

The Fate of 1,3,6,8-Tetrachlorodibenzo-p-dioxin,
a Trace Contaminant in the Herbicide 2,4-D,
in a Model Aquatic System

by

Robert L. Corbet

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ABSTRACT

A series of model outdoor pools were treated with ^{14}C -1,3,6,8- T_4CDD (pool 1, 980.0; pool 2, 245.0; pool 3, control; pool 5, 98.0 ng L^{-1}) and its movements and accumulation observed in air, water, hydrosol, vegetation, fish and invertebrates.

Following treatment of the pools, 1,3,6,8- T_4CDD was lost through volatilization from the pool surfaces during the first day. The remaining ^{14}C moved rapidly through the water column with 90% lost from the water phase within 96 h ($t_{\frac{1}{2}}$ values: pool 1 = 134 h, 2 = 20.8 h, pool 5 = 17.8 h). Uptake by the hydrosol occurred during the first 2 to 8 d following which the concentration present declined resulting in a $t_{\frac{1}{2}}$ value of 16.9 d (pool 2), and 9.1 d (pool 5) from the time of maximum deposition in the hydrosol. Increases in the activity present in all treatments after 34 d (in the hydrosol) were believed due to the end-of-season death of the rooted vegetation and addition of its associated ^{14}C to the hydrosol surface. In the air, water, and soil compartments, the level of treatment affected the rates of deposition and persistence in each of these compartments.

Both floating and rooted vegetation bioconcentrated 1,3,6,8- T_4CDD to levels reaching 2.5 to 3.4 $\times 10^4$ (duckweed) and 2.7 $\times 10^4$ to 2.0 $\times 10^5$ (rooted vegetation) times the surrounding water. The $t_{\frac{1}{2}}$ values for duckweed were 9.9 to 11.0 d and 28.2 to 113 for the rooted group. Similar magnitudes of accumulation were observed in the pond snail (Gyraulus parvus), Lymnea stagnalis and members of the family Notonectidae as well as in the (Pimephales promelas Rafinesque) fish population. Experiments performed on G. parvus indicate little or no clearance occurs over a 96 h test. This species also was found to accumulate

1,3,6,8-T₄CDD (BCF = 18.6 to 41.8 x 10³) and retain it for 32.6 to 35.1 d.

1,3,6,8-T₄CDD is photolytically transformed (t_½ = 17.3 d) and this loss route is enhanced by the presence of microbes (t_½ = 11.1d). There appears to be no thermal effect on photolytic degradation even though substantial amounts of material (¹⁴C) were lost from shielded containers. Two metabolic forms were partially characterized but a large portion of the ¹⁴C transformed to a non-extractable, soluble form which was not characterized.

Metabolites were noted in the water (90 d and 426 d) but none were detected in the soil, air, vegetation, or fish.

Results from this study indicate 1,3,6,8-T₄CDD is similar to 2,3,7,8-T₄CDD in its potential for bioaccumulation in flora and fauna. It is not as persistent as 2,3,7,8-T₄CDD with appreciably less material present by the second year of the study. Though capable of being metabolized, the degree of metabolism and metabolites formed were few or undetectable with the procedures used.

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ABBREVIATIONS

ppt	parts per trillion (ng/L)
ppb	parts per billion (ug/L)
PCDD	polychlorinated dibenzo- <u>p</u> -dioxins
T ₄ CDD	tetrachlorodibenzo- <u>p</u> -dioxins
T ₃ CDD	trichlorodibenzo- <u>p</u> -dioxins
H ₇ CDD	heptachlorodibenzo- <u>p</u> -dioxin
H ₆ CDD	hexachlorodibenzo- <u>p</u> -dioxin
O ₈ CDD	octachlorodibenzo- <u>p</u> -dioxin
G. C.	gas chromatography
HPLC	high pressure liquid chromatography
TLC	thin layer chromatography
t _½	half life
r ²	statistical indicator of closeness of correlation and linear fit
S. D.	standard deviation
PCDF	polychlorinated dibenzofuran
uL	-microlitre
• Å	Angstrom (10 ⁻⁸ cm)
ng	nanogram (L) ⁻⁹ grams)
pg	picogram (10 ⁻¹² grams)
LC ₅₀	concentration of exposure at which 50% mortality occurs
P ₅ CP	pentachlorophenol
BCF	bioconcentration factor
Y	Year
LSC	liquid scintillation counting

I. INTRODUCTION

The dioxin family of polycyclic organic compounds were recognized as early as 1872, yet no real investigations into these compounds occurred until the 1950's (Esposito et al., 1980). Much of the interest in dioxins developed because of their unique chemical stability in biological systems, the significance of anthropogenic origins, extreme toxicity to animals, hydrophobic characteristics which give them high capacities to bioaccumulate, and the widely diverse characteristics of the 75 isomers. The first published characterizations were by Buu-Hoi (1971) where mass spectra of several dioxins were described. Following closely, similar compounds were isolated as impurities in herbicides (Woolson et al., 1972) and industrial chemicals (Rappe and Nilsson, 1972; Jensen and Renberg, 1972).

The environmental input of dioxins, in Canada, has been approximated at 150 tonne y^{-1} (NRCC, 1981b) of which a major portion is from industrial sources. Industrial sources include the generation of dioxins in the synthesis of chlorophenols and their derivatives used in industry (P_5CP) and agriculture (2,4-D, 2,4,5T and other chlorophenol derived herbicides), generation from combustion processes and the release of contaminants from waste disposal of these products. Dioxin isomers are found in many parts of the environment, water supplies, fish and beef products and, recently, dioxin compounds have been detected in human tissue. Dioxins can enter the food chain in many ways; agricultural chemical application, as herbicides and pesticides, may provide one route of entry for their associated dioxin contaminants.

Dioxin isomers in several phenoxyacetic herbicides have been

noted since the early 1950's (Woolson et al., 1972) but it wasn't until 1980 that Cochrane et al., (1980) determined specific isomer forms and quantities in various 2,4-D formulations. In this study, they found that the major isomer was 1,3,6,8-tetrachlorodibenzo-p-dioxin (1,3,6,8-T₄CDD) which was detected at levels of 20 to 1800 ng/kg of technical product. Phenoxy herbicides such as 2,4-D are heavily used in western Canada with sales exceeding 3.2×10^6 kg in 1980 (Wheland, 1980). It is therefore probable that they contribute through runoff, groundwater, or directly in aquatic macrophyte control, to the environmental deposition of dioxin isomers into aquatic systems.

The knowledge regarding toxicity, bioaccumulation, and mobility in soil or water of 1,3,6,8-T₄CDD is unavailable at this time and without this data the relative environmental risk presented by this isomer is difficult to assess. The objective of this study was to determine the fate of 1,3,6,8-T₄CDD in various biological and chemical compartments in a series of outdoor artificial water impoundments.

Specially constructed pools were treated with 3 levels of universally labeled ¹⁴C-1,3,6,8-T₄CDD and the movement of ¹⁴C was followed in air, water, hyrosoil, rooted and floating vegetation, fathead minnows and invertebrates (pond snail) by HPLC, LSC, and GC. The project included the examination of the photodegradation of 1,3,6,8-T₄CDD, accumulation in biotic and physical media, volatile losses, persistence in biotic and nonbiotic media of parent compound and the possible appearance of degradation products.

II. LITERATURE REVIEW

1.0 PROPERTIES

Dibenzo-p-dioxins are a family of polycyclic compounds with a triple ring structure consisting of two benzene rings linked to each other by two oxygen bridges. There are eight possible sites for substitution by halogens, hydrogen or organic radicals (Figure 1.0). The combinations of substituents and the degree of substitution gives rise to a wide range of uniquely different compounds which vary in such aspects as toxicity, routes of degradation, solubility and presence in the environment (Esposito et al., 1980). Only a limited amount of information has been published on 1,3,6,8-T₄CDD (Rappe et al., 1978; Cochrane et al., 1980), therefore, this review will deal with chlorinated dioxins in general with specific reference to information on 2,3,7,8-T₄CDD which has received the greatest amount of study. There is no documented evidence that chlorinated dioxins are biosynthetically formed; however, it has been established by several authors that they are formed during certain combustion processes (Olie et al., 1977). Each isomer has a distinct set of properties. Generally mono-, di-, and trichloro forms are assumed to be more readily degradable because of the availability of ring carbons to chemical and biological attack.

Being planar and the placement of the chlorine substituents at the ends of the molecule, appear to be responsible for the extreme toxicity and recalcitrant degradation of 2,3,7,8-T₄CDD.

Like most halogenated hydrocarbons, PCDD's have properties which include the following:

1. They each have a stable aromatic nucleus.
2. They are relatively inert to acids, bases, oxidation,

 CHLORINATED DIOXINS

1-chloro	1,2,3,4-tetrachloro	1,2,3,4,6-pentachloro
2-chloro	1,2,3,6-tetrachloro	1,2,3,4,7-pentachloro
1,2-dichloro	1,2,3,7-tetrachloro	1,2,3,6,7-pentachloro
1,3-dichloro	1,2,3,8-tetrachloro	1,2,3,6,8-pentachloro
1,4-dichloro	1,2,3,3-tetrachloro	1,2,3,6,9-pentachloro
1,6-dichloro	1,2,4,6-tetrachloro	1,2,3,7,8-pentachloro
1,7-dichloro	1,2,4,7-tetrachloro	1,2,3,7,9-pentachloro
1,8-dichloro	1,2,4,8-tetrachloro	1,2,3,8,9-pentachloro
1,9-dichloro	1,2,4,9-tetrachloro	1,2,4,6,7-pentachloro
2,3-dichloro	1,2,6,7-tetrachloro	1,2,4,6,8-pentachloro
2,7-dichloro	1,2,6,8-tetrachloro	1,2,4,6,9-pentachloro
2,8-dichloro	1,2,6,9-tetrachloro	1,2,4,7,8-pentachloro
1,2,3-trichloro	1,2,7,8-tetrachloro	1,2,4,7,9-pentachloro
1,2,4-trichloro	1,2,7,9-tetrachloro	1,2,4,8,9-pentachloro
1,2,6-trichloro	1,2,8,9-tetrachloro	1,2,3,4,6,7-hexachloro
1,2,7-trichloro	1,3,6,8-tetrachloro	1,2,3,4,6,8-hexachloro
1,2,8-trichloro	1,3,6,9-tetrachloro	1,2,3,4,6,9-hexachloro
1,2,9-trichloro	1,3,7,8-tetrachloro	1,2,3,4,7,8-hexachloro
1,3,6-trichloro	1,3,7,9-tetrachloro	1,2,3,6,7,8-hexachloro
1,3,7-trichloro	1,4,6,9-tetrachloro	1,2,3,6,7,9-hexachloro
1,3,8-trichloro	1,4,7,8-tetrachloro	1,2,3,6,8,9-hexachloro
1,3,9-trichloro	2,3,7,8-tetrachloro	1,2,3,7,8,9-hexachloro
1,4,6-trichloro		1,2,4,6,7,9-hexachloro
1,4,7-trichloro		1,2,4,6,8,9-hexachloro
2,3,6-trichloro		1,2,3,4,6,7,8-heptachloro
2,3,7-trichloro		1,2,3,4,6,7,9-heptachloro
		Octachloro

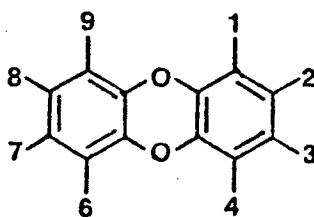


Figure 1.0. List of Chlorodioxin Isomers and General Structure (from Esposito *et al.*, 1980)

Table 1.0 Properties of Various Chlorodioxins (Firestone, 1977)

Chlorodioxin	Mol. wt	MP °C	"p" value	Est. vapor pressure	Molar refraction	UV max (CHCl ₃), nm
2,7-D ₂ CDD	253.08	-	0.76	6.0 x 10 ⁻⁶	-	302
2,3,7-T ₃ CDD	287.53	162	0.86	3.6 x 10 ⁻⁶	-	305
2,3,7,8-T ₄ CDD	321.87	306	0.51	1.7 x 10 ⁻⁶	71.4	310
1,2,4,7,8-P ₅ CDD	356.42	206	-	-	72.6	307
1,2,3,7,8-P ₅ CDD	356.42	241	-	-	-	308
1,2,4,6,7,9-H ₆ CDD	390.86	240	0.94 ^d	6.6 x 10 ⁻⁷	-	310
1,2,3,6,8,9-H ₆ CDD	390.86	-	-	-	-	281.1
1,2,3,6,7,8-H ₆ CDD	390.86	285	-	-	-	317
1,2,3,7,8,9-H ₆ CDD	390.86	243	-	-	-	317
1,2,3,4,6,7,9-H ₇ CDD	425.31	-	0.90	3.0 x 10 ⁻⁷	85.9	-
1,2,3,4,6,7,8-H ₇ CDD	425.31	-	0.90	-	-	-
1,2,3,4,6,7,8,9-O ₈ CDD	459.75	331	0.90	1.8 x 10 ⁻⁷	90.7	318

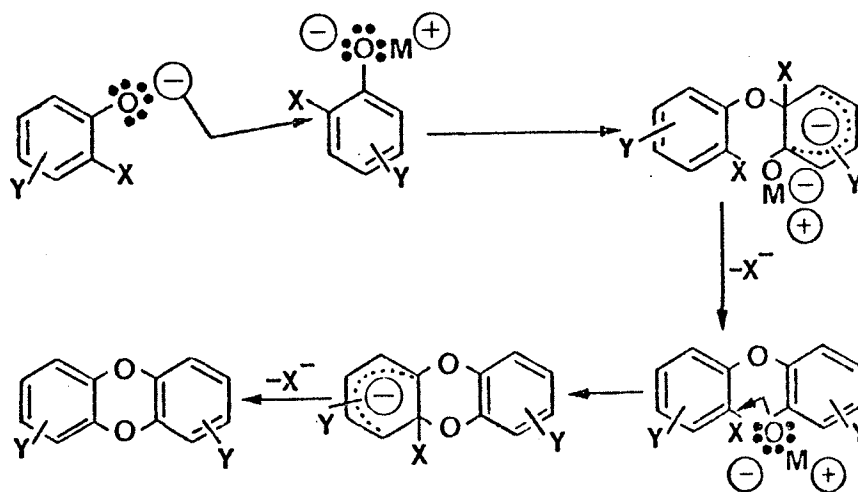
reduction and heat.

3. Similar qualitative toxic responses are believed to be elicited by specific congeners in each group.
4. There is increased environmental stability and lipophilicity with increasing halogen content.

1,3,6,8-T₄CDD is a white crystalline solid, highly lipophilic, being virtually insoluble in water, and only slightly insoluble in hydrated organic solvents (i.e., acetone: 0.01 g/L) (Esposito et al., 1980). The solubility of PCDDs in water has been estimated to be 200 ng/L and, recently, that of 1,3,6,8-T₄CDD has been determined to be 317 ng/L (Webster et al., 1983 α). Extreme hydrophobicity results in bioconcentration in biotic compartments (Isensee et al., and Jones, 1975) and is a concern to the food chain (McCall et al., 1982). The decomposition temperature of 1,3,6,8-T₄CDD is 700°C and therefore, not surprisingly, it has been detected and persists in extreme conditions such as those found in incinerators (Rappe, 1978; Esposito et al., 1980) (refer to Table 1.0).

2.0 FORMATION: GENERAL MECHANISMS

Chlorinated dioxin synthesis involves complex exothermic reactions which are still being evaluated (Esposito et al., 1980). The chlorine substituents can be affected by radiation (sunlight or γ radiation) (Crosby et al., 1971; Buser et al., 1978) and the extent of generation or degradation varies between isomers but is considered to be relatively slow (Lamparski and Nestruck, 1980; Matsumura and Benezet, 1973). A generalization of one synthetic route is described in Figure 2.0. The necessary precursors contain an ortho substituted benzene ring where one substituent is oxygen. This would include the



X=leaving group
 (e.g. Cl, F, Br, I, NO₂, SO₃R)
 M=alkali metal cation
 Y=any substituent group

Figure 2.0 Generalized Synthetic Route for Chlorodioxin
 Formation (from Esposito *et al.*, 1980)

range of phenol compounds and associated aromatic precursors. In a chlorophenol, the hydroxyl provides the oxygen and releases the H^+ to react with the available chlorine (X-). This reaction usually occurs when using a potassium or sodium salt of an ortho-chlorinated phenol (Esposito et al., 1980). An example of this would be the use of 2,3,5-trichlorophenol as the precursor. The reaction in the presence of heat progresses in two steps. Two 2,3,5-trichlorophenol units condense to form a phenoxyphenate of diphenyl ether (pre-dioxin). The second step is ring closure (Figure 2.0) (Jensen and Renberg, 1972; Buser, 1978). In many cases the final form is only generally predicted because of the occurrence of a Smiles rearrangement in which one of the rings spontaneously reverses to its mirror image at the instant of ring closure (Gray et al., 1975).

Several competing reactions occur in any laboratory or environment-based synthesis and are influenced by the precursor utilized and the reaction conditions. If a hydroxy group condenses with a meta chlorine instead of a para chlorine, an iso-predioxin may result (Jensen and Renberg, 1972) (Figure 3.0). If, at this point a chlorine is lost, a dibenzofuran could be generated. Predioxin formation is transitory and, for the most part, the reaction continues to a dioxin or reverts to the precursors. Formation is stimulated in the presence of heat between 145 to 400°C (Buser et al., 1978), and, for some precursors, pressure is necessary.

Dioxins can also be formed by photolytic, catalytic and free radical reactions (Buser, 1976).

