1}	0.0	0.0

1. Burkhard and Kuehl (1986)
2. Friesen et al. (1985)
3. Rordorf, personal communication; Rordorf et al. (1986)
4. Choudhry and Webster (1988)
5. Chapter II
6. Miller and Zepp (1987)
7. Muir et al. (1985c), Miller and Zepp (1987)

Table 18. The fixed solution for the NRC Persistence model[1] predictions.

	Fractional Retention		Fractional Degradation		Retentive Capacity
T_4CDD					
	Water	<0.01	Volatilization	0.00	4 years
	SS[2]	0.02	Photolysis	1.00	
	Sediment	0.98	Hydrolysis	0.00	
	Biota	<0.01	Biota	0.00	
O_8CDD					
	Water	<0.01	Volatilization	<0.01	6000 years
	SS	0.01	Photolysis	>0.99	
	Sediment	0.99	Hydrolysis	0.00	
	Biota	<0.01	Biota	0.00	

1. Roberts et al. (1981).

2. Suspended solids

could account for only a small fraction of the ^{14}C lost (e.g. <0.5% of T_4CDD after 7 d). Direct photolysis is predicted to be the major degradative pathway for both T_4CDD and O_8CDD in lake enclosures. Corbet et al. (1988) showed that T_4CDD had a half-life of 6.3 d when exposed to sunlight in autoclaved pond water and concluded that photolysis was a major route of degradation in ponds. Although photolysis is predicted to be the major route of removal of PCDDs from the lake enclosures the actual rates of photolysis may only be significant during the first few days of the experiment when the truly dissolved concentrations are relatively high. The large quantities of both congeners which could be accounted for after 2 years (55-57%) indicates that removal rates are minimal (Table 19).

PCDDs are generally considered to be extremely persistent in sediment with half-lives >10 years (Miller and Zepp 1987; Muir et al. 1985c). The rates of transformation (microbial) in the sediment compartment are therefore usually considered to be zero. Because most of the PCDDs are associated with the sediments the predictions of the model are very sensitive to even small rates of transformation out of the sediment compartment. Even if the half-life of O_8CDD is assumed to be as high as 100 years, transformation in the sediment is the dominant degradative pathway and the predicted retentive capacity is reduced to 250 years. If the half-lives of PCDDs in sediment are assumed to be 10 years, the predicted retentive capacities are reduced to 3 and 13 years, for T_4CDD and O_8CDD, respectively.

Even if the PCDDs degrade in the sediment, burial may be the dominant process removing them from the aquatic system. If a sedimentation rate of 0.2 to 1.0 g m^{-2} d^{-1} is assumed (Lake 304 open water, unpublished data, J. Curtis, Freshwater Institute) then the PCDD could be buried below 6 cm within 4 to 20 years. Although sedimentation rates in the littoral zones of Lake 304 are

Table 19. Mass balance of ^{14}C in aquatic mesocosms[1].

	Percent accounted for									
	O_8CDD					T_4CDD				
time	surf. water	peri/ film	plast.	sed.	total	surf. water	peri/ film	plastic	sed.	total
2 h	30	-	-	-	-	64	-	-	-	-
2/3d	41	1	1	32	76	41	<1	6	21	70
8/9d	-	<1	3	42	-	-	nd	14	43	-
24 d	2	nd[2]	-	104	106	2	nd	-	39	41
54 d	1			76	78	<1			94	95
103d	<1			56	56	<1			81	81
271d	<1			58	58	<1			50	50
360d	<1			56	58	<1			81	81
720d	<1			57	57	<1			55	55

1. corrected for extraction efficiency and assuming 67 m^3 water, 20 m^2 surface area, 39 m^2 polyethylene and 30 kg sediment (6 cm).

2. not detectable

likely lower than in the open water, burial of PCDD in the sediments may be relatively rapid compared to removal by transformation or volatilization. Astle et al. (1987), used a fugacity-based model to predict the environmental dynamics of PCDDs in Lake Siskiwit and concluded that reaction rates would be small relative to the transport rates (sedimentation) of PCDDs to the sediment. Astle et al. (1987) also predicted that 14% of the tetra-CDDs and <1% of the O_8CDD in the water column would be truly dissolved in solution and therefore available for biological uptake or chemical reaction. These results indicate that future work should focus on determining the factors controlling the removal of PCDDs from the system by sedimentation (e.g., K_p) and eventual burial in sediments.

The NRC Persistence model (fixed solution) provides a simple first approximation of the environmental fate of PCDDs at equilibrium. The opportunity now exists to use the field data generated in this study for lake enclosures and previous data generated for ponds (Corbet et al. 1988; Marcheterre 1985) to calibrate more sophisticated (unsteady state) environmental fate models (e.g., EXAMS, Burns and Cline 1985; QWASI, Mackay et al. 1983). These two PCDDs (i.e., T_4CDD and O_8CDD) may be used as benchmarks from which to make predictions of the environmental fate of other superlipophilic compounds (e.g., PCDDs, PCDFs PCBs). An environmental fate model calibrated with field results will allow rapid evaluation of the behaviour of these pollutants in aquatic environments and provide a valuable tool with which to make sound management decisions.

Generally there is a shift in the congener pattern of PCDDs in environmental samples relative to the sources toward an enrichment of the more highly chlorinated congeners (Table 3, 4). It has been suggested that the enrichment of O_8CDD in surface sediments relative to combustion sources (i.e.,

flyash) may indicate that the less chlorinated PCDDs undergo chemical reactions (photolysis) in the atmosphere (Czuczwa and Hites 1986; Miller et al. 1987). Although experimental evidence indicates that photolysis of PCDDs is strongly retarded by flyash surfaces, this does not take into account that a large fraction of the PCDDs in the atmosphere exist in the vapour phase (Miller et al. 1987). Photolysis of 2,3,7,8-T_4CDD in the vapour phase is rapid, i.e., $t_{1/2}$ <1 h (Miller et al. 1987). Because the more highly chlorinated PCDDs have lower vapour pressures and higher affinity for organic matter, i.e., high K_{ow} (Rordorf et al. 1986; Burkhard and Kuehl 1986) they will likely be found sorbed to the organic particles in the atmosphere. The quantum yield of O_8CDD is also lower than the other congeners (Choudhry and Webster 1988). The enrichment of O_8CDD in the atmosphere may be due to a larger fraction of this congener being sorbed combined with reduced transformation (photolysis) on air particulates/flyash.

Although enrichment of the more highly chlorinated PCDDs in lake sediments may be due to higher inputs, selective retention of these congeners in aquatic systems may be a contributing factor. The lower availability of the higher chlorinated PCDDs for chemical or biological transformation in the water column and the rapid removal (sedimentation) to the sediments may lead to relatively higher retentive capacity of these congeners in aquatic systems. However, the observation that both congeners in the lake enclosure were retained in similar amounts indicates that the movement to the sediment is so rapid that surficial sediments directly reflect inputs into the system. The selective retention of higher chlorinated congeners combined with the higher atmospheric inputs of these congeners (Czuczwa and Hites 1986), may explain the pattern of increasing concentrations of PCDD congeners in sediment with increasing chlorine substitution observed in the Great Lakes and other aquatic

environments (Czuczwa and Hites 1986; Ballschmiter et al. 1986a; Hagenmaier et al. 1986a).

CHAPTER V

The Chemical Limnology of Polychlorinated Dibenzo-p-dioxins in Lake Enclosures: 2. Bioavailability.

PCDDs are extremely hydrophobic compounds which partition rapidly to organic matter in aquatic environments (Chapter I-IV). The concentration of PCDD congeners in sediments in most aquatic environments generally increase with increasing chlorine substitution (Czuczwa and Hites 1986; Ballschmiter et al. 1986a; Hagenmaier et al. 1986). This is likely caused by larger inputs and long retentive capacity of the higher chlorinated congeners (Czuczwa and Hites 1986; Chapter IV). However, this pattern is not directly reflected in the concentration of PCDD congeners found in aquatic biota (Stalling et al. 1983). There is no consistent pattern in the concentration of PCDD congeners found in biota except that 2,3,7,8-T_4CDD is often the most dominant congener (Rappe et al. 1987; Kuehl et al. 1987a). Other congeners, including O_8CDD, are also commonly found in aquatic biota (Stalling et al. 1983; Miyata et al. 1987a; Clement et al. 1987; Kuehl et al. 1987a).

A simple extrapolation of the relationship between K_{ow} and BCF described by Veith et al. (1979) predicts extremely high BCFs for the higher chlorinated PCDDs (10^5 to 10^6). However, several authors (Muir et al. 1985a; Bruggeman et al. 1984; Gobas and MacKay 1987; Chapter II) have reported a decline in the BCFs of PCDDs and other compounds with log K_{ow}s greater than approximately 6.5. This decline in BCF has been at least partly explained by sorption of the compound to POM or DOM in the test solution (Muir et al. 1985a; Chapter II). Sorption of PCDDs to organic matter and/or rapid sedimentation appears to

reduce their availability directly from the water. The extremely low concentrations of PCDDs in true solution and the relatively high concentrations in the sediments, especially for the more highly chlorinated PCDDs, may result in a detrital based food chain transfer rather than direct equilibrium partitioning from the water column.

The objective of this study was to examine the bioavailability of two PCDDs representing the extremes in the physical/chemical properties of the environmentally significant PCDDs, under natural field conditions simulating lakes. The bioavailability of two PCDD congeners, 1,3,6,8-tetrachloro- (T_4CDD) and octachlorodibenzo-p-dioxin (O_8CDD), were studied in large lake enclosures at the Experimental Lakes Area in Northwestern Ontario. The environmental fate of these congeners in these lake enclosures has been reported previously (Chapter IV). The concentration of the two isomers in caged benthic invertebrates and fish held in the water column or on the sediments were compared. A compartmental model developed by Thomann and Connolly (1984) was used to predict the relative importance of direct partitioning from water versus a detrital based food chain transfer in explaining the concentration of PCDDs found in biota.

MATERIALS AND METHODS

Five 5 m diam. x 2 m deep (40 m^3) enclosures were constructed in the littoral zone of Lake 304 in the Experimental Lakes Area in Northwestern Ontario (Johnson and Vallentyne 1971). The construction and chemical characteristics of the enclosures have been reported in more detail elsewhere (Chapter IV). On June 12, 1985, two enclosures were treated with ^{14}C-T_4CDD

(24.16 mCi/mM) and two with ^{14}C-O_8CDD (20.58 mCi/mM) at a nominal concentration of 58-59 ng/L. A fifth enclosure served as a control. PCDDs were added to the enclosures as a sediment slurry to simulate inputs occurring from runoff into the littoral areas of the lake.

Benthic invertebrates

Several species of benthic invertebrates were exposed in cages at the sediment-water interface for 10 day intervals several times during the experiment (viz., days 0-10, 14-24, and 94-104). Cages were made from 500 mL Nalgene bottles with 2/3 of the walls cut away and covered with 0.45 mm Nitex mesh. One species (Hyalella azteca) was also held in cages hung in the water column during the same time periods. Benthic invertebrates were collected from each bottle at the end of each exposure period and frozen. Benthic invertebrates were blotted dry with a paper towel, weighed then lyophilized. One to four organisms were pooled together then oxidized using a Packard 306 Sample Oxidizer (Packard Instruments Co. Downers Grove, IL). The ^{14}C was trapped in 2-methoxyethylamine, diluted in scintillation fluor and assayed by LSC (LC 7500, Beckman Intruments, Irvine, CA). The concentration of PCDDs in benthic invertebrates on each date was compared ($p<0.05$) using a two way analysis of variance (Kleinbaum and Kupper 1978).