Other procedures or mechanisms noted in the synthesis of dioxin isomers include catachol based reactions, substitution, photo-dechlorination, metal substrate catalyst reaction and radical reaction,

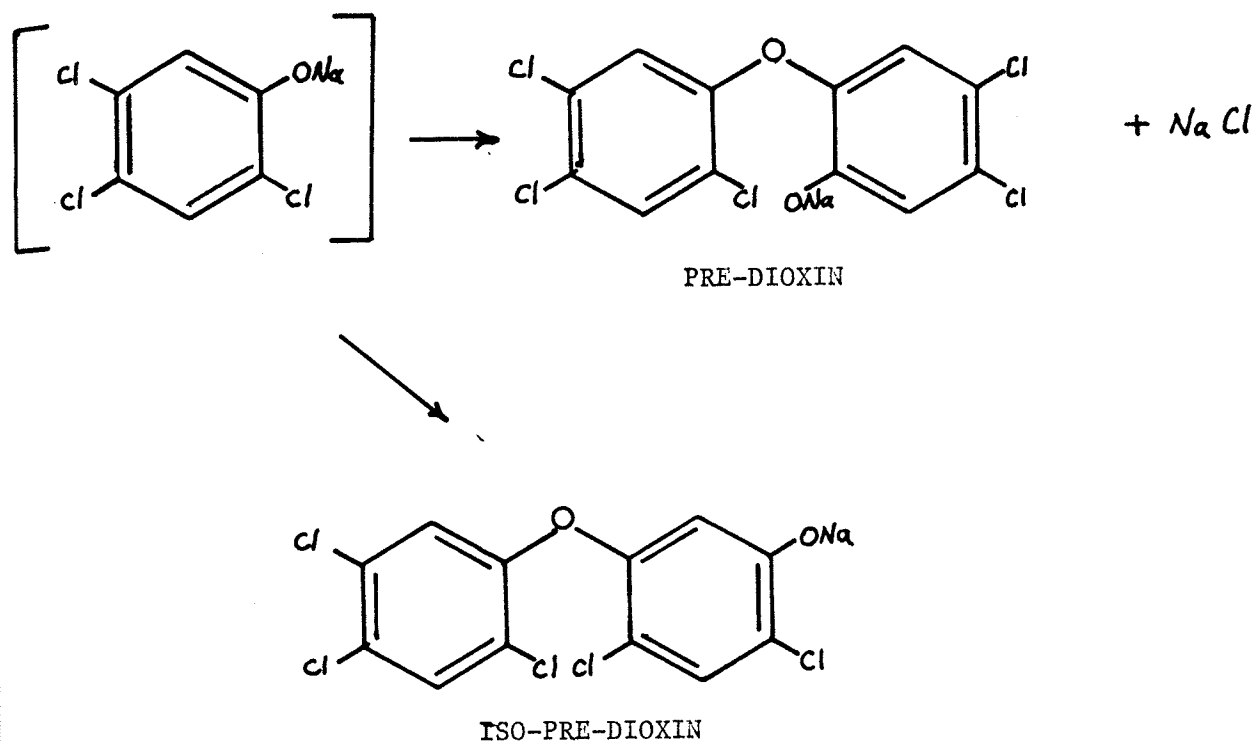


Figure 3.0. Pre-dioxin and Iso-pre-dioxin Formation

(from Esposito et al., 1980)

all of which produce a range of isomers with varied degrees of success (Esposito et al., 1980). The Ullman condensation is a procedure found to be successful in synthesizing 1,3,6,8-T₄CDD and is another example of a mechanism in dioxin formation (Buser, 1975). The dioxin is formed through the pyrolysis of alkali metal salts or ortho-halophenols at 290°C for 1-4 hours in the presence of copper salt. The mechanism involves the nucleophilic attack of the phenoxy ion on a second phenolate ring followed by expulsion of the halide to give rise to a predioxin (Figure 3.0). An intramolecular aromatic substitution occurs followed by halide loss which results in a dioxin.

3.0 SOURCES

3.1 Industrial

Industrial sources of dioxins are primarily associated with chlorophenol manufacturing (Table 2.0). Jones (1981) has noted that Canada consumes 3.4×10^6 kg of chlorophenols annually.

There are 19 different chlorophenols of which 13 are commercially produced for use directly or as precursors for use in organic syntheses of products such as fungicides and bactericides (Jones, 1981). The tri- and tetrachlorophenol products are used as preservatives for wood, latex and leather while pentachlorophenol (P₅CP) is widely used as a preservative for wood, burlap, and glues and is used as a herbicide and even as a cosmetic preservative (Esposito et al., 1980).

Varied amounts of dioxins have been found in P₅CP (Table 3.0), (3700 ppm O₈CDD and 870 ppm H₇CDD) and significant quantities have been detected in other chlorophenols (Buser 1975; Woolsen, et al., 1972; Crummett and Stehl, 1973; Buser and Bossehardt, 1976; and Jensen and Renberg, 1972; Villanueva et al., 1973). Refer to Table 3.0 for isomer

TABLE 2.0 POLYCHLORODIBENZO-p-DIOXINS IN MONO-, DI-, TRI-, TETRA-, AND PENTACHLOROPHENOLS BY ELECTRON CAPTURE GAS CHROMATOGRAPHY (Firestone et al, 1972) (from Jones, 1981)

Sample	Date Recd	Dioxin	Found (ppm)
1 2-CP	4/67	none	-
2 2,4-DCP	4/70	none	-
3 2,6-DCP	-	none	-
4 2,4,5-TCP-Na	9/67	none	-
5 2,4,5-TCP-Na	6/69	2,7-dichloro	0.72
		2,3,7,8-tetrachloro	1.4
6 2,4,5-TCP	6/69	1,3,6,8-tetrachloro	0.30
		2,3,7,8-tetrachloro	6.2
7 2,4,5-TCP	7/70	pentachloro	1.5
8 2,4,5-TCP	7/70	none	-
9 2,4,5-TCP	7/70	2,3,7,8-tetrachloro	0.07
10 2,4,6-TCP	-	2,3,7-trichloro	93
		1,3,6,8-tetrachloro	49
11 2,3,4,6-TCP	-	hexachloro	15
		hexachloro	14
		heptachloro	5.1
		octachloro	0.17
12 2,3,4,6-TCP	3/67	hexachloro	4.1
13 2,3,4,6-TCP		none	-
14 PCP-Na	9/67	hexa	14
		hepta	5.4
		hepta	9.1
		octa	3.8
15 PCP-Na	6/69	hexa	20
		hepta	1.3
		hepta	10
		octa	3.3
16 PCP	5/70	hexa	0.96
		hexa	38
		hepta	10
		hepta	39
		octa	15
17 PCP	7/70	hexa	35
		hepta	23
18 PCP	3/67	hexa	0.03
		hexa	0.14

TABLE 3.0 . . DIOXIN CONGENERS IN COMMERCIAL PCP
(Firestone, 1977) (from Jones, 1981) ⁵

No Smiles rearrangement	With Smiles rearrangement	Relative % isomers found	PCP, ppm ^a
1,3,6,8 (100%)	1,3,6,8 (25%) 1,3,7,9 (75%)	none	ND
1,2,4,7,9 (75%)	1,2,4,7,9 (31.25%)		
1,2,3,7,9 (25%)	1,2,3,7,9 (25%) 1,2,4,6,8 (43.75%)	none	ND
1,2,3,6,8,9 (50%)	1,2,3,6,7,9 (31.25%)	40-50	
1,2,3,6,7,8 (25%)	1,2,3,6,8,9 (18.75%)		
1,2,4,6,7,9 (25%)	1,2,4,6,7,9 (12.5%) 1,2,4,6,8,9 (12.5%) 1,2,3,7,8,9 (18.75%) 1,2,3,6,7,8 (6.25%)	20-40 trace 20-40	ca 15
1,2,3,4,6,7,9 (75%)	1,2,3,4,6,7,9 (75%)	ca 60	ca 200
1,2,3,4,6,7,8 (25%)	1,2,3,4,6,7,8 (25%)	ca 40	
1,2,3,4,6,7,8,9 (100%)	1,2,3,4,6,7,8,9 (100%)	100	ca 1000

specific levels (Jones, 1981).

PCDDs can be generated via the production of phenoxy radicals from the decomposition of polychlorocyclohexadiene produced by the over-chlorination of tri-, tetra- and pentachlorophenol (Firestone, 1977; Esposito et al., 1980). The radicals cause the formation of phenoxyphenols which cyclize to chlorodioxins (Jones, 1981). Nilsson (1977) and Buser (1976b) defined the industrial routes of PCDD formation as: 1) direct chlorination of isomer present, 2) condensation of chlorocatechols and chloronitrobenzene, 3) pyrolysis and dimerization of chlorophenates, and 4) cyclization of chlorinated-2-phenoxyphenols. The presence of heat, pressure and/or alkali conditions enhance these processes.

PCDDs have appeared in wood preservatives at levels reaching 6 to 15 ppm O_8 CDD and 1.3 to 39 ppm H_7 CDD (Nilsson and Renberg, 1974; Jensen and Renberg, 1972; Dobbs and Grant, 1981). Recent work has indicated that O_8 CDD can be formed on wood treated with pentachlorophenol and exposed to light (Lamparski et al., 1980). Lamberton et al., (1979) also found PCDD isomers in the circulating preservative solution in several wood-pulp plants. Another form of industrial contamination occurs as the dioxins are distributed locally to air, and soil and become associated with the industrial plants themselves. It has been estimated that there is as much as 100 ppb of 2,3,7,8- T_4 CDD in the soil surrounding the Dow plant in Midland, Michigan, U.S.A. and 1-3 ppb of assorted isomers in the air (Baughman and Meselson, 1973; Nestricks, 1981). Industrial contamination occurs due to off-site losses from storage and direct industrial dumping (Thibodeau, 1982).

Localized dissipation of dioxins has occurred via industrial

accidents. The most dramatic incident occurred in 1976 in Seveso, Italy. In this case, 2 to 3 kg of 2,3,7,8-T₄CDD is estimated to have been released over an area of 2000 hectares with most of the contamination being found within 500 m of the plant. No mortalities were recorded; however, numerous clinical manifestations on victims were noted. Vegetable and animal stocks in the exposed area had to be destroyed and the area evacuated (Pocchiari, 1978; Di Domenico et al., 1980). There have been thirteen serious accidents in 2,4,5-TCP plants alone during which workers were exposed to PCDDs. In one case in Holland, the plant was so heavily contaminated that it was encased in concrete and sunk in the Atlantic Ocean (Kriebel, 1981).

The formation and presence of PCDDs and PCDFs in transformer fluids and associated electrical equipment has been documented (Chittim et al., 1982; Smith et al., 1981). Though the initial levels of PCDD's in Arochlors, and associated Pyranol, Inerteen and Chlorextrol are low, long term use and heat have been shown to generate chlorodioxin contaminants.

As noted earlier, contaminants can enter several areas from chlorophenol-precursor-based synthesis. At present, the EPA has indicated the PCDD generation may occur in the production of over 82 industrial chemicals (Esposito et al., 1980; Jensen and Renberg, 1972).

3.2 Agriculture

Chlorinated dibenzo-p-dioxins are known to be present in trace amounts in insecticide and herbicide products derived from chlorophenols (Esposito et al., 1980; Cochrane et al., 1980; Woolson et al., 1972). Presently, there are over forty products which contain or are likely to contain PCDD isomers (Table 4.0).

Table 4.0 Listing of Agricultural Chemicals Likely to Contain PCDDs.

Bifenox*	2,3,5-T + esters + Salts*
Chloranil*	Bromoxynil + esters*
2,4-D esters + salts*	Carbophenothion*
2,4-DB + salts*	DCPA
Dicamba + diethylamine salt*	Dichlone
Dicapthon*	Dinitrobutylphenol + ammonia salt
Dichlofenthion*	Ioxynil
Sesone*	Lindane*
2,4-DP	MCPA*
Erbon*	MCPB*
Hexachlorophene*	Mecoprop*
Isobac 20	Parathion*
Nitrofen*	PCNP*
P ₅ CP + salts*	Piperalin
Ronnel*	Propanil*
Silvex + esters + salts*	Tetradifon*

* = Actively used in Canada.

This listing was made by Esposito et al., (1980) and is based partially on the structural likelihood of dioxin production during synthesis and viability of the chemical as a dioxin precursor. Several studies have confirmed the presence of dioxins in phenoxy acid herbicides (Woolson et al., 1972; Fishbein, 1973; Cochrane et al., 1980). Due to the nature of the product reaction sequence, the prediction of the synthesized dioxin isomers is difficult and heavily influenced by complex secondary reactions (Fishbein, 1973). The use of 2,4,5-trichlorophenol

as a starting material in the manufacture of 2,4,5-T, Silvex, and Ronnel, yields the highest levels of dioxin contaminants in these products. In 1970, 2,4,5-trichlorophenoxy acid was implicated as a teratogen in rats (Courtney et al., 1970) but it was not until further tests that it appeared that the teratogenesis may have been due to the 27±8 ppm of the 2,3,7,8-T₄CDD contaminant in the herbicide (Edmunds et al., 1973). Forty percent of the 2,4,5-T samples taken by Edmunds et al., (1973) had 0.5 ppm of 2,3,7,8-T₄CDD. Rappe (1978) found 1,3,6,8-T₄CDD, 1,3,7,-T₃CDD as well as 2,3,7,8-T₄CDD in his analysis of Agent Orange components. It is estimated that the use of the defoliant, "Agent Orange" in Vietnam dispersed between 0.1 to 47 mg of 2,3,7,8-T₄CDD per kg of technical material (Rappe, 1978).

In 1981, Yamagishi et al. found 150 and 30 ppm of T₄CDD and P₅CDD, respectively, in diphenyl ether herbicides*. The same contaminants were found in local fresh water fish with 0.2 ppb of 1,3,6,8-T₄CDD being present. Of the six T₄CDDs in the formulations, the major form found was also 1,3,6,8-T₄CDD.

Similarly, Cochrane et al. (1980) analyzed 16 formulations of 2,4-D amine and ester products. Eight of nine ester formulations and 4 of 7 amine formulations contained D₂-, T₃- and T₄CDDs (Figure 4.0). The 2,7- and 2,8-D₂CDD reached levels of 104 to 4,200 ppb. T₃CDD ranged from 346 to 2,000 ppb and the T₄CDD ranged from 226 to 1,700 ppb. The main isomer was 1,3,6,8-T₄CDD and Cochrane believed that it was generated from the production based dimerization of 2,4,6-trichlorophenol.

- * CNP formulations (1,3,5-Trichloro-2-(4-nitrophenoxy) benzene)
 NIP formulations (nitrofen, 2,4-dichloro-1-(4-nitrophenoxy) benzene)
 X-52 formulations (chlomethoxynil, 2,4-dichloro-1-(3-methoxy-4-nitrophenoxy) benzene)

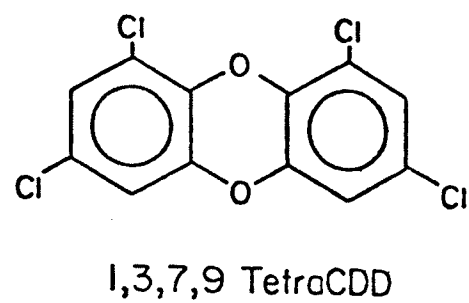
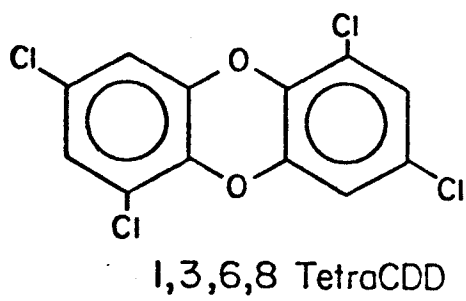
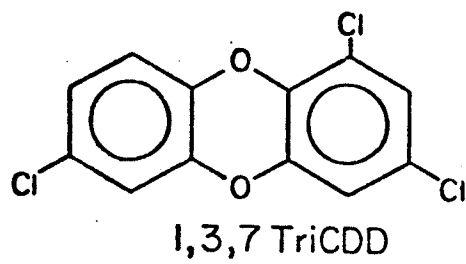
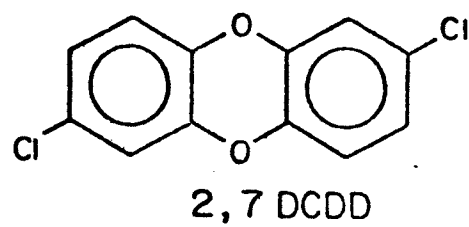


Figure 4.0. Dioxin Isomers Noted in 2,4D (Cochrane *et al.*, 1981)

There appears to be no possibility of dioxin generation while herbicides are in storage after factory production.

3.3 Combustion

An increasing number of references refer to the generation of dioxins from the incineration of municipal wastes and the burning of wood, and industrial waste. Stehl et al., (1973), Aniline (1973) and Buser (1975) pyrolyzed paper and chlorophenols to generate trace amounts of T₄⁻, P₅⁻, H₆⁻ and O₈CDD. Stehl and Lamparski (1977) found that only 1.2 to 5 x 10⁻⁵ percent by weight of 2,4,5-T was converted to 2,3,7,8-T₄CDD. There were however much higher quantities of H₇⁻ and O₈CDDs present. Many reports confirm the presence of dioxin isomers in fly ash and flue gases. Buser et al., (1978) reported that a Swiss municipal incinerator was generating flue gases with 0.2 ppm of PCDD and fly ash containing 33 separate isomers. Buser et al., (1978) found 37 of the 75 possible isomers in fly ash from an electrostatic precipitator of a power plant in Switzerland. The major T₄CDD found were the 1,3,6,8- and 1,3,7,9- isomers. Olie et al., (1977; 1980) confirmed this work by isolating 17 isomers from fly ash in a Netherlands plant. In 1978, Tiernan and Taylor detected seven T₄CDD isomers including 2,3,7,8- and the 1,3,6,8- isomers in fly ash from an American incinerator.

Choudhry et al., (1980) suggested three routes for the presence of dioxins in incinerators as follows:

- 1) the PCDDs might be present in trace amounts in the original waste,
- 2) thermal formation of homologues might occur from precursors in the waste,
- 3) de novo synthesis from elements in the waste.