Unionid clams

Unionid clams (Elliptio complanata) were collected from Lake 377 (ELA, Chapter III) and held for 2 wk before being placed in cages in the enclosures 1 h after the PCDD addition. In each enclosure, one cage was held on the sediment and a second cage was allowed to float at the surface. Cages were constructed with a wooden frame (0.6 x 0.6 x 0.9 m) covered 56% with 1 mm mesh

and 44% with 5 mm mesh (bottom and sides). Ten clams (16.4±5.3 g soft tissue wet weight) were collected on day 10 or day 24 as described above. The shell was removed from each clam and the soft tissues weighed and then lyophilized. Each individual clam was homoginized using a Polytron in 50 mL of toluene. Samples were centrifuged at 2000g for 10 min and the supernatant removed. A 1 mL aliquot was diluted with scintillation fluor (Atomlight, New England Nuclear) and assayed by LSC. The unextractable residue was air dried and a 0.2 g subsample combusted on a Packard Sample Oxidizer. Selected extracts were further assayed by HPLC with a Waters model 6000A solvent delivery system using a μBondapak C_{18} column (Waters Scientific, Milford, MA). The mobile phase was methanol (1.5 mL/min) and 0.5 min fractions were collected and assayed by LSC. The T_4CDD and O_8CDD had retention times of 4.3 and 6.2 min respectively. Extracts were shaken with one mL of concentrated H_2SO_4. The toluene was removed, rotoevaporated under a vacuum to <5 mL, further reduced to near dryness under a stream of N_2, and finally made up to 0.5 mL in chloroform. All solvents were distilled in glass grade supplied by Caledon Laboratories Inc. (Georgetown, ONT). The concentrations of PCDDs in clams held in the water column and clams held on the sediments were compared ($p<0.05$) using a two way-analysis of variance on each date (Kleinbaum and Kupper 1978).

Bioconcentration factors (BCF) were determined as:

$$BCF_a = C_b / C_w \qquad [13]$$

where BCF_a is the apparent bioconcentration factor, C_b is the concentration in biota (ng/kg) and C_w is the time weighted mean water concentration (ng/L). The predicted concentration in biota was determined by rearranging equation [13] and assuming a true BCF of 5017 for the T_4CDD and 705 for O_8CDD. These

BCFs represent the highest values reported in rainbow trout in synthetic water (Chapter II). Truly dissolved water concentrations were determined as the time weighted mean multiplied by the percentage in true solution determined during the first 48 h in Chapter IV, i.e., 15% for T_4CDD and 1% for O_8CDD.

Fish

White suckers (Catostomus commersoni) collected from a nearby lake were obtained from a local bait fisherman and held in Lake 304 for 48 h before being placed in the enclosures 1 h after the PCDD addition. Ten fish (12.5±3.6 g wet weight) were held in each of the cages described above (i.e., one on the sediment and one at the surface) and sampled on day 10 and day 24. The gills and the gut were removed from seven fish from each cage and each tissue (gill, gut, carcass) weighed, lyophilized and then reweighed separately. The carcass and gills were homogenized using a Polytron in toluene (15.0 or 4.1 mL respectively). Samples were centrifuged at 2000g for 10 min and the supernatant removed. A 2 to 5 mL aliquot was diluted with scintillation fluor and assayed by LSC. A single extraction removed greater than 90% of the extractable PCDDs. The extracted sample was air dried and the entire sample (gills) or a 0.1 g subsample (carcass) combusted on a Packard Sample Oxidizer. The gut samples were combusted directly without freeze-drying or extraction. Selected extracts were also assayed by HPLC as described above. The concentrations of PCDDs in fish held in the water column and on the sediments were compared ($p<0.05$) using a two way-analysis of variance on each date for each tissue (Kleinbaum and Kupper 1978).

Ten white suckers were also added directly into each enclosure and as many as possible retrieved on days 104-106 using small minnow traps. Fish were analyzed as described above. Tissue concentrations of PCDDs from fish

held in the water column or on the sediments were compared ($p<0.05$) using a two way-analysis of variance on each date (Kleinbaum and Kupper 1978).

RESULTS

The concentration of the T_4CDD in benthic invertebrates was higher (11.1-22.3 ng/g) than O_8CDD (<5.9 ng/g) on day 10 (Table 20). By day 24 the concentration of the T_4CDD in benthic invertebrates had declined to levels similar to those for O_8CDD (i.e., <4.3 ng/g). By day 371 the concentration of the T_4CDD in the one species examined was similar to other benthic invertebrates on day 24 while O_8CDD was not detectable. However, the detection limits were relatively high (0.6-3.0 ng/g) because of the small sample weights. Hyalella azteca held in the water column had significantly higher concentrations of the T_4CDD compared to those held on the sediments at the same time (Appendix F).

The unionid clams showed a similar trend in that the T_4CDD was 20-30 ng/g on day 10 and declined to concentrations similar to those of O_8CDD by day 24 (i.e., 0.2-2.0 ng/g) (Fig. 31). Clams exposed in cages held in the water column versus sediments had slightly higher mean concentrations (on day 10 and 24) but the results were not significantly different ($p>0.05$). All of the ^{14}C found in clams was extractable in toluene and HPLC analysis indicated that >95% of the ^{14}C-T_4CDD remained as the parent compound.

The relative concentration of PCDDs in caged fish also showed a similar trend (Fig. 32). The concentration of the T_4CDD in both the gills and carcasses of fish were significantly higher than those of O_8CDD on day 10 but declined to concentrations similar to those of O_8CDD by day 24. The

Table 20. The concentration of PCDDs in caged benthic invertebrates from lake enclosures (ng/g ±S.D.).

		T_4CDD mean (S.D.)	N	O_8CDD mean (S.D.)	N	detection limit
DAY 10 (0-10)						
Hyalella azteca	water	22.9 (8.9)	12	3.2 (1.7)	10	2.0
	sediment	11.1 (4.2)	13	nd[1]	9	2.0
Limnephilus sp.		14.3 (3.9)	9	5.9 (2.5)	8	1.5
Ephemerella sp.		20.3 (5.4)	6	nd	6	3.0
Amnicola limosa		19.0 (3.5)	6	2.8 (0.5)	6	1.1
Crangonyx laurentianus		18.5 (2.8)	11	nd	11	0.6
DAY 24 (14-24)						
Hyalella azteca	water	nd	5	nd	5	2.0
	sediment	nd	5	nd	5	2.0
Limnephilus sp.		4.3 (1.7)	8	4.8 (1.1)	8	1.5
Ephemerella sp.		trace	4	nd	4	3.0
Amnicola limosa		3.9 (0.6)	2	nd	2	1.1
DAY 371 (361-371)						
Crangonyx laurentianus		6.4 (3.8)	36	nd	36	2.5

1. not detectable

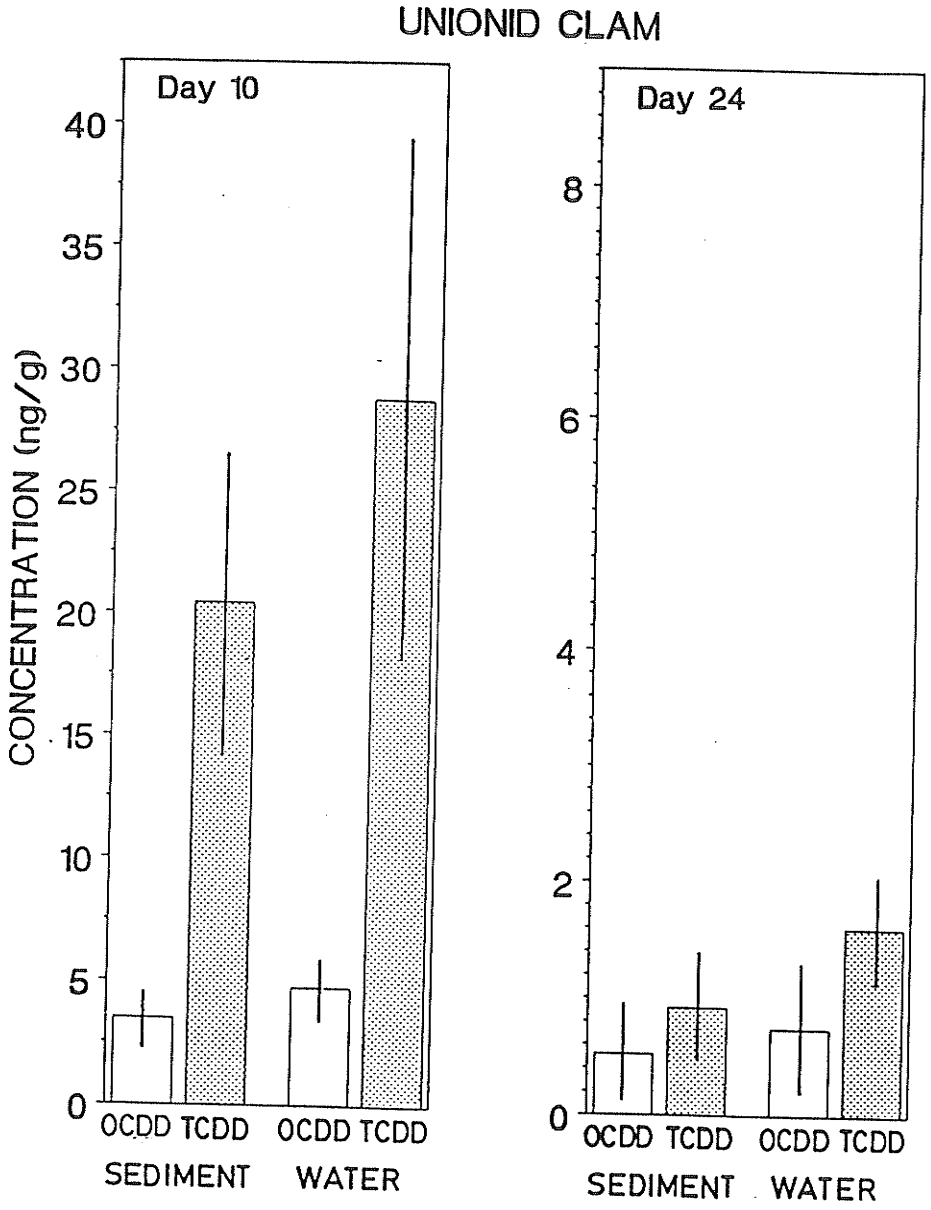

Figure 31. The concentration of PCDDs in unionid clams held in lake enclosures. Bars represent SD.

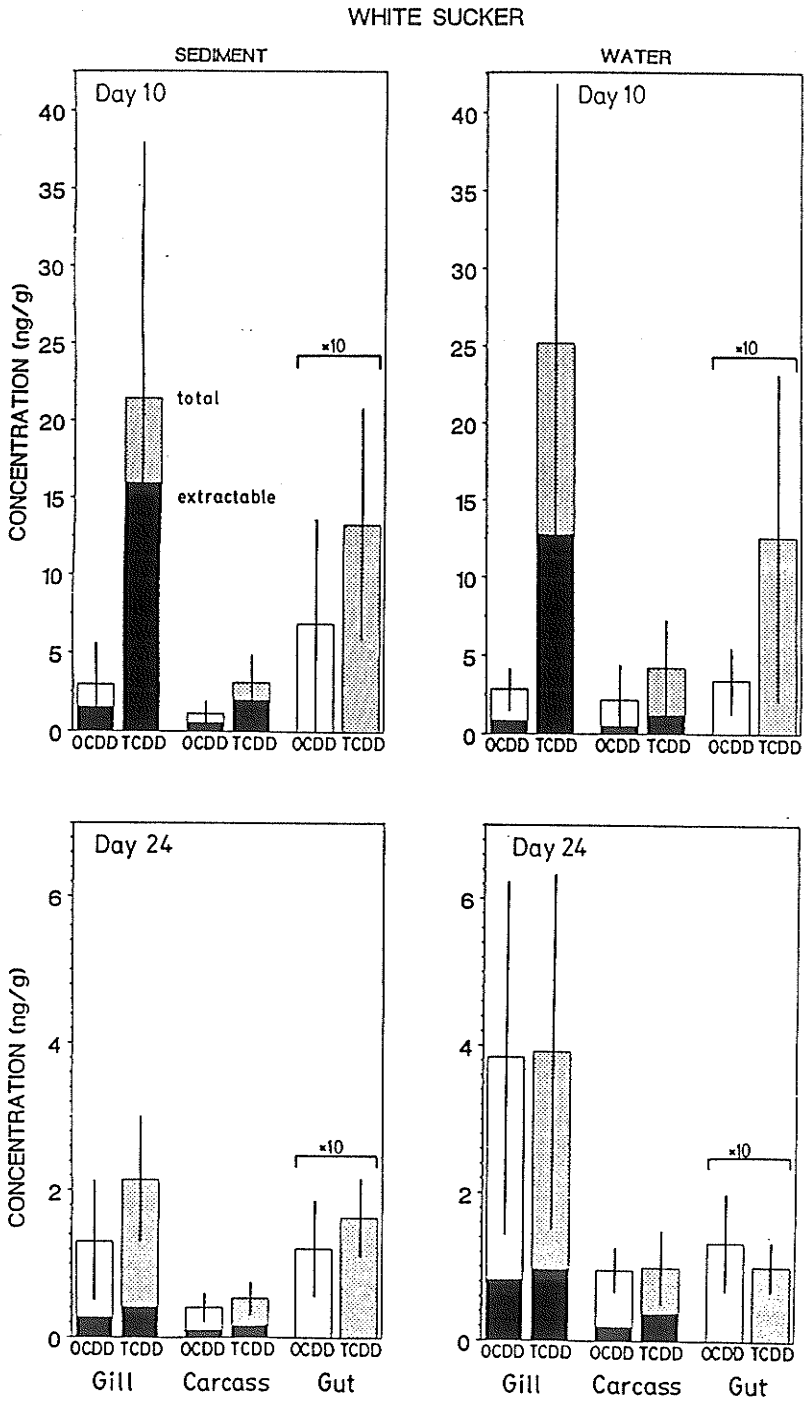

Figure 32. The concentration of PCDDs in white suckers held in lake enclosures. The concentrations in gut contents are 10 x scale. Bars represent SD.

concentration in the gut differed such that O_8CDD was higher on day 10 but similar to T_4CDD by day 24. The concentration in the gut was approximately 10 times higher than the other tissues on both dates. Again, the concentrations of PCDDs in fish held in the water column were slightly higher than those in fish held on the sediment, but were not significantly different from each other with one exception: the fish exposed in the water column had higher concentrations in the carcass on day 24. The concentrations of both the T_4CDD and O_8CDD in the gill and carcass of fish which were released into the enclosures and retrieved on day 104 were relatively low (0.1-0.5 ng/g) but similar (Table 21). The concentrations in the gut were still 2-10 times higher than those in the other tissues. On day 10, greater than 50% of the ^{14}C-T_4CDD in gills was extractable in toluene while on day 24 less than 25% was extractable (Fig. 30). Sixty-eight percent of the ^{14}C-T_4CDD in extracts analyzed by HPLC remained as the parent compound. The percentage of ^{14}C-T_4CDD extractable in the carcass was slightly less than in the gills on day 10 and this percentage also decreased on day 24. ^{14}C-OCDD also showed a similar pattern.

DISCUSSION

The concentrations of the T_4CDD in caged biota exposed in lake enclosures on days 0-10 were approximately 5 times higher than that for O_8CDD (Fig 31, 32, Table 20). The higher concentration of the T_4CDD may reflect the higher availability of the T_4CDD in the enclosures compared to O_8CDD. The percentage of the total water concentration in true solution, determined using reverse phase cartridges, was 10-15% for the T_4CDD compared to less than 1%

Table 21. The concentration of PCDDs in free swimming fish (white sucker) from lake enclosures (ng/g±S.D.) collected on day 104-106.

	T_4CDD n=4	O_8CDD n=6	detection limit
gill	3.5 ± 2.0	2.8 ± 1.4	0.20
carcass	1.8 ± 1.4	2.7 ± 1.8	0.01
gut	50.3 ± 38.1	13.9 ± 14.8	0.01

for O_8CDD (Chapter IV). Although O_8CDD is more lipophilic (i.e., log K_{ow} = 8.60 for O_8CDD and 7.13 for T_4CDD) and is therefore expected to have a higher BCF, the strong affinity of O_8CDD for organic matter in the water column may limit its bioavailability relative to the T_4CDD. Hydrophobic compounds bound to POM and DOM are generally considered not to be bioavailable (Landrum et al. 1987; McCarthy 1983; Chapter II, III). The apparent BCFs for the T_4CDD measured for biota in the lake enclosures on day 10 and 24 were 231 to 1598 which is similar to those reported for invertebrates (794-2846) and fish (1400-5840) in the laboratory (Muir et al. 1985b; 1986; Chapter II). Apparent BCFs for O_8CDD on day 10 were considerably lower (viz., 78-565) but were comparable to those reported from the laboratory for invertebrates (42-160) and fish (34-2226) (Muir et al. 1985b; 1986; Chapter II). Apparent BCFs in lake enclosures were also similar to those reported for the T_4CDD in small ponds for snails (1860-4180) and for O_8CDD in small ponds for fish (38-1300) (Corbet 1983; Marcheterre 1985).

The concentration of the T_4CDD detected in biota on both day 10 and 24 were within the range predicted by its BCF measured in the laboratory (Table 22). The large decline in the concentration of the T_4CDD in biota on day 24 relative to day 10 was due to the rapid decline in the total water concentrations (40.2 to 0.6 ng/L) as the T_4CDD partitioned into the other environmental compartments, primarily the sediment (Chapter IV). In contrast, the concentration of O_8CDD in biota declined only slightly between day 10 and day 24 and the concentration of O_8CDD in biota could not be accounted for by its BCF (Table 22). Concentrations of O_8CDD in biota were at least an order of magnitude greater than predicted by its BCF (measured in the laboratory). In addition, because the fraction of O_8CDD in true solution was assumed to be 1% and the actual percentage was somewhat less, the predicted biota

Table 22. The bioconcentration factors (BCF) and predicted biota concentrations for PCDDs in lake enclosures.

	T_4CDD		O_8CDD	
	Concentration	BCF	Concentration	BCF
Day 0 - 10				
Water (ng/L)[1]	13.4 (40.2-2.7)		14.0 (26.1-6.1)[2]	
Biota (ng/g)				
predicted[3]	10.1	5017	0.1	705
invertebrates[4]	17.7	1322	2.0	141[5]
unionid clams[6]	21.4	1598	2.9	207
white suckers-gill[6]	21.4	1598	2.9	207
white sucker-carcass[6]	3.1	231	1.1	78
Day 14 - 24				
Water	1.0 (1.4-0.6)		2.3 (3.1-1.6)	
Biota				
predicted	0.75		0.02	
invertebrates	1.6	1640	0.6	261
unionid clams	0.9	900	0.5	217
white sucker-gill	2.1	2100	1.3	565
white sucker- carcass	1.0	500	0.4	174

Table 22 continued.

Day 0 - 104				
Water	0.1		0.3	
Biota				
predicted	0.07		0.002	
white sucker-gill	3.5	35000	2.8	9333
white sucker-carcass	1.8	18000	2.7	9000

1. Time weighted mean water concentration (total ^{14}C corrected for extraction efficiency.
2. Range in water concentration.
3. Based on the BCF (highest measured in Chapter II) x mean water concentration x fraction in true solution, i.e., 0.15 for T_4CDD and 0.01 for O_8CDD.
4. Mean concentration of total ^{14}C in benthic invertebrates.
5. Based on the mean concentration in biota / time weighted mean water concentration.
6. Sediment exposed animals.

concentration may have been overestimated. Based on the relationship between K_{OC} and K_{OW} developed by Karickhoff (1981),

$$K_{OC} = [\text{sediment}] \,/\, ([\text{water}]\ f) = 0.41\ K_{OW} \qquad [14]$$

the predicted truly dissolved water concentration of O_8CDD in the enclosures would be only 0.004% of the total water concentration. This yields an even lower predicted biota concentration of <0.004 ng/g on day 10 which is only a fraction of that observed. Because the concentration of O_8CDD in the water is greater than its water solubility the truly dissolved concentrations may be equal to its water solubility, i.e. 0.4 ng/L. When DOM is added to water in the presence of excess T_4CDD the apparent solubility is increase while the concentration in true solution remains constant (Chapters I,II). If the truly dissolved concentration is assumed to be it water solubility (when the total concentration is >0.4 ng/L), then the concentration of O_8CDD found in biota on days 10 and 24 falls within that predicted by its measured BCF. On day 104, the concentration of total O_8CDD in the water was less than its water solubility and the concentration of O_8CDD in the biota could not be predicted by its measured BCF. The BCF for O_8CDD (705) used to predict the biota concentration may be an underestimate of the true BCF. Based on the relationship between K_{OW} and BCF developed by Veith et al. (1979), the BCF of O_8CDD is predicted to be 4×10^6. However, there is evidence that this relationship between K_{OW} and BCF can not be extrapolated to superlipophilic compounds. Experimental results have shown that BCF declines for compounds having log K_{OW} greater than approximately 6.5 (Muir et al. 1985a, Chapter II). Steric hindrances, solubility factors and metabolic transformation may reduce the BCFs of O_8CDD relative to that predicted by its K_{OW} (Opperhuizen et al.

1985; Gobas and Mackay 1987; Chapter II). Because the biota in the enclosures were exposed for only 10 d, they might not have reached equilibrium with the water, although fish exposed in the laboratory appear to approach equilibrium within 10 d (Muir et al. 1986; Chapter II). Hawker and Connell (1985) have shown that for superlipophilic compounds such as O_8CDD the time to reach equilibrium may be greater than the life span of the organism.

Biota (H. azteca, unionid clams and white suckers) held in cages in the water column generally had higher concentrations of PCDDs than the biota held in cages on the sediments at the same time. This may have been due to increased sorption of PCDDs to organic matter at the sediment water interface. Sediment interstitial waters from Lake 304 contain 28.8±3.6 mg/L DOC compared to only 7.8±0.6 mg/L DOC in the water column (Chapter IV). Even on day 3, PCDDs were not detectable (<0.5 ng/L) in the sediment interstitial water of the lake enclosures. Based on equation [14] the concentration of the T_4CDD truly dissolved in the sediment interstitial water would be 2.3 times lower than in the water column. If the K_{rp} measured for Lake 304 sediment interstitial water (350 L/g) is used to estimate the truly dissolved water concentration then it is 1.5 times lower than the predicted concentration in the water column on day 10 (Chapter III). The actual difference measured for the T_4CDD in caged biota on day 10 was 1.2 to 2.1 times higher in the water column. This difference was greatest for the amphipods which have a stronger association with the sediments than do fish.