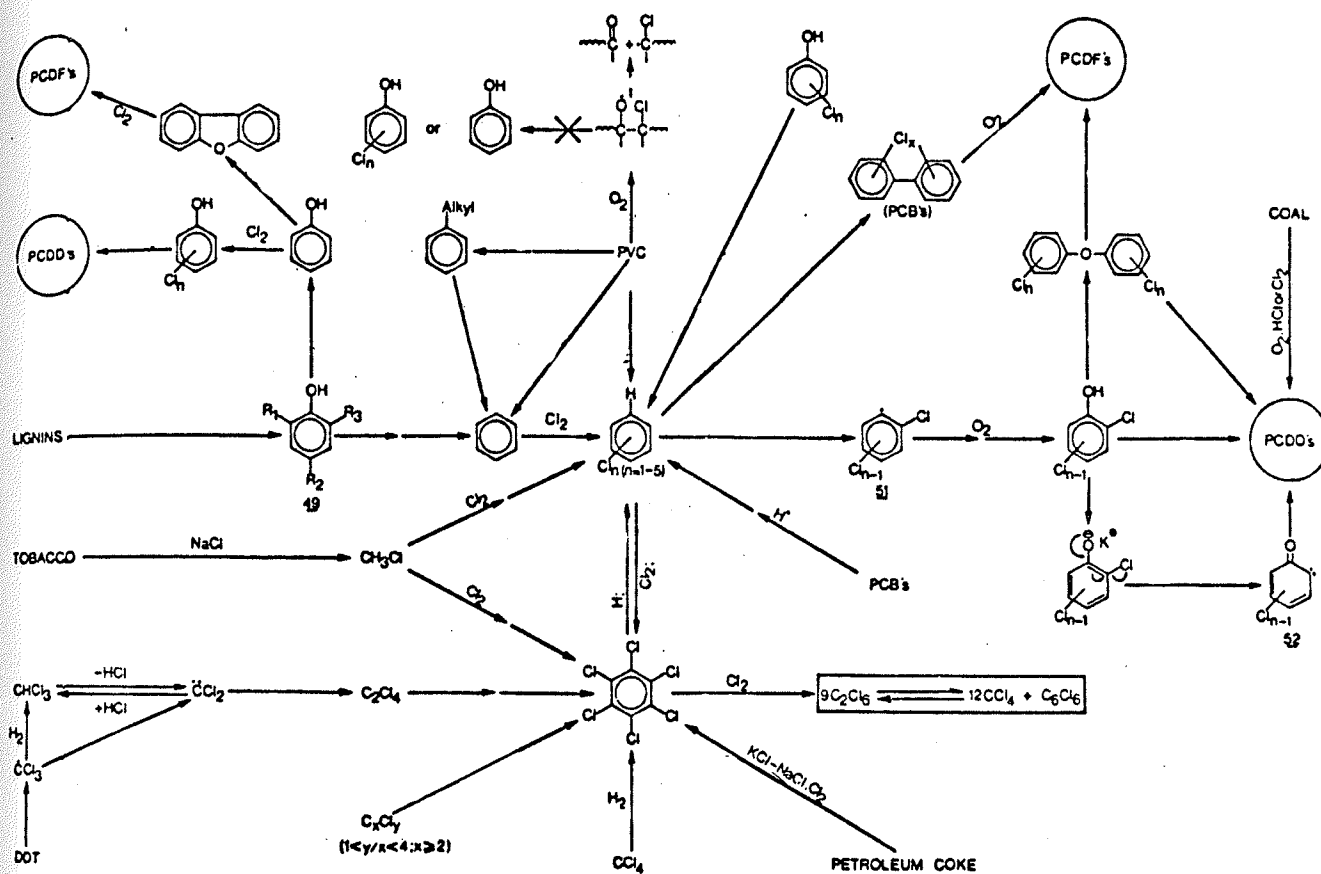


Figure 5.0. A Speculative Source of PCDD's and PCDF's Found in Incinerator Effluents (from Choudhry *et al.*, 1980).

There is validity in all three processes. Figure 5.0 denotes a series of possible de novo processes which could give rise to dioxins. Proof of route 2 is seen in Ahling et al.'s (1977) work in which 2,4,5-T was burned generating 0.05 to 3.0 mg of PCDDs per kilogram of formulation. In a later work (Ahling and Lindskog, 1980), they found that chlorobenzene precursors of PCDDs are formed from the combustion of pulp liquor which has no chlorinated hydrocarbons present. This would mean that the chlorine precursor came from inorganic sources, wood, PCBs or PVCs. The amount of dioxin formed ranged up to 240 mg/g for P₅CDD and 260 mg/g for H₆CDD. Jansson et al. (1978) combusted various chlorophenols and generated 4 to 360 mg T₄CDD per kg. Combustion was carried out at 500-600°C and Jansson found he could decrease the dioxin release by increasing the oxygen content to increase oxidation and by increasing the residence time of the phenols in the furnace. In attempts to combust pentachlorophenol (P₅CP) he generated relatively low amounts of dioxins (0.05-1.7 mg/kg. The concentrations of the homologues generated varied between sources and between sample periods (Lustenhouwer et al., 1980; Eiceman et al., 1981). One exception to the above studies was seen in Junk and Richards' (1981) work. They detected no residues in the stack particulates, however, the incinerator was operating in excess of 1200°C with abundant amounts of oxygen.

In tests by Liberti and Brocco (1980) dioxin products varied with the pre-treatment of the waste. Plants burning only vegetable matter generated little dioxin. They suggested this was due to a lack of a chlorine donor precursor which limited any reactions with a comparable phenolic or polyphenolic source.

Industrial sites provide the primary source of dioxin release

(direct or indirect) but attempts are being made to put their input in perspective by looking at other contributors. In order to explain the ubiquitous distribution of dioxin isomers in the environment, Dow Chemical Co. proposed the "trace chemistries of fire" hypothesis (1978) (Bumb et al., 1980). They proposed that dioxins were the product of micro-thermal synthesis, the variety and magnitude of which is a function of the presence of chloro-organic precursors in common waste material. These materials include packaging, plastics, wood preservatives, polymers, municipal sludge, herbicide residues (aldrin, 2,4-D) and natural precursors (catechol, vanillin). They suggested that formation was a complex operation occurring in all combustion processes and that natural processes presented a significant source for dioxin generation and dissipation e.g., forest fires, burning of natural material, wood and paper). Traces of dioxin compounds have been found in cigarette smoke, in the soil around power plants, in vehicle exhaust, in incinerator fly ash and recently, in chimneys of wood burning stoves (Bumb et al., 1980; Nestruck and Lamparski, 1982; Dow, 1978).

In Nestruck's (1982) study, he found PCDDs in chimney particulates of wood stoves in the western and central United States. He found levels of 1,3,6,8-, 1,3,7,9- and 2,3,7,8-T₄CDD of 4.6 to 24.0 ppt., 3.8 to 25.0 ppt and 6.4 to 20.0 ppt respectively. He also found H₆-, H₇- and O₈CDDs. The chimneys of the central U.S.A. were more contaminated (e.g., 1,3,6,8-T₄CDD up to 340 ppt) but their results varied greatly between chimney types. The relative significance to the loading of the environment of dioxins by these sources has yet to be assessed.

4.0 TOXICITY-TOXICOLOGY

4.1 Mode of Action

There is no confirmed mechanism for the toxic action of dioxin

isomers but there are several strong correlations noted in the literature. In a review by Neal et al., (1979), it was pointed out that 2,3,7,8-T₄CDD does not affect cell growth, replication, nutrient utilization, metabolism of reactive metabolites, covalent bonding or ATP synthesis. However, in 1976, Poland et al., described a strong correlation between the induction of aryl hydrocarbon hydroxylase (AHH) and a toxic response. It was postulated that halogenated compounds, such as PCDDs, bind to a common stereo-specific site which induces the synthesis of a variety of proteins which in turn give a toxic response (Goldstein et al., 1978). Kitchen and Wood (1979) found that 2,3,7,8-T₄CDD stimulates a number of cytochrome P₄₄₈ mediated mono-oxygenase activities in the livers of rats, mice and chickens (e.g., AHH). Such enzyme induction has been noted in lung, kidney, intestine, and the fetuses of exposed rats (McKinney et al., 1980). This localization of receptors in the cytosol of certain tissues suggests a steroid hormone-like receptor being present (McConnell et al., 1978,1979). Induction by 2,3,7,8-T₄CDD occurs at 0.27 mg/kg and has been known to occur in rats at levels as low as 0.002 mg/kg body weight. Induction can last for 31 days after exposure. How the induction and generation of microsomal components in turn triggers a toxic reaction is unknown. A current description of the induction process can be seen in Figure 6.0 (Esposito et al., 1980). Greenlee and Poland (1979) believed that dioxins bind to low capacity cytosolic receptor protein to form a non-covalently bound receptor-inducer complex which actually enters the nucleus and interacts with the P₄₈₈ genome. There are two types of inducers, the PB (phenobarbitone) type and the MC (3-methylcholanthrene) type used in describing enzyme induction mechanism. Dioxins are believed to be the MC variety (McKinney

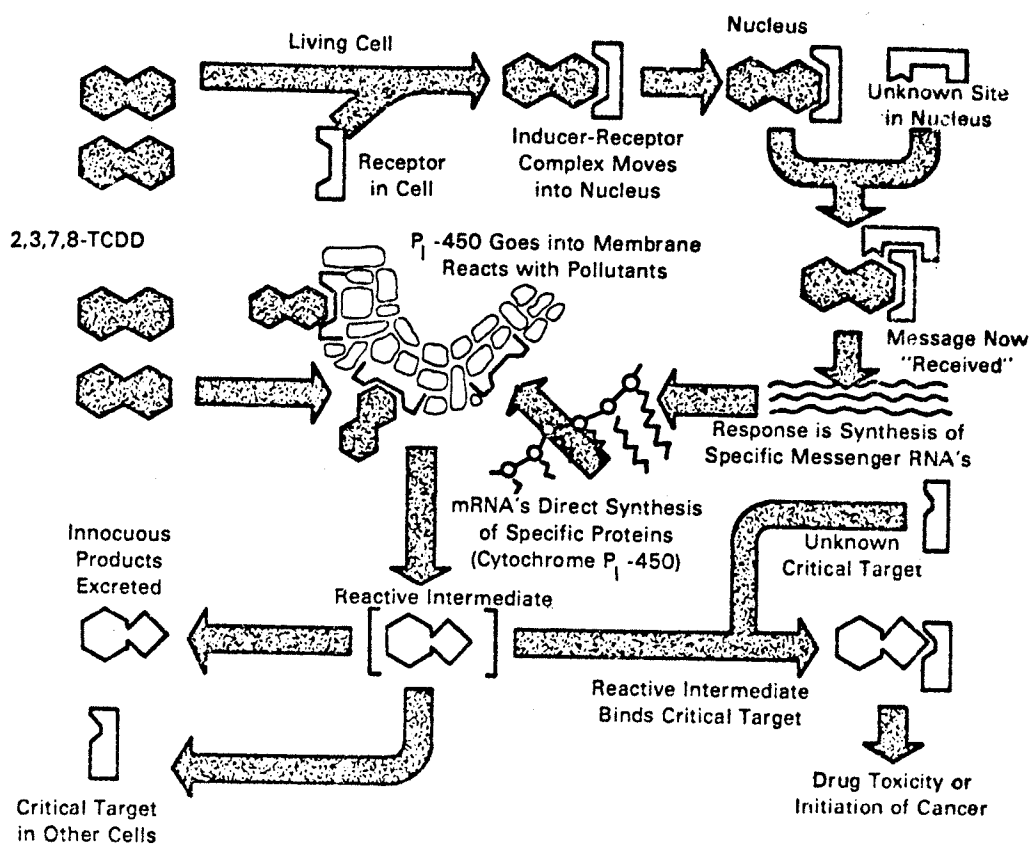


Figure 6.0. Proposed Mechanism for Induction of AHH and Toxicity by 2,3,7,8- T_4 CDD (from Neal et al, 1979).

et al., 1980). A more detailed discussion can be seen in Buckel's 1980 work.

The magnitude of the induction, and therefore, the toxicity, varies between the isomers and test species used. According to McKinney and McConnell, 1980, this variability may be symmetry based. They believed that geometry and orientation were important in the formation of the receptor-dioxin complex and that a common molecular basis existed for toxicity in aromatic compounds. They postulated that the following factors effect toxicity:

- 1) geometry - four lateral chlorines were necessary, arranged in a 30 A° box shape,
- 2) molecular thickness - planar forms are more toxic,
- 3) incumbrance area - compounds of similar rectangular area may be exchangeable and may induce at the same location (e.g., hexachlorobiphenyl and 2,3,7,8-T₄CDD),
- 4) preferential dipole distribution with halogenic buttressing which can effect the charge transfer binding at receptors.

The presence or absence of these features correlate well with what is known of isomer specific toxicity.

Bradlaw et al., (1980) used rat heptatoma cells to test the induction rates of 25 isomers. These results closely correspond to McKinney and McConnell's results (Table 4.1).

Table 4.1 Degree of Induction in Hepatoma Cells

2,3,7,8-T ₄ CDD	100%	1,2,3,4-T ₄ CDD	0%
1,2,3,4,7,8-H ₆ CDD	5-10%	1,3,6,8-T ₄ CDD	0%
1,2,3,7,8-P ₅ CDD	7%	1,2,4,6,7,9-H ₆ CDD	0%
1,2,3,6,7,8-H ₆ CDD	1%	2,3,7-T ₃ CDD	0%

4.2 Clinical Manifestations-Animals

In 1973, Helling found that two applications of 10 mg of 2,3,7,8-T₄CDD caused chloracne in test rabbits and 20-50 ug/kg body weight produced a lethal liver necrosis. At administered levels of 35-120 mg of 2,4,5-T per kg body weight, which contained 0.05 to 0.1 ppm 2,3,7,8-T₄CDD, mice developed teratogenic and embryogenic effects. One of the earliest documented toxic effects of dioxins in man or animal was chloracne. Taylor (1979) extensively reviewed this epidermal reaction. Chloracne is a refractory follicular dermatosis produced by several halogenated compounds and continued reaction may lead to systemic toxicity (Taylor, 1974).

Toxic reactions to dioxin homologues manifest themselves in many ways and the extent of the reaction is a function of route of exposure and period of exposure (Vos, 1977) (Table 6.0). Vos (1977) described the atrophy of the rat thymus when exposed to 2,3,7,8-T₄CDD. The atrophy results in a functional impairment of cellular immunity which appears to be age related. McKinney and McConnell (1980) also noted damage to the lymphatic and thymus systems in exposure studies. Other clinical manifestations occurring in test animals include histological necrosis, enzyme induction, and reproductive toxicity (Kociba *et al.*, 1982). In a study by Gasiewicz and Neal (1979) guinea pigs

developed elevated levels of plasma albumin, cholesterol, protein, iron and triglycerides in the blood. Other effects noted by Goldstein (1978) include hepatic porphyria, liver lesions, fatty liver infiltration and a slow "wasting syndrome" in mice.

Also in mice and rats, testicular degeneration, internal hemorrhage, renal hyperplasia, and, possibly, cancer have been noted (Federal Register, 1979; Fishbien, 1980). These observations were confirmed by Kociba et al., (1978) when they found that exposure to 2,3,7,8-T₄CDD increased the incidence of heptaocellular carcinomas and soft tissue sarcomas in rats. To date similar observations have not been made in studies using 1,3,6,8-T₄CDD. These symptoms and effects are similar to those seen for polychlorinated biphenyls however the relative potency is very different. The "estimated" minimal lethal dose of 2,3,7,8-T₄CDD is 3.1×10^{-9} moles per kilogram or 70 ug for a 70 kilogram man. The minimal lethal dose in guinea pigs is 150 times less than is estimated in man. This is 2000 times more toxic than strychnine (Esposito et al., 1980). In relation to 1,3,6,8-T₄CDD, 2,3,7,8-T₄CDD is 2500 times more acutely toxic but is only 5 times more active as an inducer of AHH, a possible mechanism of long term toxicity (Esposito et al., 1980; Moore, 1978). See Table 5.0 for the comparative toxicities of dioxins.

4.3 Metabolism and Deposition

In 1980, Poigner et al., fed rats 2,3,7,8-T₄CDD and noted release and metabolism. He found that hydroxylated conjugated metabolites were formed and released at a rate of 1% per day in its bile. A similar result was seen in experiments with dogs with the release rate being 4 times higher. Poigner found that the metabolized material

Table 5.0. Acute Toxicity of 2,3,7,8-T₄CDD(from McConnell *et al.*, 1978)

	Single Dose LD50 (ug per kg)
Guinea pig	0.6-2.0
Rabbit	115
Monkey	70
Chicken	25-50
Rat	22-45
Dog	100-200
Mouse	114-284
Bullfrog	1000
Hamster	1157-5051

POLYCHLORINATED DIBENZODIUXINS

Compound	LD 50 oral mg/kg b.w.			Enzyminduction Liver of chick Embryo		Chloracnegetic potential Rabbit Ear Hyperkerastosis	
	guinea pig	r a t	mouse	ALA	AHH	Response	Dose ppm
Dichloro: 2.7; 2.8	> 300'000	> 1'000'000	> 2'000'000	—	—	—	> 1'000'000
Trichloro: 2.3.7. 1.2.4.	30'000		> 3'000	++ —	++ —		
Tetrachloro: 1.2.3.4. 1.3.6.8. 1.3.7.9. 2.3.7.8.	0,6 - 2	100'000 22 - 45	340 > 5'000 280	— — ++++	— — ++++	— — + +	50 50 500 0,004 0,04
Pentachloro: 1.2.3.7.8. 1.2.4.7.8.	3 1'100		340 > 5'000	++	++		
Hexachloro: 1.2.3.4.7.8. 6.7.8. 7.8.9.	73 70 - 100 60 - 100		825 1'250 > 1'440	++ —	++++ +	+	10
Heptachloro: 1.2.3.4.6.7.8.	> 600						
Octachloro: 1.2.3.4.6.7.8.9.		> 1'000'000	> 4'000'000	—	—	—	> 1'000'000

Table 6.0. Isomer Specific Enzyminduction and LC₅₀ Levels
(from Reggiani, 1980).

was 100 times less toxic than the parent compound. The main route of metabolism-based detoxification is dechlorination (Safe, 1980). Tulp (1978) suggested that metabolism was a multi-step process and that mono, dihydroxy and sulfur metabolites are formed in rats. Two routes of metabolism are noted in Figure 7.0 (Hay, 1981).

In 1979, Gasiewicz and Neal followed the movement of 2,3,7,8- T_4 CDD in guinea pigs. The dioxin moved immediately into the adipose tissue, adrenals, liver, spleen, intestine and skin. Over several weeks, fat stores were mobilized and levels increased in the other tissues. Dioxin was excreted in the feces and urine at a linear rate for the first 23 days ($t_{1/2} = 30.2 \pm 5.8$ days). In a study on mice Nau et al., (1980) found that the dioxin was transmitted to the young in the mother's milk and the age of the embryo at exposure influenced the extent of the toxic manifestations and responses such as cleft palate and an enlarged renal capsule. Studies by Helder (1980) on rainbow trout and pike indicated severe effects to development at very trace levels (0.1 to 10 ppt). At levels of 0.1 ppt, growth retardation and skeletal malformations resulted and inclusion bodies were found in the liver, stomach and pancreas of juvenile fish exposed at 10.0 ppt of 2,3,7,8- T_4 CDD.

4.4 Human Exposure-Manifestations

Between 1977 and 1980 there were 24 accidents during which workers were exposed to PCDDs (Hay, 1981). Exposure occurs as long term occupational exposure or as a result of an accidental release. Acute symptoms include nausea, vomiting, headaches, respiratory, eye and skin irritations (IARC, 1978). As exposure continues, an individual could develop chronic poisoning. Within two to three weeks of exposure,

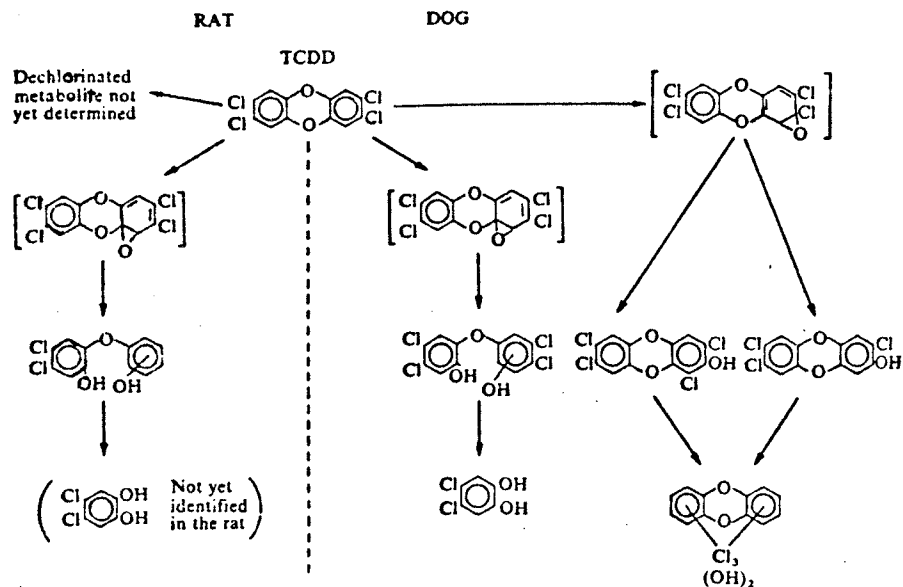


Figure 7.0. Metabolism of 2,3,7,8- T_4CDD in the Rat and Dog
(from Hay, 1981).

the first chronic symptom is chloracne. Further systematic exposure may result in hyperpigmentation, hypertrichosis, porphyria cutanea tarda (Vos, 1979; Reggiani, 1980) and may then develop into neural disorders. Other workers have noted manifestations such as swollen livers, epitaxis, neurasthenia and depression (behavioral) (Oliver, 1975; IARC, 1978). As with lab animals, changes in blood chemistry, as noted earlier, result. Several of the symptoms will dissipate in 6-8 weeks; however, several physical and emotional symptoms persist in excess of two years (Kimborough, 1980). Several of the long term risks include chromosomal damage, heart attacks and cancer (Hay, 1981). Table 7.0 itemizes the various effects of dioxin isomers on man (Reggiani, 1980; Kociba *et al.*, 1978). One of the major problems is quantifying the sublethal effects of dioxins in the industrial setting of most exposures. It is often difficult to assess the true causal agents of deleterious effects (Jirasek, 1978).

Studies by Thiess (1981) and by Hardell and Sundstrom (1978) have confirmed the carcinogenic effects of 2,3,7,8- T_4 CDD. Hardell's study of 2,4,5-T spray crews in Sweden indicates a significant number of the group having a higher than normal incidence of soft tissue sarcomas. Though these trends have been noted in field and lab studies, disagreement exists as to the effect of dioxin as a long term hazard and more data is being collected. To date no symptoms or acute reactions have been reported in similar studies, with 1,3,6,8- T_4 CDD.

5.0 ANALYSIS-GENERAL

Because of the ultra trace nature of analysis of environmental matrices for dioxins, the methods of extraction and characterization are limited to procedures and instrumentation which are capable of

TABLE 7.0 Clinical Problems Associated with TCDD Exposure *

Skin Complaints

Chloracne
Hyperpigmentation
Hirsutism

Systemic Effects

Mild fibrosis of liver
↑ serum glutamic oxalacetic transaminase (SGOT)
↑ serum glutamic pyruvic transaminase (SGPT)
uroporphyrinuria
hypercholesterolemia
hypertriglyceridemia
cardiovascular, urinary tract, respiratory and pancreatic disorders
digestive disorders (flatulence, nausea, vomiting, diarrhoea)
loss of appetite and weight loss
muscular aches and pains
joint pain
reduced primary immune capability

Neurological Effects

polyneuropathies
lower extremity weakness
sight disturbance
loss of hearing, taste and smell
headaches

Psychiatric Effects

depression
loss of energy and drive
sleep disturbance
uncharacteristic bouts of anger

*(from Reggiani, 1980)

working quantitatively at the low ng to pg per gram level. The complexity of extraction and cleanup for a variety of media have been reviewed by Lustenhouwer et al., (1980); McKinney (1978); Rappe et al., (1979); Baker et al., (1981); and Harless et al., (1980). Several procedures have been developed for ppt, isomer specific analysis by Lamparski and Nestruck (1981); Nestruck et al., (1979) and Albro and Corbett, (1977) in materials such as fish, fly ash, and sediment.

Extraction procedures include the use of soxhlet extractors (16-36 hr.) (Lustenhouwer et al., 1980; Olie et al., 1977), ultrasonic probe (Kimble and Gross, 1980; Eiceman et al., 1980) and saponification or hydrolysis (reflux) extraction (Mitchum et al., 1980; Harless et al., 1980). Extraction is usually conducted using an organic solvent (toluene, benzene or acetone/hexane) followed by a single or double column clean-up and/or partitioning of the extract to remove co-extractives and impurities. The use of strong bases in extraction is not recommended as it is known to degrade the higher chloro-dioxins (Firestone, 1977). Dioxin isomers are resilient to extreme conditions; therefore, many procedures are designed to extract all compounds, then to remove the interfering compounds.

Chromatographic cleanup has been conducted using florisil (Langhorst and Shadoff, 1980; Hummel, 1977), acidified silica (Lustenhouwer et al., 1980), or NaOH - silica and AgNO_3 - silica (Lamparski and Nestruck, 1980) columns. A second column of neutral alumina has been used to separate PCB₅ and chlorobenzenes (2% methylene chloride-hexane) from dioxins and dibenzofurans (50% methylene chloride-hexane).

Nestruck and Stehl (1970) have used a AgNO_3 - silica column to remove DDE, chlorinated aliphatic hydrocarbons and sulfides, followed

by a basic alumina column to separate ethers and PCB₅.

Analysis and detection has been conducted using three methods, gas chromatography (ECD-GC) (Buser and Rappe, 1980; Buser, 1979), gas chromatography mass spectrometry (GC-MS). Mitchum et al., Hass et al., (1979) and high pressure liquid chromatography followed by gas chromatography. (HPLC-GC) (Lamparski and Nestricks, 1981; Nestricks et al., 1979; Pfeiffer et al., 1978). Each method has specific capabilities and advantages.

5.1 ECD-GC

Electron capture gas chromatography (Buser, 1980) used capillary columns coated with OV-17 and OV-101 (25.0 m) to resolve D₂⁻, T₃⁻ and T₄CDD. Similarly, Buser and Rappe (1980) used OV-101, OV-17 and Silar 10C stationary phases in capillary columns to resolve the 22 tetrachloro dioxin isomers. The sensitivity of electron capture-GC allows for the quantification of a clean extract at levels as low as 1-5 pg of dioxin.

The use of capillary chromatography improved quantification by increasing separation through enhanced resolution. Open tubular and fused silica columns with plate numbers of 80,000 to 120,000 enable better ease of separation with a small volume of sample. The use of ECD-GC necessitates extensive cleanup procedures to reduce interfering co-extractives (Buser, 1976; Rappe, 1978).

5.2 GC-MS

This means of analysis is the most widely used as it allows good resolution and isomer specific confirmation in the pg/g range. Usually a capillary gas chromatograph is directly attached to a mass spectrometer (Harless and Lewis, 1980). Variations in the mode of mass fragmentation used involves the use of electron impact (Buser and Rappe,

1980), negative chemical ionization (Hass, et al., 1979) and atmospheric pressure negative chemical ionization (Mitchum et al., 1980). A detailed explanation of these methods can be found in Webster et al., (1983b).

5.3 HPLC-GC

High pressure liquid chromatography used in conjunction with gas chromatography or mass spectrometry has proven to be a highly successful technique for organic analysis (Pfeiffer et al., 1978; Nestrick et al., 1979). HPLC aids in the isolation, characterization and preconcentration of the compounds of interest. For example, Lamparski and Nestrick (1980) fractionated an extract on a pair of reverse phase Zorbax-ODS columns, in series, run isocratically in MeOH at 2.0 mL/min. The detector was a UV detector set at 235 nm. As a dioxin compound was eluted, it was collected off-line. The fractions were then refractionated on a two column system of Zorbax-SiL in series (normal phase). The system was run at 2.0 mL/min. with hexane. The re-resolved fractions were then injected on a packed gas chromatography column (60% OV-17 and 40% poly S-179 on 80/100 mesh Permabond). The column in turn was connected to a mass spectrometer for ECD-GC for confirmation (Nestrick, 1981).

HPLC is restricted to U.V., refractometer and fluorescence detectors unless coupled to another instrument which may restrict the sensitivity of HPLC quantification. The method allows for selective polarity or solubility separation with variation in column type and mobile phase composition. Compounds can therefore be repeatedly resolved in a non destructive manner enabling a high degree of enhancement and resolution.

6.0 PHOTOLYTIC DEGRADATION

Photolysis is a major route of degradation for polychlorinated aromatic compounds. This topic has been reviewed in several papers and the predominant route for dioxins involves dechlorination of substituent groups while leaving the basic tricyclic structure intact (Plimmer, 1978; Crosby et al., 1971). Dechlorination can occur by the dissociation of an excited molecule to a free radical or, nucleophilic displacement involving a donor (H+) from the solvent or other available species (Nestrick et al., 1980).

Originally, Crosby et al. (1971) found that dechlorination of O_8 CDD was slower than that of 2,3,7,8- T_4 CDD in an organic solvent under lab and natural light (Figure 8.0). He believed no degradation occurred when he performed the same experiment with soil. In 1978, Plimmer exposed 2,3,7,8- T_4 CDD to light on thin layer chromatographic plates. After 20 hours, 57% of the compound had degraded. When he repeated the experiment with soil, no metabolism occurred, yet 32% of the material was missing. He attributed the losses to volatilization. Plimmer noted that, in cyclohexane, 2,3,7,8- T_4 CDD absorbed at 294 nm. However, when silica was added, the absorbance band widened and shifted to 288 nm.

Liberti et al. (1978) confirmed Plimmer's results but also noted that temperature was a significant factor in 2,3,7,8- T_4 CDD photolysis. As is seen in Figure 9.0 dioxin degradation losses were almost 95% in 80 hours at 30°C while at 23°C the losses were 40% for the same period. Liberti et al., (1978) also postulated that 2,3,7,8- T_4 CDD was inherently persistent once in the soil, and not mobile. The required presence of a H+ donor was noted for dechlorination to occur

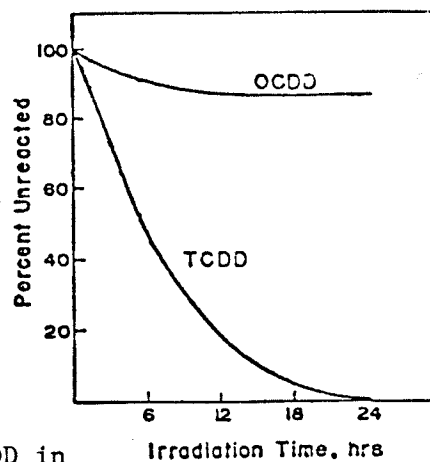


Figure 8.0

Photoreduction Rates of T₄CDD and O₈CDD in Methanol (from Crosby *et al.*, 1971).

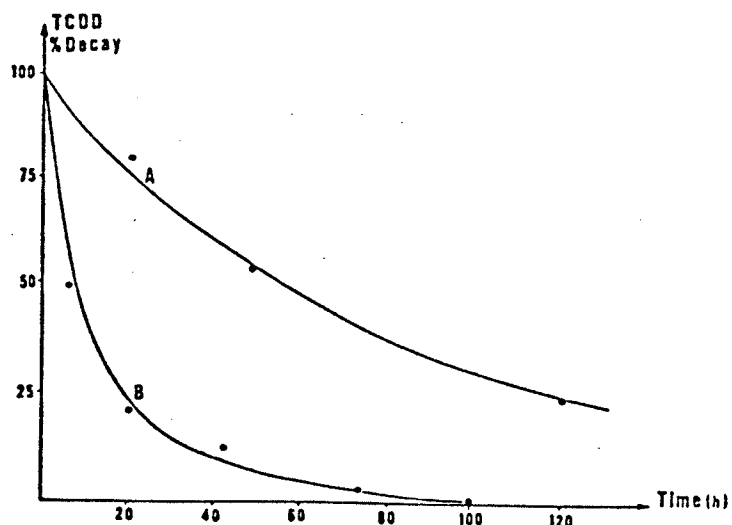


Figure 9.0. Thermal Effect on Reaction Rate. 2,3,7,8-T₄CDD on Glass Plates. A, 23°C. B, 30°C. (from Liberti *et al.*, 1978).

(Crosby et al., 1971). Hydrogen donors include alcohols, ethers, hydrocarbons and such materials as waxes and natural surfactants. Dobbs and Grant (1979) have shown that the meta chlorines are more susceptible to attack than those in ortho positions. This factor was further discussed also by Arsenault (1976) and Wipf et al. (1978).

Zepp et al. (1981 and 1977) postulated that photochemical oxidation of organic compounds by singlet oxygen may be a significant route in natural waters. In this mechanism, light is adsorbed by a sensitizer which transfers energy to a ground state oxygen to form a singlet radical which then attacks organic compounds. The highest rates of singlet radical generation occur in water containing high concentrations of fulvic acids which is frequently found in stillwater enclosures.

Dobb and Grant (1979) photolyzed H_7^- , H_6^- and O_8CDD and found that the more toxic isomers degraded faster than the other isomers. O_8CDD degraded to a H_6CDD which, in turn, degraded. The rate of degradation may effect the time during which toxic isomers are present before transformation to more innocuous forms. The following schematic describes Dobbs and Grant's (1979) results and the determined photolytic rates (Figure 10.0).

Other workers have described the generation (photolytic) of lower chlorinated isomers from higher chloro-isomers. Arsenault (1976) suggested it as a source of dioxins in treated wood. Photolytic studies by Buser (1979) to generate standards of lower chlorinated isomers indicated several products from this form of dioxin decomposition. The result can be seen in Figure 11.0.

In 1980, Nestricks, Lamparski and Townsend performed photolytic tests on 28 isomers. They postulated that degradation followed

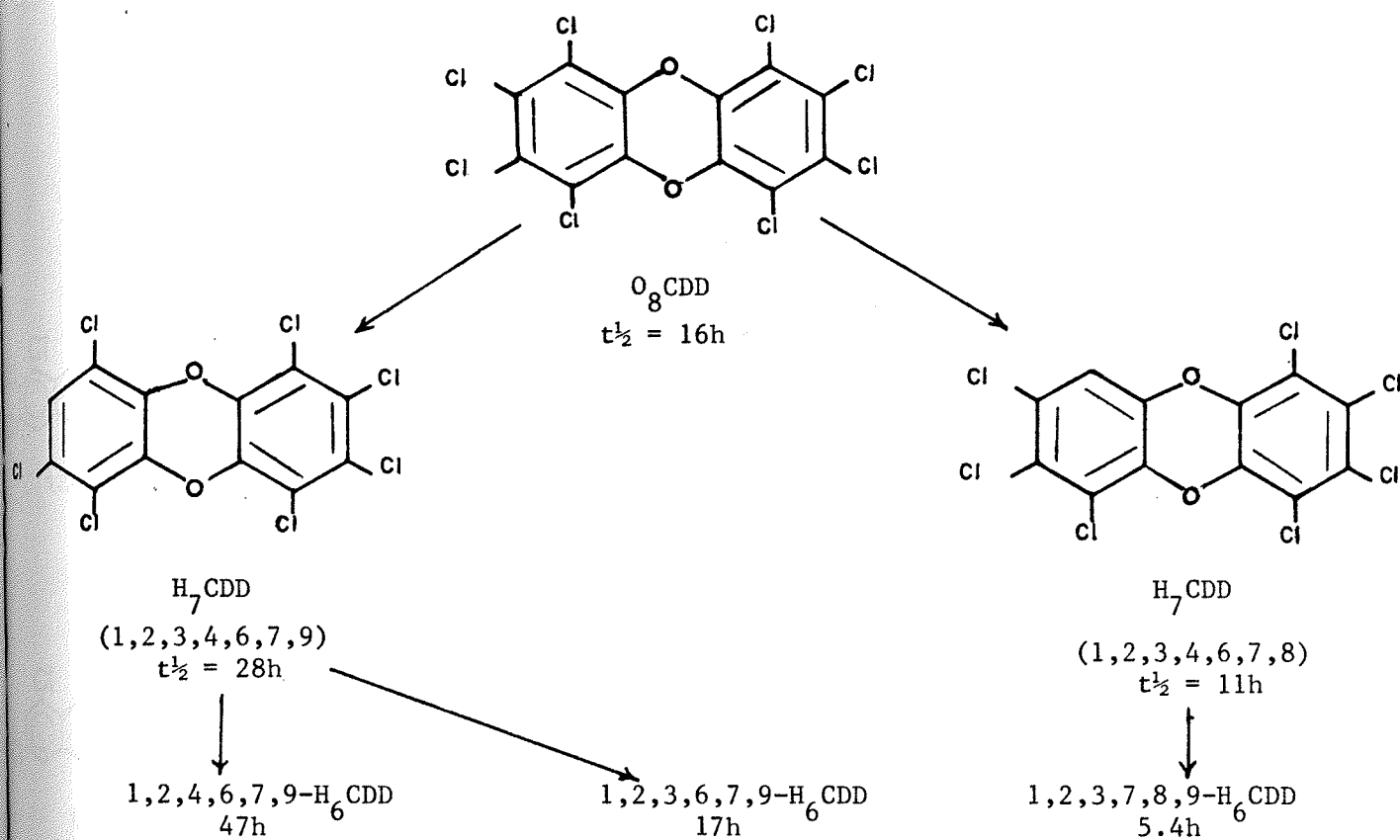


Figure 10.0 Photolysis Rates of O_8CDD to H_7 - and H_6CDD s.
 (from Dobbs and Grant, 1979)