Numerous studies have reported a reduction in the bioavailability of hydrophobic compounds in the presence of POM or DOM (McCarthy 1983; Landrum et al. 1987; McCarthy and Jimenez 1985; Chapter II, III). The amount of the hydrophobic compound bound to organic matter has been shown to be directly related to the organic matter concentration in solution (Landrum et al. 1984;

McCarthy et al. 1985, Morehead et al. 1986; Chapter I-III). The organic matter-contaminant complex is apparently too large or too polar to penetrate the respiratory membranes or the contaminant does not have significant time to diffuse out of the organic matter complex and interact with the biological membranes (Landrum et al. 1987). Even when the measured truly dissolved water concentrations are used to predict the BCFs of PCDDs in the laboratory, they are considerably lower than would have been predicted by their K_{ow} alone; i.e., extrapolation of the relationship presented by Veith et al. (1979) between BCF and K_{ow} (Muir et al. 1985a; Chapter III). Failure to fully explain the divergence from the predicted relationship may be at least partly due to the difficulty of measuring the truly dissolved water concentrations of extremely hydrophobic compounds such as O_8CDD. Steric hindrances may also be important in controlling the uptake of PCDDs. Opperhuizen et al. (1985) predicted that hydrophobic molecules with minimum internal cross sections (MIC) greater than 9.5 Å would not permeate the polar holes of the epithelial membranes. H_6CDD, H_7CDD and O_8CDD all have MICs of 9.8 A (Chapter III). Gobas et al. (1987) predicted that the activity coefficients of solutes in membranes differs from 1-octanol and that the activity coefficients become larger with increasing solute size likely as a result of the increased energy of cavity formation in the membranes. The reduced uptake of O_8CDD in the enclosures might have been due to several factors (including sorption to organic matter) which limited its uptake relative to that of the T_4CDD. The relative importance of these factors in controlling the bioavailability of PCDDs has not yet been adequately answered although sorption of PCDDs to organic matter plays an important role (Chapter II, III).

The failure of the BCFs (measured in the laboratory) to predict the observed concentrations in biota may be due to technical problems associated

with determining BCFs. A possible alternative hypothesis is that the O_8CDD in biota was derived from the food and not the water. As the truly dissolved water concentrations of both PCDDs in the water column declined there may have been a shift in the route of uptake from direct equilibrium partitioning to a detrital based food chain transfer. Removal of the PCDDs to the sediments may have effectively eliminated the water column as a source of PCDDs for biota. This shift may have occurred after day 24 for T_4CDD, but the extremely low truly dissolved water concentrations of O_8CDD may have limited its bioavailabily from the water column even immediately after the additions to the enclosures when the total water concentrations were relatively high. The similarity in the concentrations of O_8CDD on day 10, 24 and 104 may be due to the relatively constant concentration of O_8CDD available to biota via the food vector. Both congeners were extremely persistent in the sediments which may have been the base of the food chain (Chapter IV). The concentration of both T_4CDD and O_8CDD in the gut of white suckers was similar on each date and the concentrations were similar on day 24 and 104 (Fig. 32, Table 21). The concentration of both congeners in the gut were also approximately 10 times higher than the concentration in any other tissue. This indicates that fish were feeding on material that had relatively high concentrations of PCDDs. Although the food items were not enumerated the gut contents contained zooplankton, chironomids and possibly sediment.

The apparent BCFs on day 104 for free swimming white suckers (released into the enclosures on day 1) were 18000 to 35000 for T_4CDD and 9000 to 9333 for O_8CDD. This is much higher than the BCFs observed in caged fish on days 10 or 24 in the enclosures or in the laboratory (Muir et al. 1986; Chapter II). There is also a decrease in the ratio of the concentration of T_4CDD in the gills to the carcass between day 10 (>6) and day 104 (<2). These results

suggest a shift in the route of uptake of the T_4CDD from the water to the food while the route of uptake of O_8CDD may have been food throughout the study.

Crossland et al. (1987) predicted a similar shift in the route of uptake of 2,5,4'-trichlorobiphenyl for fish in outdoor ponds. After the initial spike, when the water concentrations were high, the concentration of the PCB in rainbow trout and grass carp were similar. After 14 days, the concentration of PCB in the carnivorous (invertebrates) rainbow trout was higher than in the herbivorous (vegetation) grass carp indicating that uptake had shifted to accumulation from the food. Using a steady state food chain model, Thomann (1981) predicted that the body burden of PCBs in top predators was almost entirely due to transfer from contaminated prey. Thomann and Connolly (1984) using a similar model predicted that >99% of the concentration of PCBs in the lake trout of Lake Michigan was due to exposure through the food chain.

The four compartment food chain model of Thomann and Connolly (1984) was used to predict the relative importance of food chain versus water uptake of PCDDs in an aquatic environment. The model is based on food chain leading to lake trout as the top predator in Lake Michigan and consists of four compartments: detritus/phytoplankton, filter-feeding crustaceans (Mysis relicta), small fish (alewife) and preditory fish (lake trout). At steady state the concentration of the PCDD in each trophic level (CF_i) is given by:

$$CF_i = k_{ui}\, CW / K_d + \alpha\, F_i\, CP_{i-1} / K_d \qquad [15]$$

where k_{ui} = uptake rate constant (L kg^{-1} d^{-1}) from water, CW = water concentration of PCDD (ng/L), α is the assimilation efficiency from food, CP_{i-1} = concentration (ng/kg) in prey at one lower trophic level and k_d is the

depuration rate constant. Values for many of the parameters are available or can be predicted from the literature (Table 23). The steady state solution assumes there is a single age class, and no change in the feeding rates growth rates or kenetic parameters within each trophic level. These assumptions are unrealistic but allow a simplistic comparison of the relative distribution of PCDDs in food chains (Thomann 1981). The relative concentrations of PCDDs derived from the water or food were predicted based on the concentrations of PCDDs measured in the sediment and water in the enclosures (Chapter IV). Uptake and depuration rate constants were taken from Muir et al. (1985b) for invertebrates and Chapter II for fish. The K_d for large fish was assumed to be one half that for small fish. Assimilation efficiencies were taken from Muir et al. (1986). Feeding rates at each trophic level were taken directly from Thomann and Connolly (1984).

The hypothesis that there is a shift in the route of uptake of the T_4CDD in the lake enclosures is supported by the food chain transfer model. As the water concentration declines, the model predicts a shift from accumulation from water to food (Fig. 32). This shift occurs sooner for the higher trophic levels and for O_8CDD relative to T_4CDD. On days 0-10 the model predicts that >80% of the residues in filter-feeders are from the water while 98% of the residues in fish are from the food. By day 24 the water concentrations are much lower and only 25% of the T_4CDD in filter-feeders is derived from the water. Because the uptake rates of O_8CDD from water are low, a larger fraction of the O_8CDD is predicted to be derived from food, even in the filter-feeders on day 10.

The food chain transfer model was also used to predict the relative distribution of PCDDs in biota in Lake Huron. The concentrations of PCDDs in detritus were taken from surficial sediment (0-1 cm) in Lake Huron reported by

Table 23. The food chain model prediction for the concentration of PCDDs in the biota of Lake Huron.

Compound and compartment	C_w[1] (pg/L)	k_u[2] (d^{-1})	k_d[2] (d^{-1})	α[3] (%)	F[4] ($gg^{-1}d^{-1}$)	Concentration from water (pg/g)	Concentration from food (pg/g)	Concentration from food (%)
T_4CDD								
1. Detritus/ periphyton	0.009	-	-	-	-	1[5]		
2. Crustacea/ filter-feeder		1200	0.06	6	0.105	<0.1	10.5[6]	>99
3. Small fish		285	0.12	13	0.017	<0.1	19.3	>99
4. Large fish		285	0.06	13	0.009	<0.1	37.7	>99
O_8CDD								
1. Detritus/ periphyton	0.1	-	-	-	-	1300		
2. Crustacea/ filter-feeder		60	0.12	2	0.10	<0.1	25.0	99
3. Small fish		30	0.08	3	0.017	<0.1	21.3	>99
4. Large fish		30	0.04	3	0.009	<0.1	19.2	>99

1. Water concentration estimated from K_{OC} value and the concentration in

Table 23 continued.

surficial sediment (Czuczwa and Hites 1984).

2. Uptake and depuration rate constants estimated from Muir et al. (1985a) and Chapter II.
3. Assimilation efficiency estimated from Muir and Yarechewski 1988.
4. Feeding rates taken from Thomann (1981), ignoring age class differences.
5. Estimated concentration in surficial sediments based on concentration (0-1 cm) in mid-Lake Huron reported by Czuczwa and Hites (1984).
6. Predicted based on total ^{14}C.

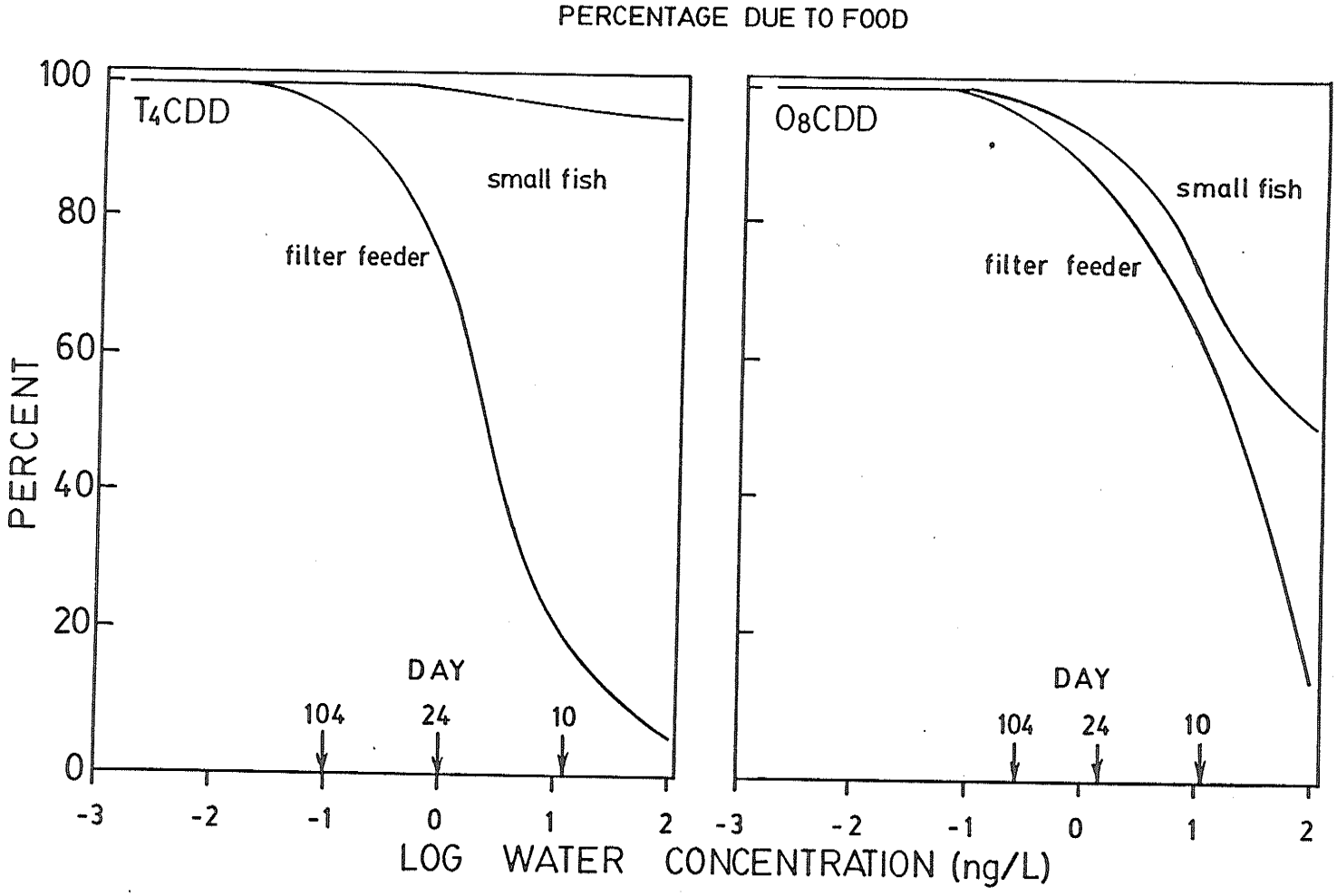

Figure 33. The effect of water concentration on the predicted contribution of uptake from food to the concentration of PCDDs found in biota. Predictions based on the food chain model using parameters in Table 22 and a sediment concentration reported in lake enclosures of 120 ng/g dry weight (Chapter IV). Arrows represent the water concentration in lake enclosures on days 10, 24 and 104.