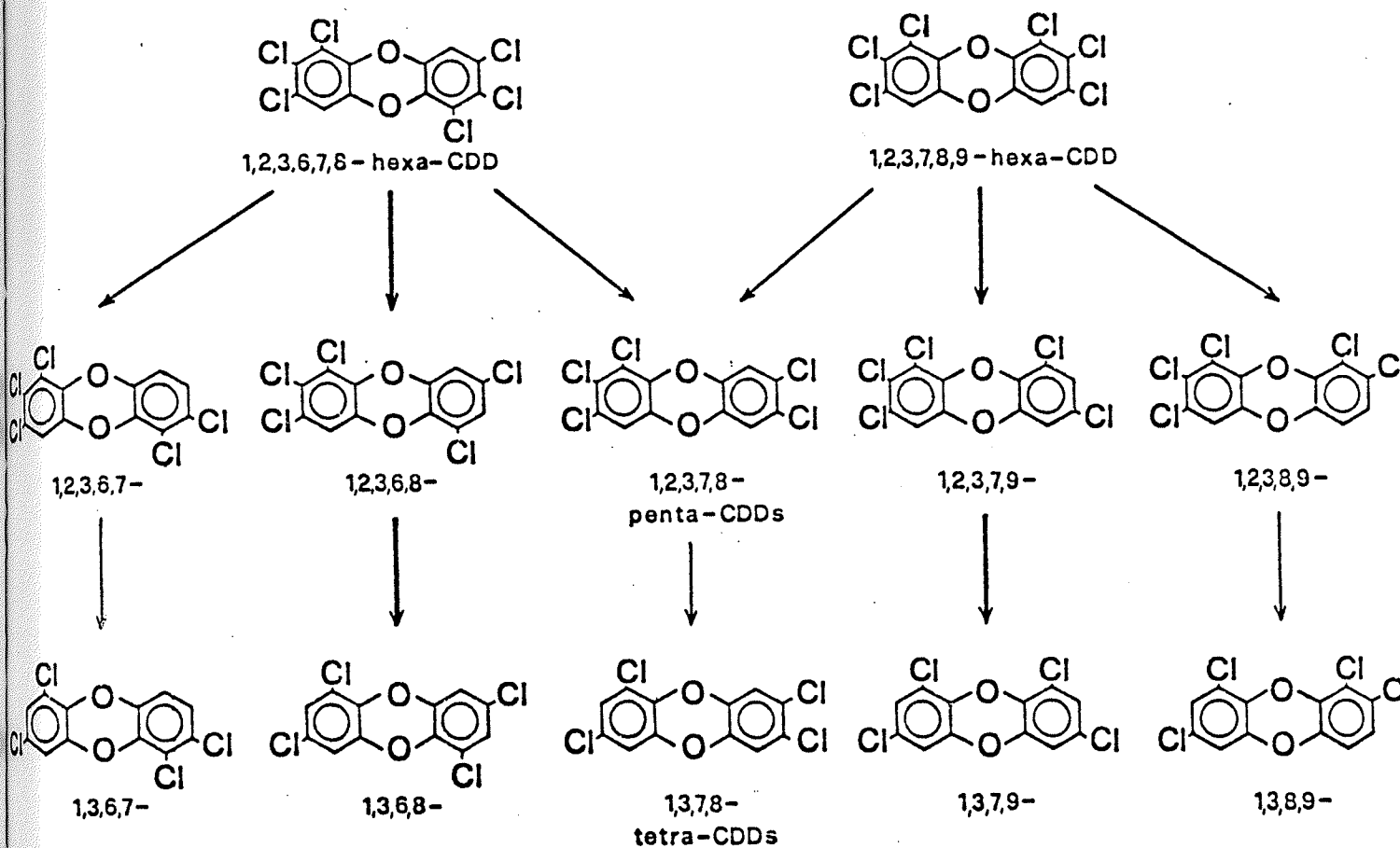


Figure 11.0. Major Photolysis Pathways leading to T₄CDD and P₅CDDs from 1,2,3,6,7,8- and 1,2,3,7,8,9-H₆CDD (from Buser, 1979).

first order kinetics and that the photolytic half life could be expressed as:

$$t_{\frac{1}{2}} = \frac{t \ln 2}{\ln (a/x)} \text{ where } t_{\frac{1}{2}} \text{ is}$$

the half life of the compound in solution, t is time, "a" is the initial concentration and x is the final concentration. They found that the rate of decomposition was inversely related to the degree of chlorination with preferred loss of the 2,3,7 and 8 chlorines. Also, as was seen in earlier work, exposure as films on soil, glass or in water (distilled) proved to result in only negligible degradation (photolytic). Selected data from their work is included in Table 8.0.

Table 8.0 Photolytic Degradation Rates of Select PCDDs

isomer	Exposure in Solution (hexadecane)		Exposure on Glass Surface	
	$t_{\frac{1}{2}}$ absolute	$t_{\frac{1}{2}}$ rel. to 2,3,7,8-T ₄ CDD	$t_{\frac{1}{2}}$ absolute	$t_{\frac{1}{2}}$ rel. to 2,3,7,8-T ₄ CDD
2,3,7,8	56.8 min	1.0	8400	1.00
1,3,6,8	499.0	8.79	264	0.031
1,3,7,9	509.0	8.93	169	0.020
hexa (1,2,3,6,7,8)	379.0	-	44100	-
hepta (1,2,3,4,6,7,8)	1800	-	3140	-
octa	1460	-	48900	-

In one of the few field studies, Nash (1978) found that the half life of 2,3,7,8-T₄CDD applied in an emulsified and granular formulation of fenoprop was 7.65 days and 13.5 days respectively.



7.0 ECOSYSTEM STUDIES-DIOXIN

Few ecosystem studies have been attempted using dioxin isomers and no previous work of this nature on 1,3,6,8-T₄CDD had been reported.

Kearney et al. (1972) exposed several soil types to 2,7-D₂CDD and 2,3,7,8-T₄CDD to determine the effect of soil type on degradation rate and to isolate possible metabolites. He found that 2,3,7,8-T₄CDD persisted (56-63% remaining after 1.0 year) and that only 2,7-D₂CDD appeared to degrade. In 1971, Isensee and Jones exposed oats and soybean plants to two concentrations of ¹⁴C-2,7-D₂CDD and ¹⁴C-2,3,7,8-T₄CDD. They found that though there appeared to be some uptake initially, once the plants reached maturity, the oats contained 8% of the 2,7-D₂CDD and 1% of the 2,3,7,8-T₄CDD. Similarly the soybeans had 1.5% of either dioxin. There was no significant accumulation or translocation of foliar applied material.

Nash and Beall (1977; 1980) reported studies performed in microagroecosystems and field plots where ³H-2,3,7,8-T₄CDD had been applied with emulsified and granular Silvex three times on turf. Approximately 80% of the 2,3,7,8-T₄CDD remained in the top 2.0 cm of the soil with trace movement to 8-15 cm. Seven weeks after the last application, trace amounts (0.05 to 0.06 ppb) of dioxin appeared in leachate samples.

In 1975, Isensee and Jones continued with studies of ¹⁴C-2,3,7,8-T₄CDD behavior in aquaria to simulate exposure of organisms in a food chain. They found that bioaccumulation occurred in all levels of the food chain (fish, invertebrates, flora). The degree of accumulation was directly related to the concentration in the water. The rate of accumulation included 2.0-2.6 x 10⁴ times for snails,

mosquito fish and zooplankton and $4-9 \times 10^3$ times in duckweed and catfish. No metabolites were detected.

Matsumura and Benezet (1973) also ran a series of aquaria studies on pesticides (DDT) and 2,3,7,8- T_4 CDD. They found the dioxin to be immobile in soil and only 5 of 100 strains of microbes were capable of degrading 2,3,7,8- T_4 CDD. They noted that differential bioaccumulation occurred and that the rates in fish and invertebrates were less for 2,3,7,8- T_4 CDD (121 x in shrimp) than for DDT (689 x).

Ward and Matsumura (1978) and Ward (1976) expanded model studies with a series of lab experiments of water-sediment incubations with ^{14}C -2,3,7,8- T_4 CDD. They noted several trends and came to four major conclusions as follows:

- 1) most of the parent compound was bound to the sediment where it was relatively unavailable to microbes.
- 2) water mediated evaporation of 2,3,7,8- T_4 CDD occurred.
- 3) degradation of parent compound occurs in a limited fashion in the water column.
- 4) binding occurs with unknown factors which make identification of transient metabolites difficult.

Tsushimoto et al. (1982) compared laboratory and outdoor systems in an experiment to describe the movement of ^{14}C -2,3,7,8- T_4 CDD. The enclosures, though of concrete construction, contained soil, fish, aquatic flora and water compartments. Tsushimoto et al. found that the radio-label equilibrated in the aquatic plants in 1 month, the fish in 2 months and in the sediment in 6 months. He noted significant variability in his vegetal data as the plants would suddenly die shifting the spatial deposition of the label. The 2,3,7,8- T_4 CDD persisted: 49.7% and 29.4% of the label remained after 12 and 25 months respectively.

Metabolites were detected in the plants and water (approximately 40% of the original ^{14}C). (Matsumura and Ward (1978) had suggested that the sediment released metabolites to the water over time). The remaining 2,3,7,8- T_4CDD was found in the organic layer of the sediment. No metabolites were isolated or identified; however, they postulated that degradation occurred due to direct and indirect light.

8.0 Volatilization: estimated and predicted flux:

Volatilization of environmentally hazardous compounds has been indicated as a significant route of removal (McCall *et al.*, 1982). This is based upon the hydrophobic nature of non-polar compounds such as PCB, PCDDs and PCDFs (Mackay and Leinonen, 1975). In assessing the magnitude of this mechanism, the gas-liquid exchange interface must be described. This zone is described as a two film system in a homogeneous environment. The film model and flux estimates are described as

$$F = K \Delta C \quad (1)$$

where ΔC is the concentration difference across the layer of thickness Z , and K is the exchange constant in units of velocity. K is dependent on the degree of turbulence in both phases, the chemical reactivity of the chemicals and the "resistance" of the specific compound to transfer.

Smith *et al.* (1981) described volatilization from water as a first order kinetic process. In a two film mass transfer model he found that volatilization rate coefficient, K_v , could be expressed as

$$K_v = \frac{1}{L} \left(\frac{1}{K_1} + \frac{RT}{H K_g} \right)^{-1} \quad (2)$$

where: L = depth (cm),
 K_1 = the liquid mass transfer coefficient (cm h^{-1})
 R = the gas constant ($\text{L atm mol}^{-1} \text{K}^{-1}$)
 T = temperature ($^{\circ}\text{K}$)
 H = Henry's constant ($\text{atm m}^{-2} \text{mol}^{-1}$)
 K_g = gas mass transfer coefficient (cm h^{-1})

The rate is then expressed as in equation (1). The mass transfer coefficients were estimated by Liss and Slater (1974) in which

they correlated movement with Henry's constant. The factor K_1 relates to the evaporation of water from a natural surface as a function of wind speed (1.8 cm h^{-1}) (the liquid coefficient). The gas coefficient, K_g , relates the movement of oxygen from the surface (2100 cm h^{-1}). Compounds volatilized from this surface are related by their molecular weight to ideal K_1 and K_g values.

In all calculations we assume that diffusion or mixing is fast enough in the liquid phase so that the concentration at the interface reflects the water concentration. Similarly, the workers had to assume that there were no other sources of loss (eg. adsorption).

Mackay and Leinonen (1975) expressed the rate of the evaporation process, from a water body, of a low solubility compound. They described it in terms of the bulk liquid concentration (C_i) and the atmospheric partial pressure (P_i) of the compound. The flux (N_i) was expressed as in equation (3).

$$N_i = K_{iL} (C_i - P_i/H) \text{ ng m}^{-2} \text{ h}^{-1} \quad (3)$$

where: C_i = the concentration at time x .

H = Henry's constant

$$K_{iL} = \left(1/K_L + 1/(H K_g/RT)\right)^{-1}$$

In calculating the flux I've assumed P_i to be insignificant due to 1,3,6,8- T_4 CDD's low estimated solubility therefore equation (3) can be simplified to this form.

$$N_i = K_{iL} (C_i) \quad (4)$$

The volatilization rate (K_v) of equation (2) is a depth

dependent rate constant while the flux (N_i) value of equation (5) is independent of depth.

Claith et al. (1980) measured the loss of pesticides volatilizing from a flooded field. They estimated the vapor concentration with a series of polyurethane foams mounted above the water surface and noted the density gradient produced.

The expression of flux ($P\uparrow$) they derived was based on an aerodynamic equation.

$$P\uparrow = K^2 \Delta c \Delta u / \phi^2 [\ln (z_2 - z_0) / (z_1 - z_0)]^2 \quad (5)$$

where:

- K = the von Karman constant (0.4)
- Δc = the vapor density between height z_i and z_x ($\mu\text{g m}^{-3}$)
- Δu = wind speed differential (cm s^{-1})
- z_x = height above water (cm)
- ϕ = stability correction term to correct for atmospheric (unitless) instability on flux and reflects changes in atmospheric lapse rate.

The stability term is expressed as

$$\phi = (1 + 16 R_i)^{\pm 0.33} \quad (6)$$

where:

- R_i = Richardson gradient number derived from
- $R_i = g (\overline{\Delta T} / \Delta z) / \bar{T} (\Delta u / \Delta z)^2 \quad (7)$

where:

- g = acceleration by gravity (cm sec^{-2})
- \bar{T} = average air temperature ($^{\circ}\text{C}$)
- $\overline{\Delta T}$ = differential air temperature between z_i and z_x ($^{\circ}\text{C}$)

Equation (4) represents a predicted flux based upon chemical characteristics, while equation (5) is based on field data.

III. MATERIALS AND METHODS

1.0 STANDARDS AND CHEMICALS

Solvents - All solvents were Caledon pesticide grade distilled in glass and included hexane, acetone, dichloromethane, and toluene. Fisher brand methanol (HPLC grade) was used in the HPLC work.

Standards - The universally labeled ^{14}C -1,3,6,8-tetrachlorodibenzo-p-dioxin (^{14}C -1,3,6,8- T_4CDD) was manufactured by New England Nuclear to a purity of 97%. The isomer has a specific activity of 14.8 mCi/millimole. Upon its arrival the standard was dissolved in benzene and made up to 50.0 mL in a volumetric flask as a primary standard. The standard was characterized by capillary GC, TLC (5% H_2O -MeOH) and HPLC (7% chloroform-MeOH). Two contaminants were found to exist accounting for 3.8% of the label (possibly a phenol from the synthetic processes and 1,3,7,9- T_4CDD , the Smile's rearrangement product).

Reagents - Silica gel: 60-200 mesh, Fisher brand. Sodium Sulfate: anhydrous granular Fisher brand. Silanized Glass Wool: fine, Fisher brand. 4-Methoxy-ethanolamine: (CO_2 -M-Met) oxidizing agent from Amersham Radiochemicals. PCS cocktail: scintillation fluor, Amersham Radiochemicals. Atomlight: scintillation fluor, New England Nuclear.

2.0 POOL CONSTRUCTION - SITE PREPARATION

Experimental Area - The study was performed at the University of Manitoba Agriculture field station at Glenlea, Manitoba. The area was located 20 km south of Winnipeg on Highway 75. Four pools were used in the study (Figure 12.0).

The four pools were constructed similarly to those described

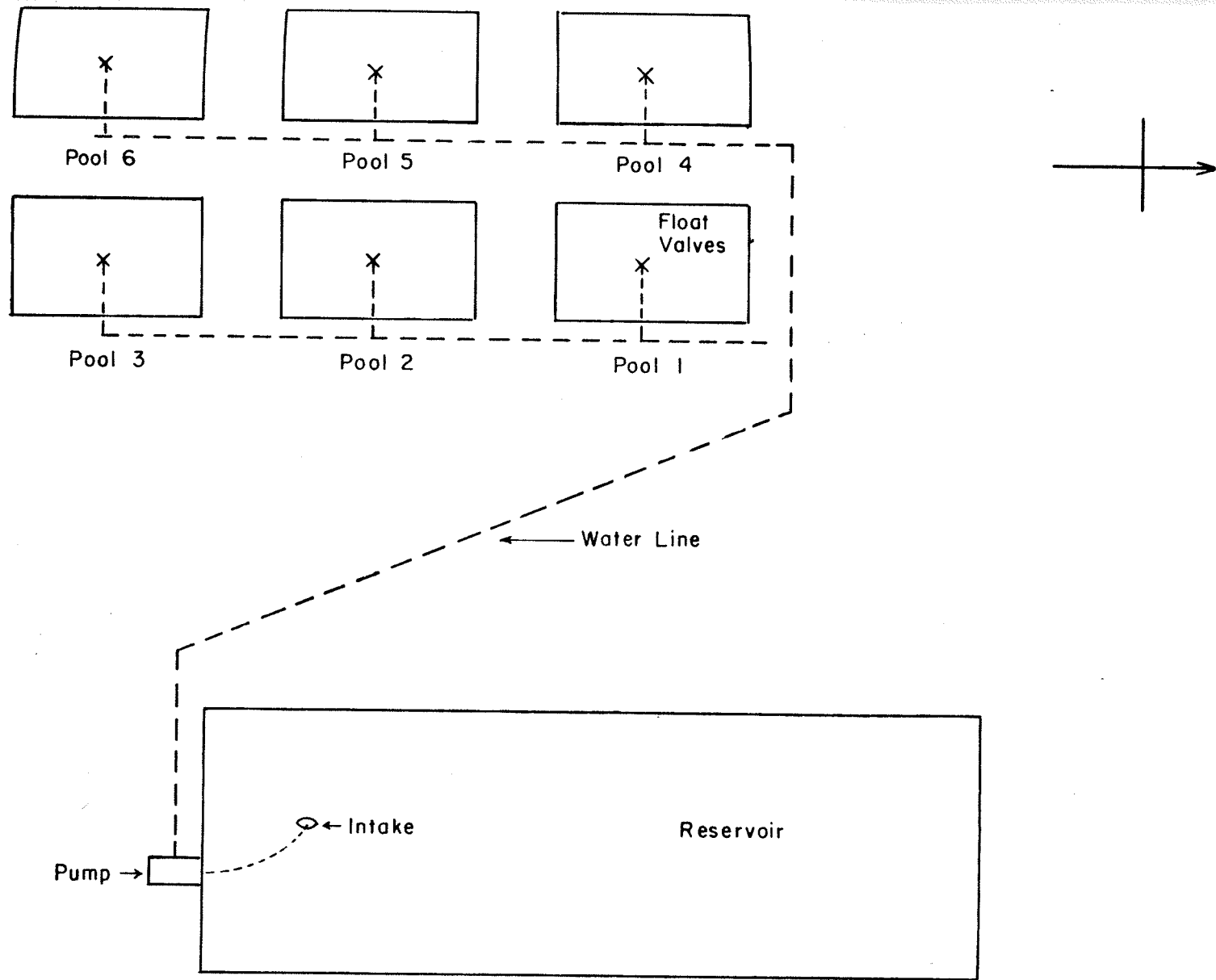


FIGURE 12.0 Schematic of the Pool Site, Pools and Reservoir

by Madder, 1978. Excavation was performed by a Drott 440 excavator with a wrist articulating arm. The holes measured 5.5 m by 4.5 m with 35-40° sloping walls leading to flat base 2.0 m x 1.6 m at a depth of 0.9 m. The walls were then hand smoothed and 5 cm of mortar (Figure 12.1) sand laid over the bottom and compacted. A layer of 10 mil polyethylene plastic was laid down (Figure 13.0) and the bottom lined with 5.0 cm of sand. The side walls were overlaid with two offset 5.0 cm layers of clay base sod. Each pool required approximately 115 square yards of sod. The bottom was then covered with a 10.0 cm layer of excavated earth and the pools filled to a depth of 50 cm. The final dimensions of the pools were 4.9 m by 3.8 m. Due to the heavy clay composition of the site, clay colloid expansion occurred during the overwintering period stabilizing the pool depth at 50.0 cm.