Czuczwa and Hites (1984). The concentration of the T_4CDD in sediments was assumed to be 1 pg/g and the concentrations in water were derived a described by Muir and Yarechewski (1988) from equation [14].

The food chain model predicts that food chain transfer (>99%) is the major route of uptake of PCDDs in Lake Huron (Table 23). This is due to the extremely low truly dissolved concentrations predicted in the water column. However, even if the concentrations were as high as 1 pg/L, >98% of the PCDD found in fish would be derived from the food. Although this is a very simplistic model and makes numerous assumptions and omissions (i.e., no growth) it is very useful as a tool to help conceptualize the relative distribution of PCDDs found in biota (Thomann 1981). The model does predict concentrations in fish which are similar to that reported by Stalling et al. (1983) in Lake Huron and Saginaw Bay (see Table 6).

The lower chlorinated PCDDs are predicted to biomagnify up the food chain. Therefore only small quantities of T_4CDD need to be available in the detritus before detectable amounts will be found in the biota at the top of the food chain. However, this is based on the assimilation efficiency of T_4CDD being 13%. Tetrachlorodibenzo-p-dioxin isomers other than 2,3,7,8-T_4CDD, including 1,3,6,8-T_4CDD are metabolized and the assimilation efficiency of the parent compound is low (Muir and Yarechewski 1988; Niimi and Oliver 1986). The assimilation efficiency of extractable 1,3,6,8-TCDD is <4% and it may be lower because not all of the extractable ^{14}C remains in the fish as the parent compound (Muir et al. 1986). If this value is used in the model then T_4CDD does not biomagnify and the predicted residues in top pedators is less than 0.1 pg/g. 2,3,7,8-T_4CDD has a relatively high assimilation efficiency of 34-36% (based on data from Hawkes and Norris 1977; Kleeman et al. 1986a) and a low depuration rate constant (Branson et al. 1985; Mehrle et

al. 1988). Therefore, the 2,3,7,8-T_4CDD isomer will likely biomagnify up the food chain and become detectable in fish although the concentrations in detritus are very low. This might explain why 2,3,7,8-T_4CDD is often the only tetra congener found in fish (Kuehl et al. 1987; Rappe et al. 1987). Rappe et al. (1987) has reported finding non 2,3,7,8 substituted PCDDs in crabs. This may indictate that some invertebrates do not have the ability to metabolize PCDDs and they may bioaccumulate in these organisms. Unionid clams in this study did not appear to be able to metabolize the 1,3,6,8-T_4CDD congener. Almost all of the ^{14}C-T_4CDD was determined to remain as the parent compound while only a small fraction of the ^{14}C-T_4CDD in white suckers remained as the parent compound (Fig. 31, 32). This may have important implications for monitoring programs.

In contrast to 2,3,7,8-T_4CDD the hexa, hepta and octa congeners are predicted to not biomagnify (Muir and Yarechewski 1988; Table 23). However, the concentration of PCDD congeners found in sediments (i.e., detritus) generally increases as the degree of chlorination increases (Czuczwa and Hites 1986). This may lead to detectable amounts of the higher chlorinated dioxins in biota despite their low assimilation efficiencies. The concentrations of the higher chlorinated PCDDs in the environment are so high that even a small amount of tranfer up the food chain may result in detectable quantities in the top predators.

The concentrations of PCDDs found in biota are not directly correlated to the concentrations in the sediments, although the sediments appear to be the source of PCDDs in biota and therefore the key to predicting residue levels in the biota. Because PCDDs are extremely persistent in the environment they will remain in the surficial sediments for a very long time depending on the physical characteristics of the system. This implies that

even if the sources of PCDDs to aquatic environments are reduced, residues of PCDDs in biota will remain high for a very long period, until the PCDDs are buried in the sediments or transported out of the aquatic system.

Unfortunately there is very little information in the literature on the relative concentration of PCDDs in detritus and biota at different trophic levels from the same environments. The literature is dominated by monitoring studies which seldom include both sediments and biota. Most studies on biota have concentrated on the 2,3,7,8-T_4CDD congener and rarely report the concentrations in more than one species (usually fish) from a single environment. Because of the predicted importance of food chain transfer there is a need for a consistent data set including several trophic levels from a single environment to help clairify the distribution patterns of PCDDs observed in biota. Although it may be very difficult, an attempt should also be made to measure the truly dissolved water concentrations of PCDDs in the environment. A better understanding of the environmental dynamic of PCDDs would help to interpret the significance of residues found in biota and lead to improved assessment of the hazard they represent.

SUMMARY

This thesis represents a series of independent experiments on the environmental chemistry of PCDDs, combined into five separate chapters. Chapters I through III are laboratory studies focusing on the factors which influence the determination of K_p, water solubility and BCF. All of these parameters are important for modelling and understanding the behaviour of PCDDs in aquatic environments. Chapters IV and V discuss studies which were conductedin large lake enclosures at the Experimental Lakes Area. These field studies examined the fate and bioavailability of PCDDs under natural conditions simulating lakes. All of the studies emphasized the importance of measuring truly dissolved concentrations to understanding the environmental dynamics of superlipophilic compounds.

In Chapter I, the K_p for T_4CDD was determined for suspended sediment concentrations ranging over four orders of magnitude. The truly dissolved concentrations were estimated using conventional centrifugation techniques or the recently developed reverse-phase C_{18} cartridge and dynamic head space analysis methods. Centrifugation at either 6000g or 20000g resulted in similar negative slopes (-1.47, -1.54) between log K_p and log suspended sediment concentration. DOC concentrations increased as the suspended sediment concentration increased and may have inflated the truly dissolved concentrations estimated using centrifugation. This may have led to an overestimation of the K_p, especially at high suspended sediment concentrations. However, measuring the truly dissolved concentrations did not completely eliminate the negative correlation between log K_p and log suspended sediment concentration (-0.51, -0.64). Although methodological bias may explain this negative relationship another mechanism such as particle

interaction may explain the deviation from zero slope. This has important implications for the environmental modelling of hydrophobic organic compounds.

The extent to which DOM influences the bioavailability of PCDD was determined using Aldrich humic acid or filtered lake water (L239) in Chapter II. This work extended the study of DOM-hydrophobic organic contaminant interactions to compounds more lipophilic than had been previously examined. It is also one of the few studies that attempted to measure the truly dissolved concentrations in the exposure system. The BCFs of four PCDDs congeners were determined for rainbow trout (Salmo gairdneri), using both centrifuged and reverse-phase cartridge extractable water concentrations. Addition of Aldrich humic acid to synthetic water reduced the apparent uptake rate constants by reducing the truly dissolved concentrations available to the fish. DOM from natural lake water (L239) had little effect on the apparent uptake rate constants relative to Aldrich humic acid. Measured apparent BCFs were below those predicted from their K_{OW} alone but only part of this reduction was explained by calculating the true BCFs using the estimated truly dissolved concentrations. This may be due to experimental errors leading to an overestimation of the truly dissolved concentrations, although steric hindrances, solubility factors or metabolic transformation may be important factors controlling the BCFs of PCDDs.

Based on the results in Chapter II, the interaction between T_4CDD and DOM from a wider range of natural sources was examined in more detail in Chapter III. The effect of DOM on the apparent solubility and bioavailability of T_4CDD was examined in epilimnetic and sediment interstitial waters collected from Canadian Shield lakes in the Experimental Lakes Area. Although DOM from natural sources had less effect on the behaviour of PCDDs than commercial humic acids it did have a significant influence. DOM from natural

sorces enhanced the apparent solubility of T_4CDD by as much as three times over the reference DOM-free water (DOM <0.24 mg/L DOC). The percentage of T_4CDD bound to DOM increased as the DOC concentration increased and the uptake rates constants of T_4CDD in Crangonyx laurentianus decreased with increasing DOC. The quality or site of collection of the DOM was also an important factor. Sediment interstitial waters had a greater affinity for T_4CDD than epilimnetic waters as indicated by the DOC partition coefficients. Although the DOM may enhance the apparent solubility of T_4CDD and increase its mobility, DOM may also reduce the amount of T_4CDD truly dissolved and therefore bioavailable.

Unique field experiments with PCDDs were conducted using large (40 m^3) lake enclosures at the Experimental Lakes Area. The environmental fate and bioavailability of T_4CDD and O_8CDD were studied under field conditions closely simulating lakes. This study represents the first attempt to use lake enclosures (limnocorrals) to study the environmental chemistry of such lipophilic comounds. PCDDs were added to replicate enclosures as a sediment slurry at a nominal concentration of 58-59 ng/L. Both congeners partitioned/settled rapidly to the surficial sediments where they persisted over the two years of the study. Initially the total concentrations of T_4CDD in the water were higher than those of O_8CDD, however, they declined more rapidly with $t_{1/2}$s of 2.6±0.2 and 4.0±0.3 d, respectively. Approximately 10-15% of the T_4CDD and <1% of O_8CDD detected in the water column during the first 48 h were determined to be truly dissolved using reverse-phase cartridges. The rapid partitioning of O_8CDD to organic matter in the water column and sediments limited its availability and therefore influenced its fate. Sorption of PCDDs to organic matter increased the retentive capacity predicted by the NRC Persistence model, especially for the more hydrophobic

O_8CDD.

The T_4CDD was more bioavailable to caged benthic invertebrates and fish (white sucker) than O_8CDD immediately after the addition to the enclosures. As the water concentrations rapidly declined in the enclosures the bioavailability of T_4CDD declined to a level similar to O_8CDD. Sorption of PCDDs to organic matter and rapid partitioning to sediments may have reduced the uptake of PCDDs from the water column. Accumulation of PCDDs in biota is hypothesized to shift from direct equilibrium partitioning from the water column during the first few days to a detrital based food chain transfer as the freely available concentrations in the water rapidly declined. This conclusion is supported by the results of a simple four compartment, food chain model of Thomann and Connolly (1984), based on the uptake kinetics of PCDDs from water and food measured in the laboratory.