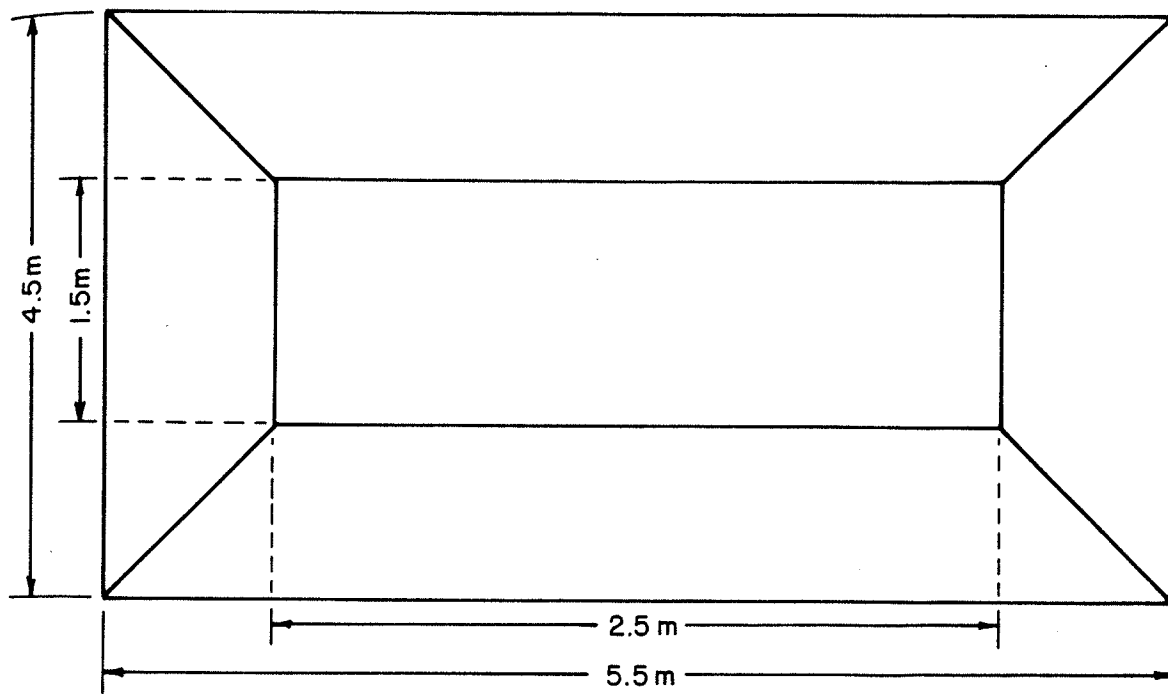
Each pool was reinforced around its edges with 2 x 4 lumber and provided with a self-leveling water system. The enclosures were left to biotically colonize and equilibrate for one year (Figure 14.0) with a variety of flora and fauna.

Water was automatically provided through a series of float valves and 3/4" PVC lines running from a pressure pump at a plastic lined isolated dugout which provided a continuous supply of non-contaminated natural water. The swing arm valves maintained the water level at 50.0 ± 1.2 cm depth over the sampling period till ice up. Weather conditions were recorded at a meteorological station near by.

All systems and field equipment were tested for 30 days previous to the pool inoculation.

3.0 POND TREATMENT

In the first week of July, 1981, three of the four pools were



POOL FORM - TOP VIEW

POOL FORM - SIDE VIEW

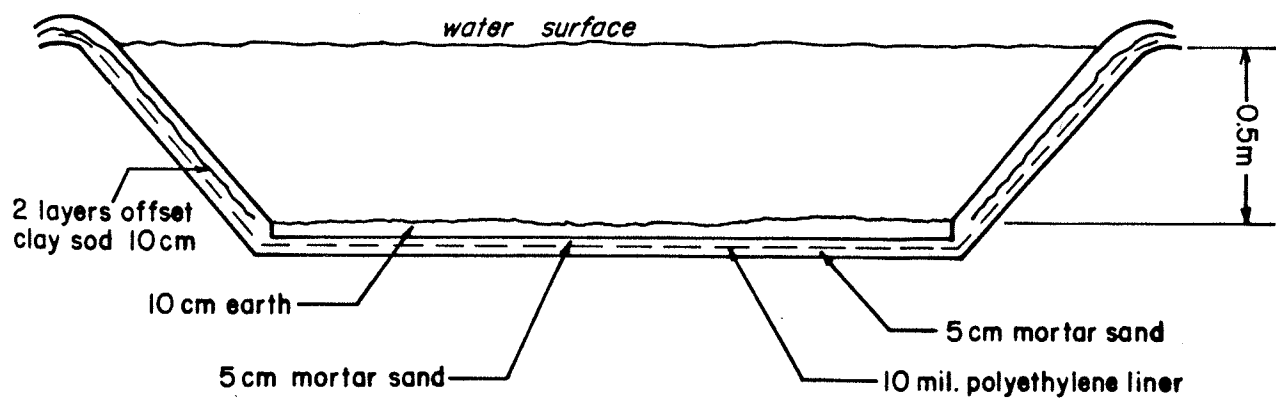


Figure 12.1 Schematic of Pond Design and Construction



Figure 13.0 Photograph of Partially Assembled Pool
with Plastic in place and Sod lining applied



Figure 14.0 Photograph of Equilibrating Completed Pool

treated with three levels of ^{14}C labeled 1,3,6,8- T_4CDD . The pools were treated at levels of 100,000 dpm/L (980.0 ng/L) (pool 1); 25,000 dpm/L (245.0 ng/L) (pool 2); 10,000 dpm/L (98.0 ng/L) (pool 5) and the fourth pool (pool 3) was maintained as a control. The treatment solution was transported in an insulated sealed flask from the lab and the stock solution added by pipet, subsurface, in a swirling motion over the entire pool surface. Each pool was then agitated manually. The label was applied in a pre-mixed solution of acetone/benzene (1:9) which disappeared from the water surface within 2-3 seconds. Sampling of the various media began immediately.

4.0 SAMPLING

The sampling schedule for all media in the field study is noted in Table 9.0.

4.1 Air

The air sampler was constructed with materials and in a design provided by Muir (1983). The apparatus consisted of a series of three glass tulip funnels (Figure 15.0) (10 cm x 5.0 cm) inverted at 5.0, 10.0 and 18.0 cm above the water surface. This system was attached to a portable vacuum pump at the pool edge. A 10.0 cm x 5.0 cm polyurethane foam was placed in each funnel and air was drawn through each foam at a flow rate of 8.0 L per min. Every 24 h for the first 3.0 days and on days 5 and 7, foams were exchanged, sealed in glass jars and returned to the lab for immediate extraction.

4.2 Hydrosol

At thirty days pre-treatment, aged pool hydrosol (two years) was excavated from nearby pools and placed into 28 Meyer's metal

Table 9.0 Field Sampling Schedule of Various Media

Time	Air	Water	Hydrosoil	Fish	Snails	Vegetation	Miscellaneous Invertebrates
0	x	x	x	x	x	x	x
2 h		x		x			
6 h		x					
12		x	x	x	x		x
24	x	x	x	x			
48	x	x	x	x			
72	x	x					
96		x	x				
103				x			
120	x						
7(day)	x						
8		x	x	x	x	x	x
15		x	x	246 h	x	x	x
17				x			
24		x	x		x	x	x
34		x	x				
57		x	x		x	x	x
90		x	x		x	x	x
426		x	x [*]		N/A	N/A ^{**}	N/A

* 10 Oakfield cores (0-5 cm) treatment

N/A Not available at time of sampling

** Rooted vegetation was available on day 426

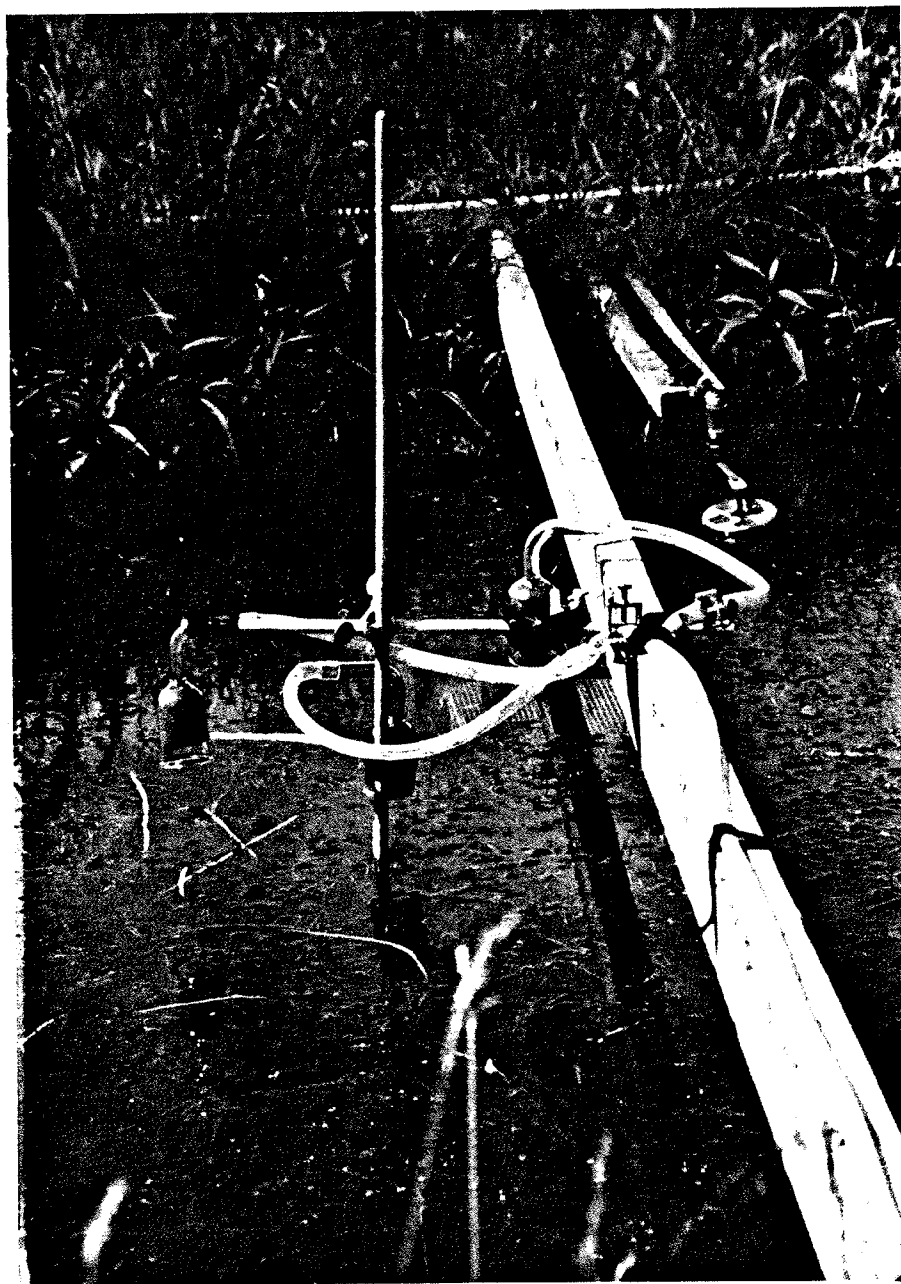


Figure 15.0 Photograph of Air Sampling and Float Valve
Water Control System

containers in each pool. Each container was 10 cm in diameter and 3.5 cm in depth with a water tight metal lid. Each can was weighed to insure a minimum of 110 g wet weight being present. An aluminum wire was attached to each open container to allow easy retrieval during sampling. At prescribed intervals (Table 9.0) replicates were taken, the cans sealed on site, labeled and placed in -36°C storage until extraction.

4.3 Water

Water samples were taken in replicate at a depth of 20-25 cm using pre-silanized amber Packers bottles with an aluminum foil-lined lid (a twin tube fill system was used so sample could be taken at the prescribed depth by tilting). Each sample contained 880 ± 16 mL of water to which 40.0 mL of dichloromethane were added at the time of sampling to preserve the sample and to initiate extraction. The samples were transported in coolers and held at 4°C until extracted.

4.4 Vegetation

The floating vegetation (Lemna sp.)^{*} was sampled by scooping available plants from the surface until approximately 3.0 to 5.0 g wet weight had been collected. Sampling was affected by the varying availability of vegetation. The rooted vegetation (Potamogeton sp.)^{*} was harvested by hand from the upper 15.0 cm of the plants. All samples were placed in silanized glass bottles and held at -36°C until extraction.

4.5 Fish

Two hundred and twenty-five minnows, Pimephales promelas, were

* Potamogeton berchtoldii Fieber var. acuminatus Fieber

* Lemna trisulca L.

placed in each pool 23 days pre-treatment. During day one, 10 fish were held in 30 x 30 x 10 cm subsurface cages which were sampled over the first 24 hours, after which baited minnow traps were used. All sampled fish were stored at -36°C until analysis. Apparent predation of the fish stocks curtailed sampling by day 15 (pool 1) and earlier in pools 2, 3 and 5.

4.6 Invertebrates

Invertebrate sampling was performed by sweep grab samples using a small mesh net. The sweeps were made up from the edge of the vegetable cover, at the pool bottom, upward to the surface along the pool wall. Sampling was continued until 30-50 individuals of the pool snail (Gyraulus parvus Say) were collected. Individual gastropods and pelagic invertebrates were sampled as available. All samples were placed in silanized glass jars and stored at -36°C until freeze dried, weighed and combusted. The main objective was to denote the presence and relative levels of ^{14}C label in as diverse a selection of pool invertebrate types (pelagic, benthic, predators, scavengers) as possible. The samples taken varied greatly as was dictated by availability over the season.

5.0 EXTRACTION AND CLEANUP

5.1 Air

Each foam was soxhlet extracted for 3.5 h with hexane. The extract was concentrated to 1.0 mL by rotoevaporation, rinsed (twice) into an amber vial and stored at 4°C . The extracted foams were reused in the field. Tests as to efficiency of the extraction procedure, carryover of ^{14}C activity in the foams and the trapping ability of the

foams were performed (Section 7.4).

There was no cleanup procedure necessary with these samples.

5.2 Hydrosol

The containers of frozen soil were lyophilized for 72 h and weighed. The soil was transferred to pre-extracted (16 h soxhlet extracted with benzene) cellulose thimbles and soxhlet extracted for 24 h with benzene. The extraction flask was rinsed and the extract concentrated by rotoevaporation to near dryness and reconstituted with 100.0 mL of pH 1.0-1.5 distilled water (acidified with 1.0 N HNO_3). The solution was then extracted three times (50, 30, 30 mL) by liquid-liquid partition with dichloromethane. The extract was dried over an anhydrous Na_2SO_4 (20.0 g), concentrated by rotoevaporation with an iso-octane keeper (1.0 mL) to near dryness and reconstituted in 5.0 mL of chloroform. Aliquots (4.0 mL) of the extracted water were counted directly by LSC using the Atomlight fluor to determine extraction losses. One hundred- μL aliquots of extract were counted by LSC directly for total activity. A 1.0 mL aliquot was eluted through a Waters C_{18} Sep-Pak to remove debris using a three tier elution system consisting of 7.0 mL of chloroform, 2.0 mL of hexane and 2.0 mL of methanol. The extract was concentrated using a stream of prepurified nitrogen to 200-300 μL in chloroform for further characterization by HPLC. Soil replicate one was exhaustively extracted as noted above, but replicate two was vigorously ground, once lyophilized, and three subsamples taken. The samples were weighed, wrapped in filter paper and oxidized for total activity present. Similarly replicate subsamples of the "extracted" soil were taken and oxidized to determine the extraction efficiency, and unextractable residue remaining.

5.3 Water

The samples were extracted in a 2 L separatory funnel by liquid-liquid partitioning with dichloromethane. The Packer jar was rinsed (40 mL) and the sample extracted three times (150, 100 and 100 mL, total 430 mL). The extract was passed through a 20.0 g anhydrous sodium sulfate bed and concentrated by rotoevaporation (bath 38°-40°C) into 1.0-2.0 mL of hexane which was then placed in 7.0 mL amber vials with teflon lined lids, prior to analysis. Aliquots of the extracted water (4.0 mL) were counted by LSC directly to determine the extraction efficiency. Similarly, 0.5 mL of the 5 mL extract was counted for activity and back calculated to ng/L of 1,3,6,8-T₄CDD material. Confirmation of the identity of the activity was done by HPLC and GC.

5.4 Vegetation

Frozen samples were allowed to thaw and air dry for 48 hours in an air circulation hood. The samples were weighed and extracted by ball mill homogenization (Grussendorf et al., 1970). The vegetation was extracted three times with hexane/acetone (1:1) %50/50). Following each extraction (60, 30 and 30 min.) on a wrist action shaker, the samples were centrifuged at 2000 rpm for 20 min. The supernatant was pipetted off and the procedure repeated. The extracted material was air dried and subsampled for combustion (oxidation) to determine the amount of unextractable label. The extract was concentrated by rotoevaporation and resuspended in 75 mL of acidified distilled water (pH 1.0-1.5) and extracted four times (50, 30, 30, 30 mL) with dichloromethane. Sampling of extracted water (4.0 mL) and direct counting was carried out to determine extraction efficiency. The extract was dried by passage through an anhydrous sodium sulfate bed and concentrated to

5.0 mL of chloroform for further analysis. Prior to the analysis, the samples were eluted through a C₁₈ Sep-Pak using the method described in section 5.2.