Sorption of PCDDs to organic matter may increase their apparent solubility and alter their predicted environmental fate (i.e., increased mobility). Although solubility enhancement by organic matter may increase the total concentration of PCDDs in the water column, only a small fraction will be truly dissolved in the water (Chapter I-III). Under most natural conditions where the environmental concentrations are low, association with organic matter will effectively reduce the concentration available for chemical or biological reaction. Since sorption of organic contaminants is considered to be a reversible process, biota may be continuously exposed to low levels of PCDDs which may otherwise have only limited spatial distribution.

The lower availability of the higher chlorinated PCDDs for chemical (e.g., photolysis) and biological transformation, or removal (e.g., volatilization) and the rapid movement to the sediments may lead to relatively

higher retentive capacities of these congeners in aquatic systems (Chapter IV). The selective retention of higher chlorinated congeners combined with higher inputs of these congeners may explain the pattern of increasing concentration of of PCDDs homologues with increasing chlorine substitution observed in the Great Lakes and other aquatic environments. Because only the PCDDs in true solution (free) are bioavailable, sorption to organic matter in the water column may reduce or eliminate uptake of PCDDs in biota via the gills (Chapter II,III). As the water concentrations decline food may become the dominant vector of accumulation of PCDDs (Chapter V). Although the concentrations of PCDDs found in biota are not directly correlated to the concentrations in the sediments (detritus), a detrital based food chain transfer may be the major route of uptake of PCDDs in biota (Chapter V). Therefore the concentration of PCDD homologues in the sediment may control the relative concentrations of PCDDs in biota. Although PCDDs, with the possible exception of 2,3,7,8-T_4CDD, do not biomagnify in aquatic food chains, the high concentration of PCDDs, escpecially the higher chlorinated congeners, at the bottom of the food chain (detritus) may serve as the source of contamination of biota at higher trophic levels. The high concentrations and retentive capacities of PCDDs in aquatic sediments means that contaminated sediments may serve as a source of PCDDs to the environment and biota long after inputs have been reduced or eliminated. Unfortunately there is very little information in the literature on the relative concentrations of PCDDs in water (total or truly dissolved), detritus, and biota at different trophic levels from the same site. In the future, a consistent and complete data set from a single or several sites may help to clarify the pattern of PCDDs observed in the aquatic environment.

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APPENDIX A

The effect of methanol as a cosolvent on the interaction of 1,3,6,8-tetrachlorodibenzo-p-dioxin with dissolved organic matter in aqueous solutions

There are numerous methods that have been employed to dissolve hydrophobic organic compounds in water for studies of their interactions with DOM. One of the most common techniques is the use of a nonaqueous cosolvent such as methanol used in Chapters I & III. However, the hydrophobic compound may come out of solution immediately upon addition to the water or the organic solvent my alter the system being studied. To evaluate the possible effect of cosolvent addition, the solubility enhancement and partitioning of 1,3,6,8-tetrachlorodibenzo-p-dioxin (T_4CDD) in the presence of DOM was examined at varying concentrations of methanol (the cosolvent used in Chapters I & III). This experiment is part of a review of methods for dissolving hydrophobics in water for studies of their interactions with DOM (Webster et al. 1987).

METHODS AND MATERIALS

Partitioning

An excess of ^{14}C-T_4CDD (specific activity 24.16 mCi/mM, Pathfinder Laboratories, St. Louis, MO) was added to the wall of a 2 L erlenmeyer flask and the cosolvent allowed to evaporate away. A solution of 20 mg/L Aldrich humic acid (0.45 µm filtered) in Milli-Q water (Millipore Corp., Bedford, Mass.) was added to the flask and allowed to equilibrate for 3 h. 20 mL of the solution was added to 25 mL Corex glass tubes, methanol added to each tube

(0, 0.05, 0.1, 0.25 2.5, 5.0 15.0% v/v) and then gently shaken for 24 h at 23°C. The percentage of TCDD bound to the DOM was determined using reverse-phase cartridges as described by Landrum et al. (1984). Each tube was centrifuged at 20000g for 30 min and replicate 4.0 mL aliquots of the supernatant diluted with scintillation fluor (Atomlight, New England Nuclear, Boston, Mass.) and assayed by LSC. Replicate four mL aliquots of the supernatant were passed through Waters C_{18} Sep Paks and the eluant counted by LSC. The T_4CDD associated with the DOM will pass through the column (Landrum et al. 1984).

Solubility enhancement

Solubility enhancement was examined using a method similar to that of Chiou et al. (1986). Sixty-five ng of T_4CDD was deposited on the wall of 25-mL Corex glass tubes. This was sufficient T_4CDD to create a solution with a concentration of 3250 ng/L, well in excess of the solubility of T_4CDD in the absence of DOM (Friesen et al. 1985). Twenty mL of a 20 mg/L Aldrich humic acid-Milli-Q water solution was added to each tube and gently shaken for 24 h at 23°C. A 4.0 mL aliquot from each tube was assayed directly by LSC.

RESULTS AND DISCUSSION

The concentration of the T_4CDD which was bound (i.e., unextractable by C_{18} Sep-Pak) was 10-15 times greater in the presence of 20 mg/L Aldrich humic acid, and this result was not affected by the presence of up to 5% methanol (Fig. 1). Aldrich humic acid enhances the apparent solubility of the T_4CDD 4 to 5 fold in the presence of 15% methanol. The levels of methanol used as a cosolvent in Chapter I & III (<0.01%) is not likely to affect the sorption of

the T_4CDD to DOM in the test solutions. Use of methanol as cosolvent appears to be a simple and valid method for introducing the T_4CDD into aqueous solutions for the examination of T_4CDD interactions with DOM. However, the cosolvent may have an effect on the biota in the system, which may influence the interpretation of any results. Although this has not been examined the extremely low concentration of the cosolvent added to system is unlikely to have a significant affect on the organisms (amphipods) studied in Chapter III.

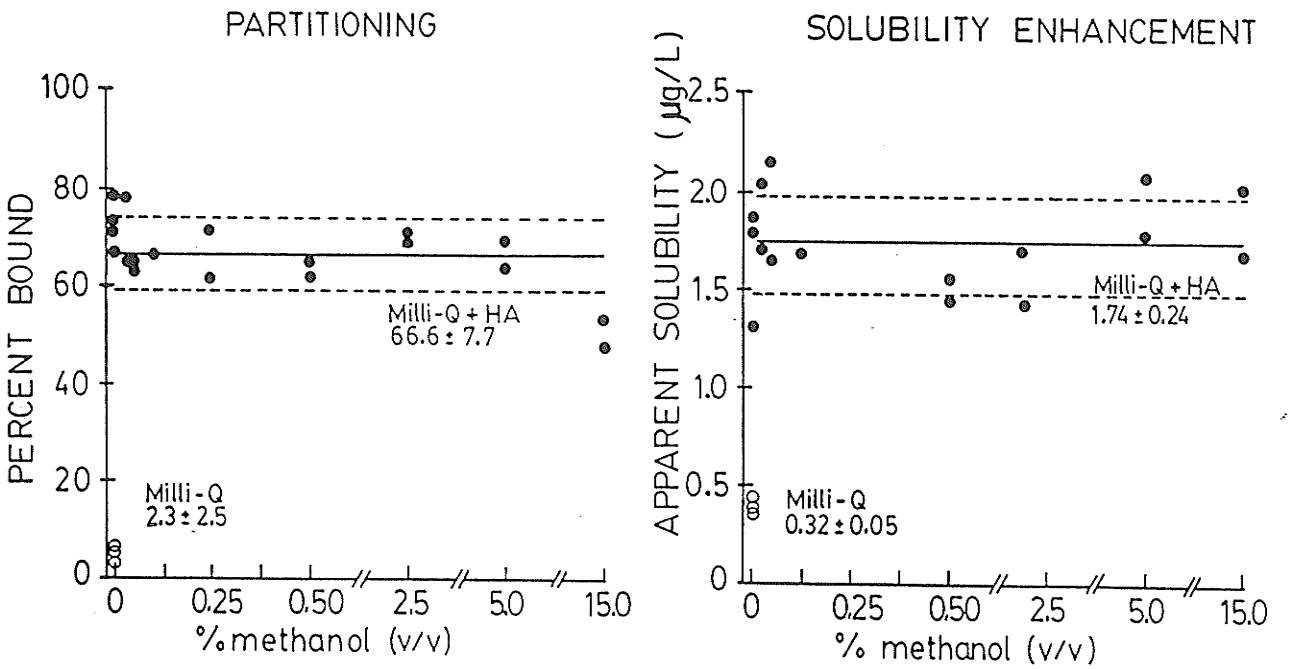


Figure 1. The effect of methanol on the interaction between T_4CDD and Aldrich humic acid (mean±S.D.).

APPENDIX B

The chemical characteristics of the reference enclosure (E) and lake site (L) during 1985.

Date	Susp. P µg/L		Susp. N µg/L		Susp. C µg/L		DOC µM/L		Conductivity µmhos		Temperature °C	
	E	L	E	L	E	L	E	L	E	L	E[1]	L[2]
15/05	13	10	142	116	1150	1080	610	730			16	16
29/05	12	7	129	104	1070	920	660	660			16	16
06/06	9	9	94	102	870	970	590	780			15	15
11/06	9	12	107	113	1160	1060	570	750			15	15
13/06	7	7	90	102	880	1030	610	680	18	19	17	17
14/06	9	10	80	91	790	980	560	690	20	20		
18/06	13	11	116	93	1040	920	550	600	19	20	17	17
21/06	19	13	98	73	1070	960	550	660	20	22	19	20
26/06	24	15	173	120	1630	1110	500	580	18	20		
02/07	12	8	112	92	970	780	590	710	22	24	19	20
05/08	32	7	354	71	3130	690	600	720	26	25	22	22
23/09			106	163	1000	1300	510	740				