5.5 Invertebrates

All samples were freeze dried (lyophilized) for 48 h and then sorted by family (Coroxidae, Notonectidae) with the exception of the pool snails (Gyraulus parvus) which were sorted to species (Clark, 1973). The samples were weighed as triplicated subsamples, wrapped in filter paper and combusted for total ¹⁴C activity. Because of the limited amounts of available insect material, exhaustive extraction was ruled out. Therefore all levels determined were expressed in terms of 1,3,6,8-T₄CDD equivalents.

5.6 Fish

The fish samples were stored at -36°C and then lyophilized for 48 h. The whole fish was weighed and extracted with 25.0 mL of toluene using the stainless steel ball mill noted in section 5.4. The samples were shaken on a wrist shaker for 1.0 h, and centrifuged for 20.0 min at 2000 rpm. The supernatant was pipetted off and the fish re-extracted two more times for 30.0 min. The composite extract was concentrated to near dryness and resuspended in 5.0 mL of chloroform for analysis by HPLC. Attempts to cleanup the samples with Sep-Pak were inefficient due to excessive amounts of fatty material in the samples, therefore, the samples were analyzed directly by HPLC.

5.7 Glassware

All glassware used in direct contact with ¹⁴C were treated with Surfasil silanizing agent, in toluene. Owing to the hydrophobic

nature of the 1,3,6,8-T₄CDD and losses found to occur in previous dioxin studies (Nestrick, 1981), silanization was believed to be prudent.

After use, all glassware were washed with Contrad detergent, rinsed with distilled water and acetone and extracted by organic solvent previous to use. Post and pre-washed glassware were periodically tested for label carryover. This was performed by the use of 50.0 mL of dichloromethane which was used to extract the used glassware (e.g., 2.0 L separating funnels after water extractions). The entire extract was concentrated by rotoevaporation and counted by LSC. The extent and magnitude of label carryover and losses in silanized and unsilanized glassware is noted in the discussion.

6.0 ANALYSIS - INSTRUMENTATION

6.1 Gas Chromatography

A Tracor 560 with a ⁶³Ni linearized electron capture detector was used. The columns used were a 30 m OV-101 SCOT capillary column and a 33 m SP 2100 SCOT column. A modified Varian 2400 with a 60 m fused silica column (OV-34) was also used.

The carrier was helium with 5% Argon/methane make-up gas resulting in a column flow of 6.5 mL/min. The standard program consisted of 8°C per minute ramp from 140°C to 270°C with a 15 minute final hold. The injector port was maintained at 220°C in the splitless mode. The injector was maintained at 210° and detector at 310°C.

6.2 High Pressure Liquid Chromatography (HPLC)

6.21 Instrument

The instrument used was a Waters Scientific system

consisting of an 6000A pump, a 440 (254 NM) U.V. detector and a Fisher recorder. The injector was a six port Valco valve with a 100 uL sample loop leading to a C₁₈ uPorasil guard column followed by two C₁₈ uPorasil columns (30 cm x 1.8 cm) in series. The mobile phase was 7.0% chloroform in methanol (distilled in glass) and the system was run at 0.9 to 1.1 mL (per minute) at a pressure of 1950 to 2300 p.s.i. (1.36×10^5 - 1.61×10^5 kpas.).

A 100 uL injection containing 30.0 ng of 1,3,6,8-T₄CDD resulted in a signal response of four times background at highest sensitivity (0.005 aufs). The result of elution tests indicate 88.0 to 94.0% recovery of injected labeled material. A 20 uL injection of 30 ng of 1,2,3,4-T₄CDD resulted in a signal response of 50% of full scale at 0.005 aufs.

6.22 Metabolite isolation

The use of HPLC to cleanup and fractionate 1,3,6,8-T₄CDD proved to be simple, and precise. The standard material had a retention time of 8.3 min for parent compound. Previous to injection, the 5.0 mL extracts were concentrated under pre-purified nitrogen to near dryness and reconstituted to 300 uL in chloroform. Often an extract would be concentrated to 100 uL and 30 to 40 uL injected. Fractions were collected every 20 s between 3.6 min (the solvent front) and 10.5-11.0 min, and the fractions counted by LSC (refer to Figure 16.0). When viable, a second injection was made, fractions collected and those fractions containing label were concentrated and injected for gas chromatographic characterization.

An arbitrary minimum acceptable level of activity for a peak was established at three times background. This would be equivalent

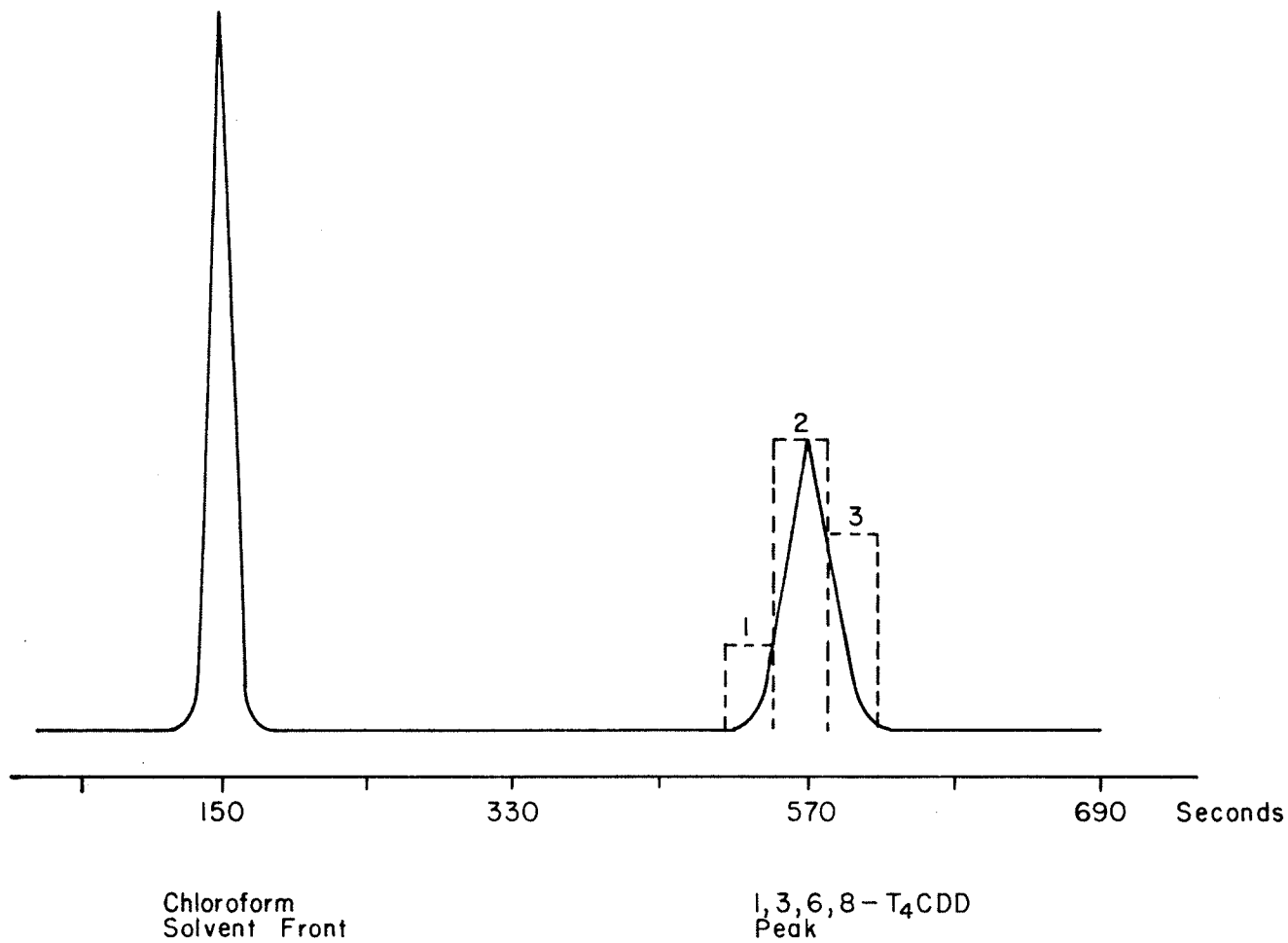


FIGURE 16.0 Representation of Fraction Collection of HPLC Characterization of 1,3,6,8-T₄CDD

to 1.5 to 3.0 ng of parent compound present based on back calculations of specific activity.

6.3 Oxidation

The instrument used as a Packard 306 Oxidizer which in the presence of oxygen burned samples for total ^{14}C activity. Evolved $^{14}\text{CO}_2$ was captured by $\text{CO}_2\text{-M-Met}^*$ and counted by liquid scintillation in PCS:xylene (2:1) fluor. Oxidation of 1,3,6,8- T_4CDD was found to be $89.1 \pm 2.2\%$ efficient with the instrument process being 97 to 100% efficient on ^{14}C standards (5000 dpm). Samples oxidized ranged from 0.05 to 0.65 g in weight.

6.4 Liquid Scintillation Counting (LSC)

A Beckman 7500 scintillation counter was used, programmed for replicate 10.0 minute counts over the entire ^{14}C energy range with a sigma error factor of 2.0%. Background counts and efficiency were automatically compensated for as well as quenching effects up to an H value of 420. As noted earlier, 2 fluor cocktails were used in counting, Atomlight with water samples and PCS with extracts.

7.0 INDIVIDUAL EXPERIMENTS

7.1 Snail Clearance

Twenty days after the pools were treated, 100 pool snails (Gyraulus parvus) from pool 1 and pool 2 were collected with a sweep-net. The bottom and surface regions were sampled separately and the snails returned live to the laboratory. Ten individuals were sampled on site for time 0, and, once in the laboratory, the remaining were placed into silanized glass pans with 2.0 L of dechlorinated aerated,

* 4-methoxy ethylamine

filtered water (pool 1: pelagic, pool 1: benthic, pool 2: pelagic, pool 2: benthic). The snails were allowed to clear accumulated material (^{14}C), and every 24 hours, 75% of the water was exchanged. Ten individuals were randomly sampled with forceps, air dried on filter paper, weighed and combusted in the oxidizer for total ^{14}C label present at times 0, 6, 12, 24, 48, 72 and 96 h.

7.2 Photolytic Experiments - Year I

A series of 24 1.0 L Erlenmeyer flasks was filled with un-treated pool water and injected with 30,000 dpm of 1,3,6,8- T_4CDD (294.4 ng) in acetone/benzene (1:9). Three sets of two replicate flasks were covered with aluminum foil.

At prescribed times (Table 10.0), over a 30-day period, flasks were removed in duplicate, preserved in the field with 40.0 mL of dichloromethane and extracted in the laboratory.

Extraction involved liquid-liquid partition with dichloromethane three times (200, 100, 100 mL). The extract was dried on a 20.0 g anhydrous Na_2SO_4 bed, concentrated by rotoevaporation with a 1.0 mL iso-octane keeper to 3.0 mL of hexane. A 100 μL aliquot was counted by LSC.

This experiment proved instrumental in the redesign of extraction procedures and experimental design for Year II, from its resulting trends.

7.3 Photolytic Study - Year II

In this experiment, a series of 33 1-L Erlenmeyer flasks was filled with pre-filtered pool water. The water was drawn through a pre-extracted glass fiber (0.45 μm) Whatman filter. Twenty of the

Table 10.0 Photolytic Experiment - Year I Sampling Schedule

Time (hours)	Unfiltered Pond Water	Field Black Bottles
0	x	x
4	x	
9	x	
24	x	
48	x	
72	x	
168	x	
336	x	x
720	x	x

Table 11.0 Photolytic Experiment - Year II Sampling Schedule

Time (hours)	Filtered Pond Water	Filtered, Autoclaved	F&A Field Black Bottles	F&A Fridge Black Bottles
0	x	x	x	
25	x	x		
48	x	x		
60	x	x		
72	x	x		
84	x	x		
96	x	x		
109	x	x		
120	x	x		
168	x	x	x	x
240	x	x		
336	x	x	x	x
672	x	x	x	x

flasks were autoclaved and sealed while the remaining 13 were not sterilized. In the field, standard sterile procedures were used during inoculation of flasks to minimize the degree of microbial colonization. The flasks were prepared in the following way:

13 flasks, filtered water, sealed and exposed to natural light.

13 flasks, filtered water, autoclaved, sealed and exposed to natural light.

4 flasks, filtered water, autoclaved, coated with a double layer of reflective aluminum foil and exposed to natural light.

3 flasks, filtered water, autoclaved, coated with foil and held at 4°C.

The inoculation was performed by injection of 1.0 mL of standard in a benzene-acetone carrier (1:1) giving each flask an application level of 164,000 dpm or 1.29 ug of dioxin. The three flasks in refrigeration were injected in the lab, and the remainder were treated in the field. Individual flasks were then sampled over a 30 d period at times noted in Table 11.0. The flasks were preserved in the field with dichloromethane (40.0 mL) and returned to the laboratory for immediate extraction.

Extraction procedures:

The pH was determined and the water extracted (usually at a pH of 8.9-9.6) three times (200, 100, 100 mL) with dichloromethane. The partitioned extract was run through a 20.0 gm Na_2SO_4 bed, and concentrated by rotoevaporation to 5.0 mL of chloroform (Fraction I). The extracted water was then adjusted to pH 1.0-1.5 with concentrated

sulfuric acid and extracted three times (100, 100, 50 mL) by partitioning with dichloromethane. The extract was dried and concentrated to 5.0 mL of chloroform (Fraction II). The water was re-extracted twice (150, 100 mL) with ethyl acetate, the extract of which was dried on a NaSO_4 bed and concentrated into 5.0 mL of chloroform (Fraction III). Aliquots of 100 μL were taken and counted by LSC. A 60 μL aliquot was then analyzed by HPLC as noted earlier. The extraction flasks were periodically re-extracted with dichloromethane to note any losses to the glass and with each extraction a 4.0 mL aliquot of water (extracted) was taken and counted directly by LSC to determine the extraction efficiencies.

7.4 Test of the Foam Air Samples

As each chemical's trapping efficiency varies, 1,3,6,8- T_4CDD had to be tested. To determine this, a procedure and apparatus developed by Grover and Kerr (1981) was used. The apparatus consisted of a U-tube in a 50°C water bath upstream of a tulip glass chamber in which two, 2.5 cm by 5.0 cm, foams were placed in series. A flow rate of 10.0 L per min through the foams was applied by vacuum (Figure 17.0).

Once the temperature and flow rates stabilized, a 200 μL aliquot of standard was added in benzene. The label was rinsed into the bottom of the submerged U-tube along a curved Pasteur pipet. The U-tube was sealed and left for 24 h during which time any volatilized label was trapped in the foams. Two trials were run at 360 pg and one at 4000 pg of 1,3,6,8- T_4CDD .

The foams were removed and extracted separately for 3.5 h with hexane by soxhlet as noted earlier for the field air foams. The U-tube was extracted with benzene and the residue counted by LSC. A

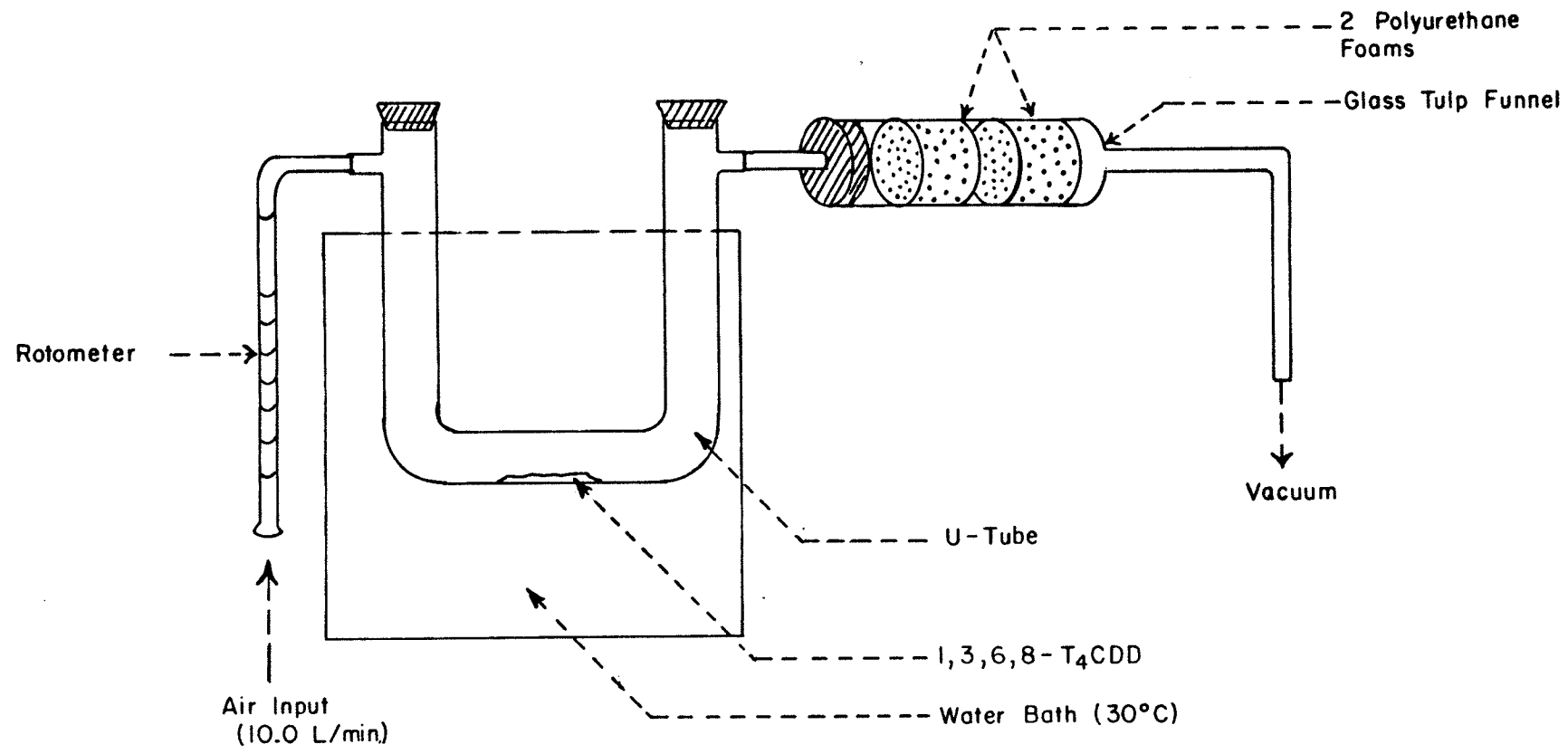


FIGURE 17.0 Apparatus for Testing Trapping Efficiency of Polyurethane Foams — From Grover & Kerr, 1975

100 uL aliquot of the concentrated foam extract (3.0 mL) was counted and a further 60 uL characterized by HPLC. The relative trapping efficiencies, losses and percent breakthrough are tabled in Section IV, 1.0 of the results.