1. reference enclosure

2. lake site

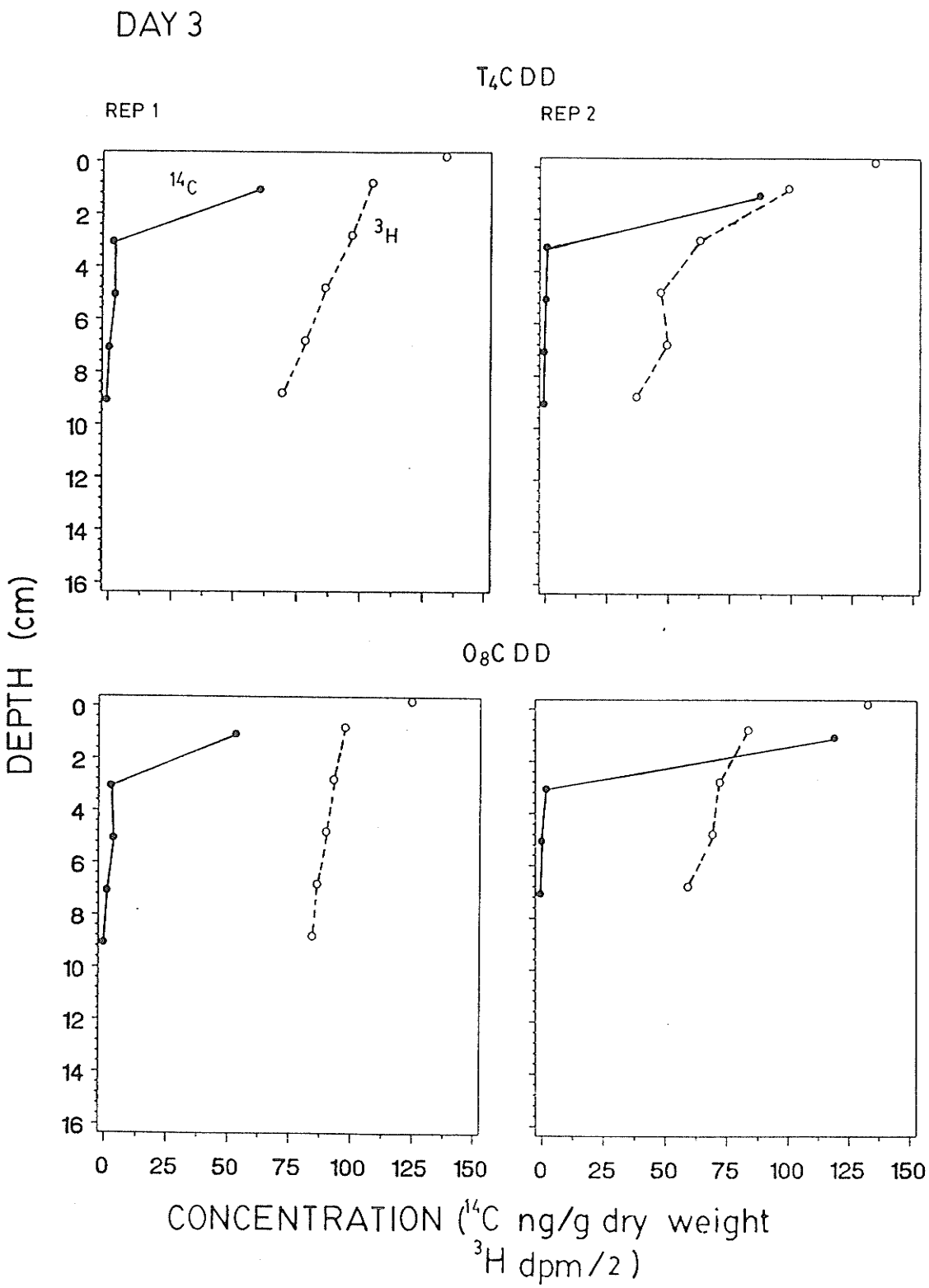
APPENDIX C

The concentration of PCDDs in replicate samples from lake enclosures on day 5 (total extractable ^{14}C). PCDDs in the reference enclosure was not detectable (<0.05 ng/L).

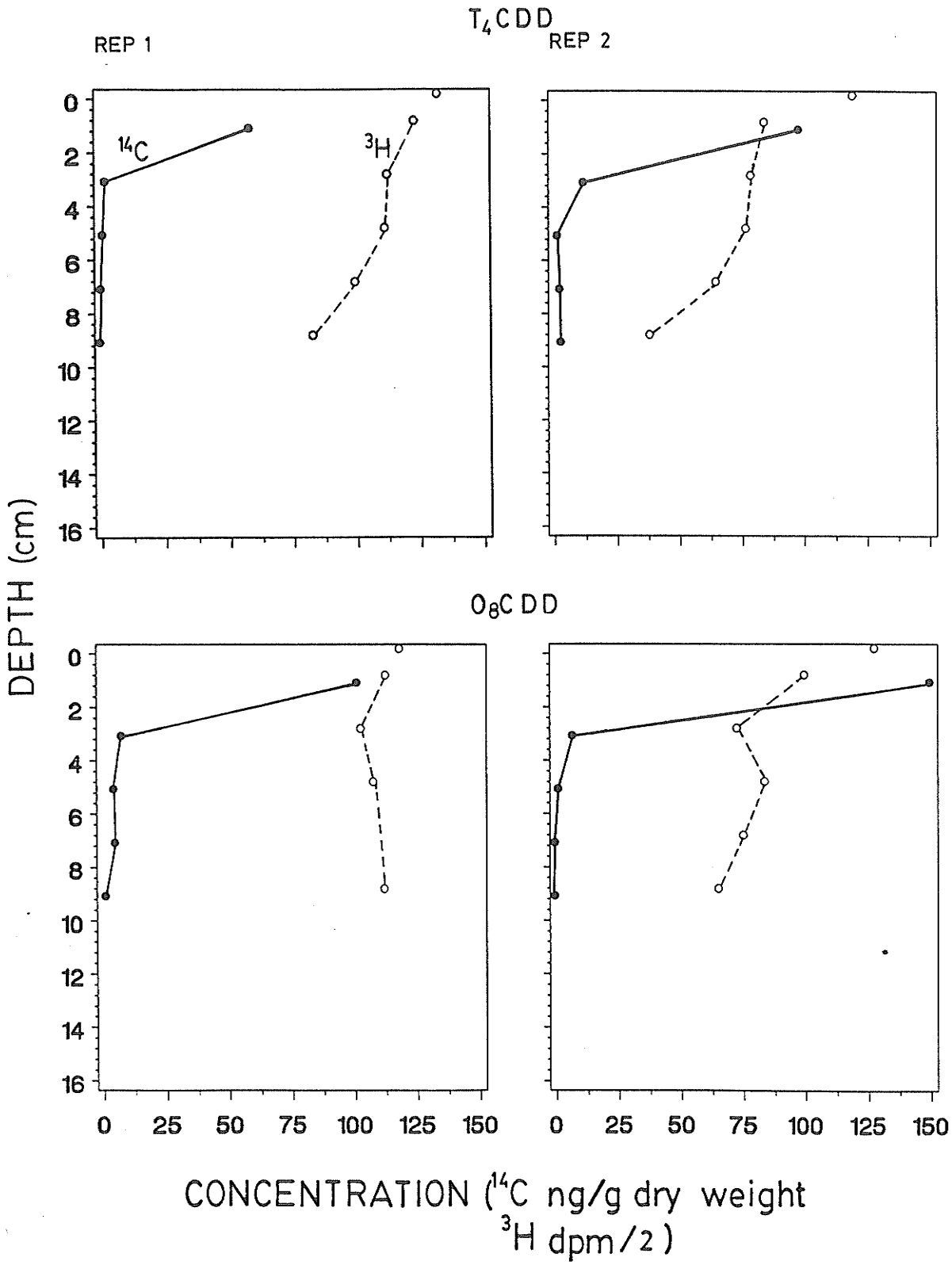
site	depth (m)	O_8CDD ng/L	T_4CDD ng/L
east wall	1	10.6	6.1
east wall-1 m	1	12.0	5.6
west wall	1	9.3	4.8
west wall-1m	1	7.1	5.2
centre	0.5	11.8	7.0
centre	1	6.3	5.6
centre	1	11.7	3.6
centre	1.5	4.6	5.3
mean±S.D.		9.2±2.9	5.3±1.0

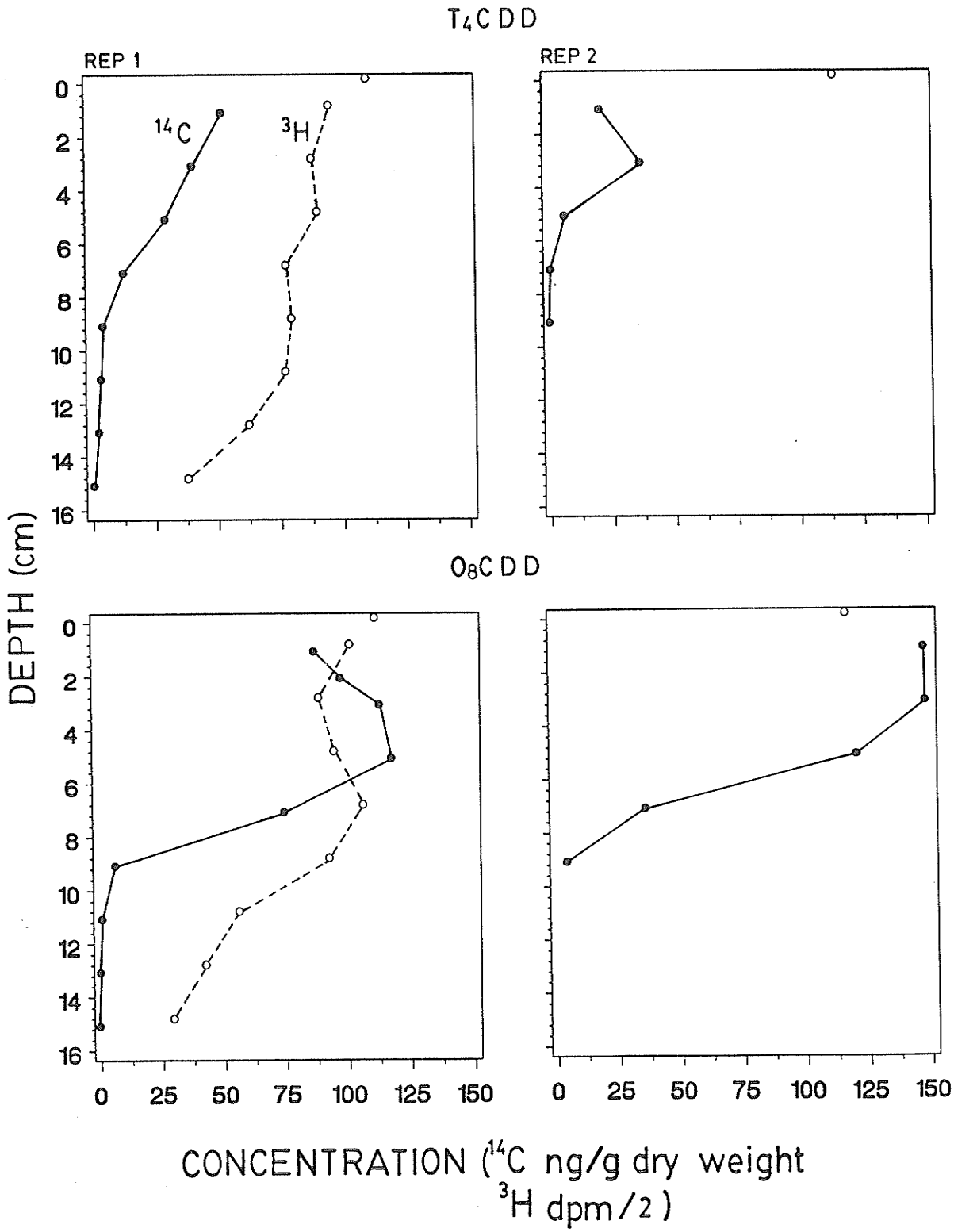
APPENDIX D

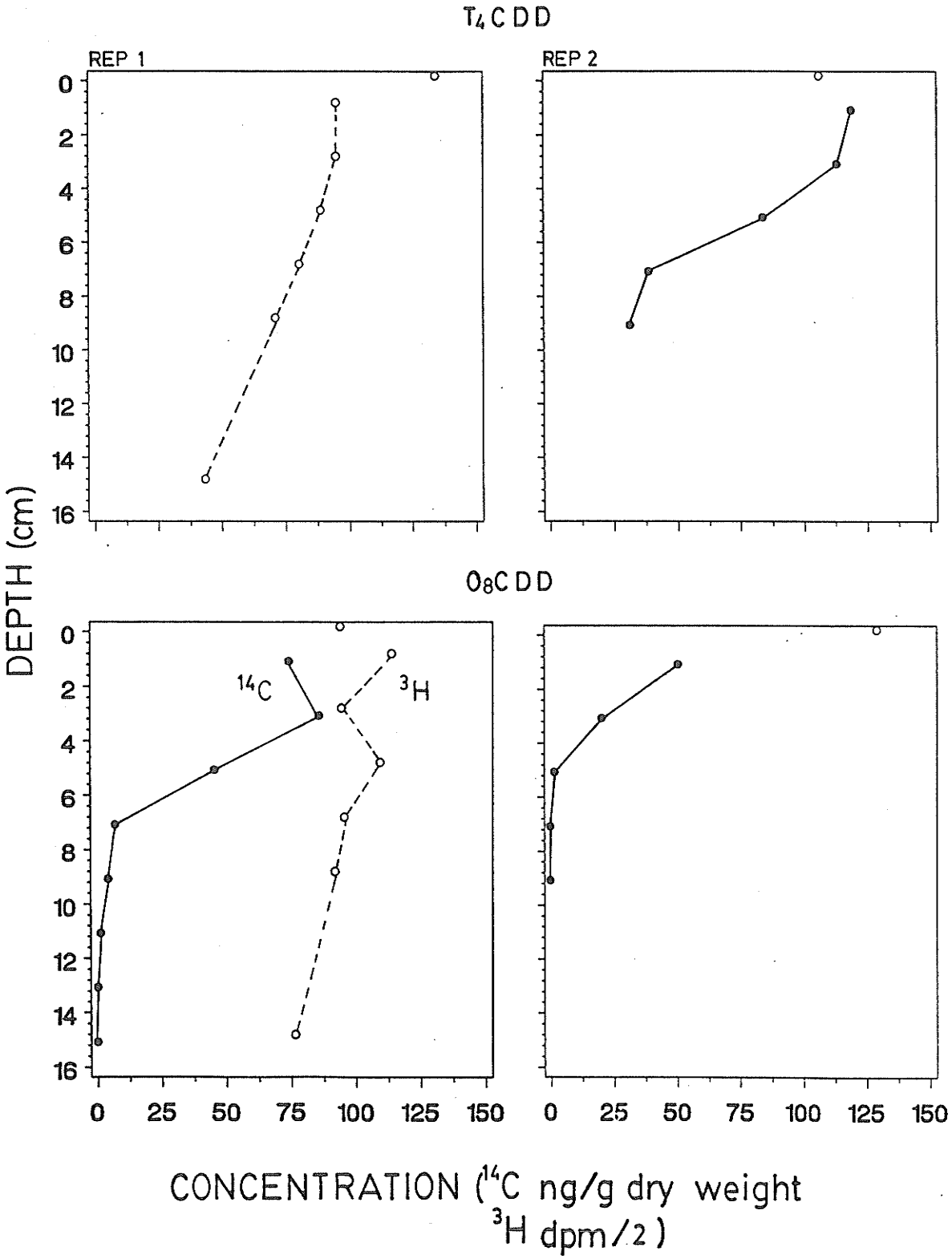
Sediment core profiles for PCDDs in lake enclosures.

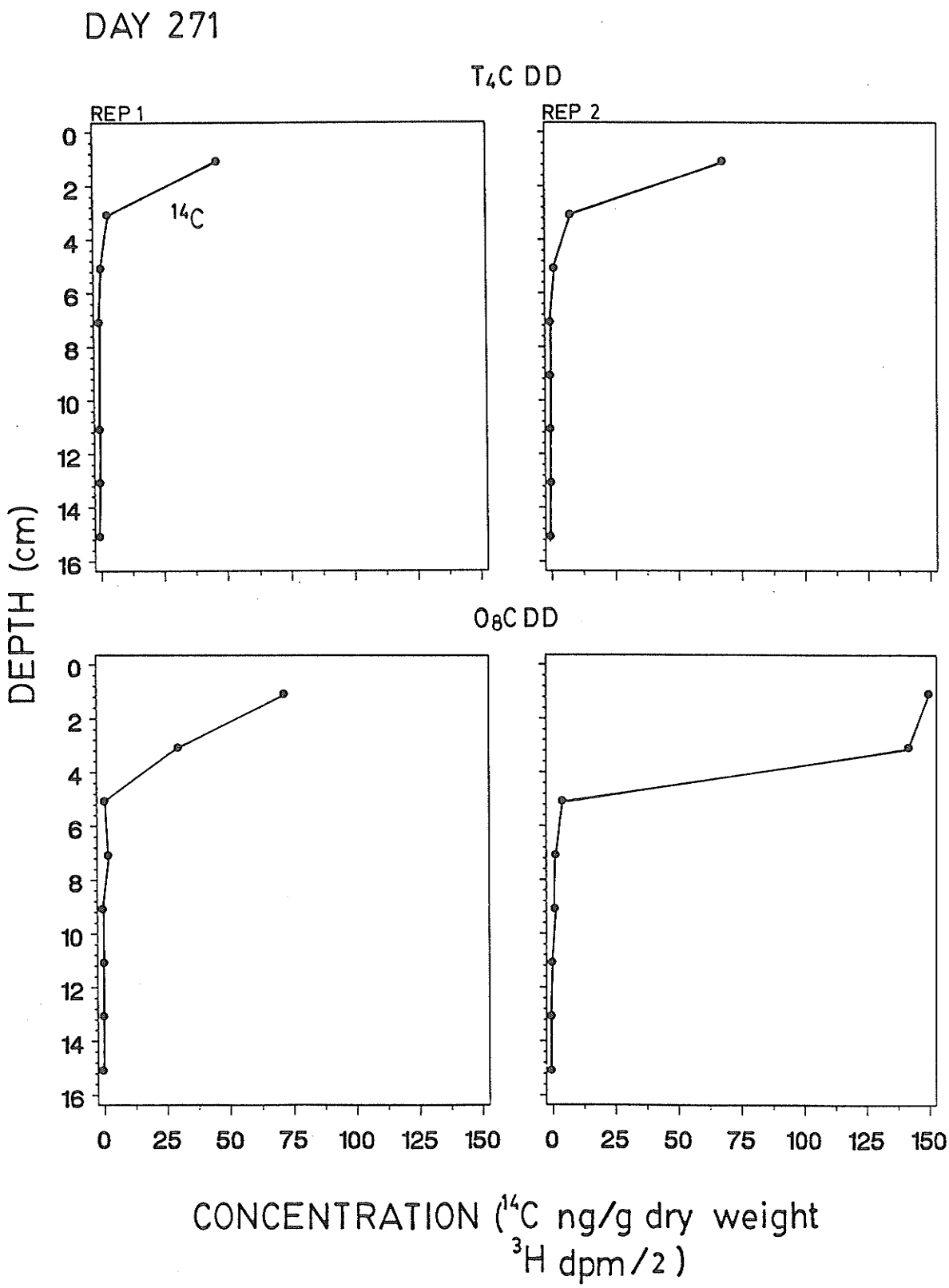


DAY 8


T4CDD
REP 1
REP 2
14C
3H
O8CDD
DEPTH (cm)
0
2
4
6
8
10
12
14
16
0
25
50
75
100
125
150
CONCENTRATION (14C ng/g dry weight
3H dpm/2)


DAY 25
T4CDD
REP 1
REP 2
14C
3H
O8CDD
DEPTH (cm)
0
2
4
6
8
10
12
14
16
0
25
50
75
100
125
150
CONCENTRATION (14C ng/g dry weight
3H dpm/2)


DAY 54
T4CDD
REP 1
REP 2
O8CDD
DEPTH (cm)
0
2
4
6
8
10
12
14
16
14C
3H
0
25
50
75
100
125
150
CONCENTRATION (14C ng/g dry weight
3H dpm/2)


DAY 271
T4C DD
REP 1
REP 2
14C
O8C DD
DEPTH (cm)
0
2
4
6
8
10
12
14
16
0
25
50
75
100
125
150
CONCENTRATION (14C ng/g dry weight
3H dpm/2)

APPENDIX E

The fixed solution at equilibrium for the NRC Persistence model (Roberts et al. 1981).

Fractional retention

$$F_i = \frac{K_i W_i}{K_1 W_1 + K_{CA} W_2 + K_S W_3 + K_F W_4 N}$$

where

F_i is fractional retention in compartment i

K_i is the partition coefficient

0 removal/degradation

1 water

2 catchall (suspended sediment)

3 sediment

4 fish

and

$K_1 = 1$ (water)

$K_{CA} = k_{21}/k_{12}$ (catchall)

$K_S = k_{31}/k_{13}$ (sediment)

$K_F = k_{41}/k_{FC}$ (fish)

k_{ij} is the transfer rate constant between compartments and

W_i is the mass in compartment i

$W_2 = CA_{ppm} \times 10^6 W_1$

W_4 = mass of each fish

N = number of fish

Fractional degradation

$$D_f = \frac{f}{k_{01}K_1W_1 + k_{02}K_{CA}W_2 + k_{03}K_SW_3 + k_{04}W_4^{-0.2}K_FW_4N}$$

where

D_f is the fractional degradation by each process

f can equal $k^vK_1W_1$, $k^hK_1W_1$, $k^pK_1W_1$, $k_{02}K_{CA}W_2$, $k_{03}K_SW_3$, or $k_{04}W_4^{-0.2}K_FW_4N$

k^v volatilization

k^h hydrolysis

k^p photolysis

k_{02} removal from catchall

k_{03} removal from sediment

k_{04} removal from fish

Retentive capacity

$$RC = \frac{K_1W_1 + K_{CA}W_2 + K_SW_3 + K_FW_4N}{k_{01}K_1W_1 + k_{02}K_{CA}W_2 + k_{03}K_SW_3 + k_{04}W_4^{-0.2}K_FW_4N}$$

where

RC is the retentive capacity

$k_{01} = k^v + k^h + k^p$

System half-life

$t_{1/2} = \ln 2\ RC$

APPENDIX F

Multiple comparisons of the mean concentrations of PCDDs in biota using least significant difference (LSD) method (Kleinbaum and Kupper 1978).

White suckers

TIME	TISSUE	T_4CDD		O_8CDD	
		water	sediment	water	sediment
10 d					
	gill	25.2^{a}	21.4^{a}	2.8^{b}	2.9^{b}
	carcass	4.2^{ab}	3.1^{a}	2.9^{b}	1.1^{b}
	gut	125.7^{ab}	133.0^{a}	33.9^{b}	68.7^{ab}
24 d					
	gill	3.9^{a}	2.1^{ab}	3.8^{a}	1.3^{b}
	carcass	1.0^{a}	0.5^{b}	0.9^{a}	0.4^{b}
	gut	10.0^{a}	16.3^{a}	13.3^{a}	12.0^{a}

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

Unionid clams

TIME	T_4CDD		O_8CDD	
	water	sediment	water	sediment
10 d	25.2^a	21.4^a	2.8^b	2.9^b
24 d	1.6^a	0.9^b	0.7^b	0.5^b

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

Benthic invertebrates

TIME	SPECIES	T_4CDD	O_8CDD
10 d			
	H.azteca water	22.9[a]	3.2[d]
	sediment	11.1[c]	trace
	Limniphilus sp.	14.3[bc]	5.9[e]
	Ephemerella sp.	20.3[ab]	nd
	A. limosa	19.0[ab]	2.8[d]
	C. laurentianus	18.5[ab]	nd
24 d			
	H. aztecawater	nd	nd
	sediment	nd	nd
	Limniphilus sp.	4.3[a]	4.8[a]
	Ephemerella sp.	nd	nd
	A. limosa	3.9[a]	nd

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