IV. RESULTS AND DISCUSSION

1.0 VOLATILIZATION: ESTIMATED AND PREDICTED FLUX:

To assess the losses of 1,3,6,8-T₄CDD from the pond surfaces, two mathematical expressions noted in Section II . 8.0 were used:

$$(a) \text{ predicted flux} = K_{iL} x(C_i)$$

$$\text{where: } K_{iL} = \left(\frac{1}{K_L} + \frac{1}{H K_g} \right)^{-1} \frac{1}{RT}$$

$$(b) \text{ estimated flux} = P \uparrow$$

$$P \uparrow = K^2 \overline{\Delta C} \overline{\Delta U} / \phi^2 [1_n (z_2 - z_0) / (z_1 - z_0)]^2$$

$$\text{where: } \phi^2 = [(1 \pm 16 R_i) \pm 0.33]^2$$

$$\text{and } R_i = g (\overline{\Delta T} / \Delta Z) / \overline{T} (\Delta U / \Delta Z)^2$$

The results of calculations of expressions "a" and "b" are listed in Table 12.1, while the data of trapped 1,3,6,8-T₄CDD are described in Table 12.0 and Figure 18.0 and 19.0. These results are approximations only and are based on the assumption of homogeneity of the air concentrations of 1,3,6,8-T₄CDD and estimates of wind velocity and lapse temperature. Sample calculations are performed on the following page.

Based upon the Claitth et al. (1980) estimates, volatilized losses of 1,3,6,8-T₄CDD ranged from 21.4 to 33.7 ng m⁻² h⁻¹ suggesting a liquid phase mass transfer mechanism (Mackay and Leinonen, 1975). Over the first 24h the flux ranged from 33.7 ng m⁻² h⁻¹ during the day to 1.40 ng at night from pool 1 and 0.72 to 21.4 ng m⁻² h⁻¹ from pool 2. After the first day the rates sharply dropped to estimated levels of 0.01 to 0.48 ng m⁻² h⁻¹. Though the daily losses are significant in

Table 12.0. Air Samples of the Volatile Release of 1,3,6,8-T₄CDD

(ng per 24h)

Height	Pool 1			Pool 2		
	5 cm	10	18	5	10	18
<u>Time</u>						
0 h	0.09	0.02	0.05	0.04	0.06	0.05
24 h	235.3	239.8	174.2	107.6	112.3	68.8
48 h	15.4	9.2	7.7	3.2	5.0	1.6
72 h	11.7	3.5	3.2	*6.1	6.5	2.0
5 d	6.4	3.4	1.7	9.9	2.2	1.2
7 d	4.7	2.3	1.9	*3.3	1.3	1.1

Blank = 37.5 dpm = 0 ng

* = indicates foams heavily contaminated with water therefore amounts found maybe non representative.

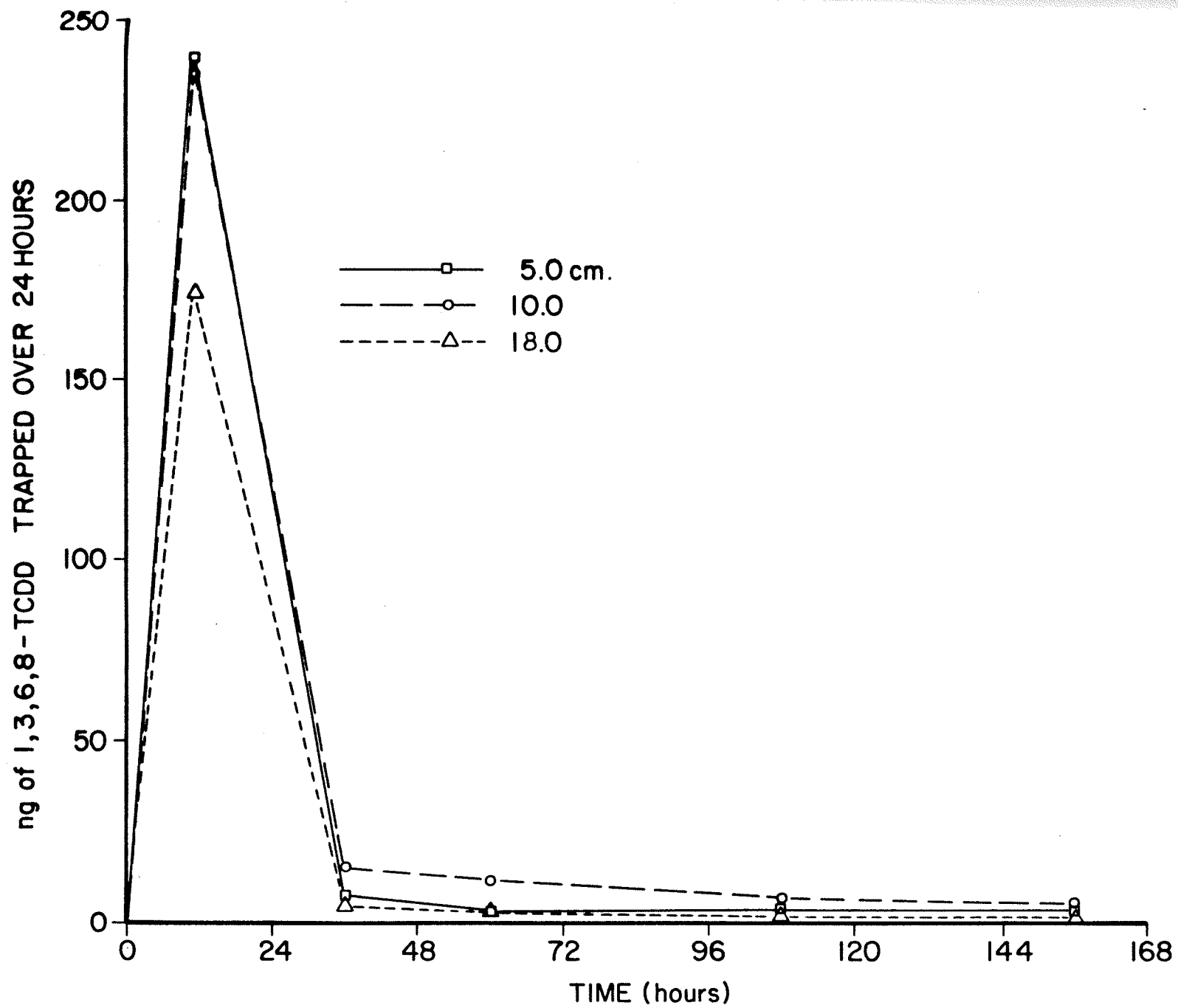


Figure 18.0 Losses of 1,3,6,8- T_4 CDD at Three Heights from Pond 1

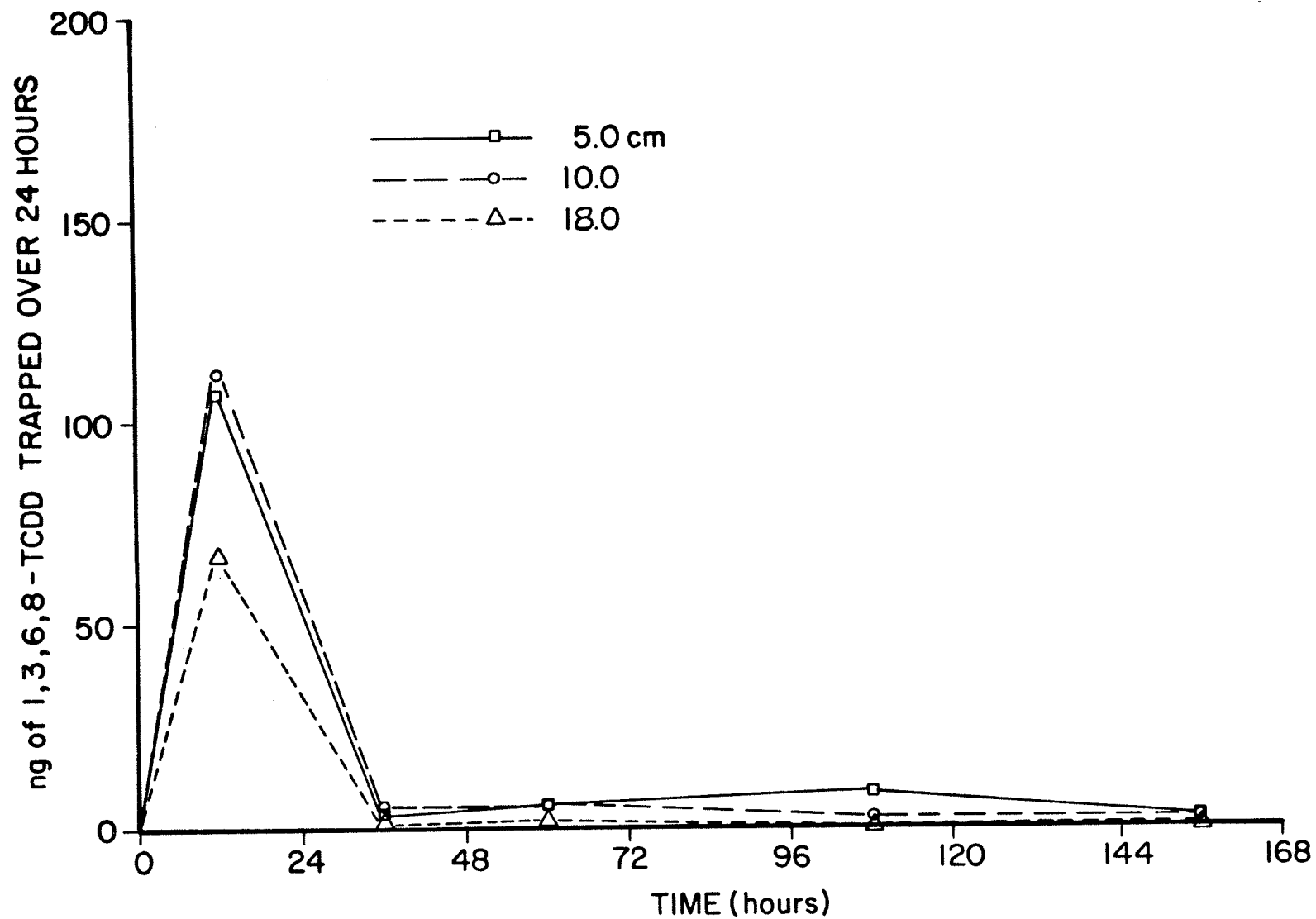


Figure 19.0 Losses of 1,3,6,8- T_4 CDD at Three Heights from Pond 2

Sample Calculations:

A) Mackay and Leinonen, 1975. - Predicted Flux

assume $t = 24\text{h}$, $T = 294^\circ\text{K}$, $C_i = 78.2 \text{ ug m}^{-3}$

$$N_i = K_{i1} \times (C_i) \quad \text{equation (4)}$$

$$= .004048 \times (78.2) = \underline{316.5 \text{ ng m}^{-2} \text{ h}^{-1}}$$

where

$$K_{i1} = \left(\frac{1}{0.0056744} + \frac{1}{\left(\frac{6.8 \times 10^{-5} \times 4.965}{8.2 \times 10^{-5} \times 294} \right)} \right)^{-1}$$

where

$$K_1 = 0.018 \times \sqrt{\frac{\text{mol. wt. O}_2}{\text{mol. wt. 1,3,6,8-T}_4\text{CDD}}} = 0.0056744 \text{ m h}^{-1}$$

$$K_g = 21.0 \times \sqrt{\frac{\text{mol. wt. H}_2\text{O}}{\text{mol. wt. 1,3,6,8-T}_4\text{CDD}}} = 4.965 \text{ m h}^{-1}$$

B) Claith et al., 1980. - Estimated Flux equation (6)

assume $t = 24\text{h}$, $k = 0.4$, $z_0 = 0 \text{ cm}$, $z_1 = 5.0 \text{ cm}$,

$z_2 = 18.0 \text{ cm}$, $u = 20.0 \text{ cm s}^{-1}$, $T = \pm 0.5^\circ\text{C}$.

$$C = 5.304 \times 10^{-6} \text{ ng cm}^{-3}$$

where

$$C = (C_{z_1} - C_{z_3}) / (11,520,000 \text{ cm}^{-3} \text{ per } 24\text{h})$$

$$R_i = 970 \times (+0.5/13) / (21 \times (20/13))^2$$

$$= 0.7506$$

$$\phi = (1 \overset{+}{-} 16 \times (0.7506))^{\overset{+}{-}.33} \text{ where "-" denotes day and a "+" denotes night estimates.}$$

$$\phi^2 = (2.33188)^2 = 5.43766$$

Therefore using equation (6) of Section III.8.0.

$$\text{Flux (P}\uparrow) = (0.4)^2 \times (5.3038 \times 10^{-6}) \times (20.0) / \phi^2 \times (\ln((18-0)/(5-0)))^2$$

$$= 5.6 \times 10^{-5} \text{ ng m}^{-2} \text{ min.}$$

$$= \underline{33.7 \text{ ng m}^{-2} \text{ h}^{-1}}.$$

Table 12.1 Estimates and Predicted Value of the Volatile Release of 1,3,6,8-T₄CDD

Time (h)	Predicted (ng m ⁻² h ⁻¹)		Estimated (ng m ⁻² h ⁻¹)	
	Pool 1	Pool 2	Pool 1	Pool 2
24 day	316.5	249.7	33.7	21.4
night			1.4	0.7
48 day	151.8	30.8	4.4	0.9
night			0.1	0.1
72 day	61.1	13.8	4.9	2.4
night			0.2	0.1
120 day	49.7	12.1	1.7	4.8
night			0.1	0.2
168 day	36.4	5.7	1.4	1.3
night			0.0 (0.04)	0.0 (0.04)

1 = Mackay and Leinonen, 1975

2 = Claith et al., 1980

relation to the concentration of the label in the water column at the specific time, the total losses were small in relation to the treatment levels (initial). The predicted values are consistently higher than the estimated values (7-9x) indicating possible weakness in the mathematical expression for Ni due to such assumptions as that no other sources of removal are present. This is unrealistic in a natural system and further suggestions by Hassett and Anderson (1982) indicate that interaction of hydrophobic compounds such as 1,3,6,8-T₄CDD with natural compounds or suspended solids may result in increased aqueous solubility. This in turn would effect the mass transfer across the water-air interface.

Using an expression derived by Mackay and Leinonen (1975), the $t_{1/2}$ of 1,3,6,8-T₄CDD in the liquid phase, based upon the effect of Henry's constant and temperature on the resistance developed across the two film transfer interface of the pond surface, (Liss and Slater, 1974) was calculated as follows:

$$t_{1/2} = 0.69 L/K_{iL}$$

where:

$$K_{iL} = \left(\frac{1}{K_1} + \frac{1}{HK_g/RT} \right)$$

where:

$$H = \text{Henry's constant } (6.8 \times 10^{-5} \text{ atm. m}^{-2} \text{ mol}^{-1})$$

$$R = \text{gas constant } (8.2 \times 10^{-5} \text{ m}^3 \text{ atm mol}^{-1} \text{ K}^{-1})$$

$$T = \text{temperature } (^{\circ}\text{K})$$

$$K_g = \text{gas transfer coefficient} = 4.965 \text{ (m h}^{-1}\text{)}$$

$$K_1 = \text{liquid transfer coefficient} = .005674 \text{ (m h}^{-1}\text{)}$$

$$L = \text{depth (m)}$$

The $t_{1/2}$ is estimated to be 85.6 h. This value may be mediated by other mechanisms that effect ideal estimates of losses (Hassett and Anderson, 1982).

The HPLC analysis and GC confirmation of foam extracts indicate that all the material lost over the 7d field test was 1,3,6,8-T₄CDD.

Limited volatile loss of 1,3,6,8-T₄CDD is indicated in this study. Also the use of polyurethane foams as a trapping medium for dioxins was found to be simple and efficient. Following the procedure described in Section III, 7.4 the trapping efficiency of the foams was tested. The data indicates (Table 13.0) that the foams are >80% effective which compares well to similar tests with other non-polar compounds (Grover and Kerr, 1977; Muir, 1983). No breakthrough from the second foam (Figure 17.0) occurred, indicating that the total trapping of the volatile, available 1,3,6,8-T₄CDD was taking place. As 18 to 20% of the label was missing it suggested that adsorption to the glass, stoppers and tubing may have occurred as suggested by Nestrick (1982).

Table 13.0 Trapping Efficiency of Foams

Treatment Initial Concentration	Foam		Residue	Trapping Efficiency
	Front	Back		
3,920 ng	3,150	14.3	5.9	80.8 %
436.1 ng	344.6	2.5	4.4	80.4
425.7 ng	341.6	5.4	4.3	82.3

2.0 HYDROSOIL

The fate of hydrophobic organic pollutants in natural water systems is dependent, to a large extent, on the sorption to sediment and suspended organic materials which effects the physical movement, degradation and the concentration of the pollutant in the solution phase (Karickhoff *et al.*, 1979).

From Table 14.0, replicate I, 1,3,6,8- T_4 CDD was deposited at a rate of $0.03 \text{ ng g}^{-1} \text{ h}^{-1}$, $0.04 \text{ ng g}^{-1} \text{ h}^{-1}$ and $0.08 \text{ ng g}^{-1} \text{ h}^{-1}$ in pools 1, 2 and 5 respectively. At day four, pool 1, the rate decreased and stabilized between 4 d and 34 d at approximately $11.6 \pm 1.3 \text{ ng g}^{-1}$ after which the levels dropped (Figure 20.0). These calculations utilized the total amount of label present, assuming that the non-extractable residue was 1,3,6,8- T_4 CDD. This assumption is based upon the absence of metabolites in the extract and observations by Ward and Matsumura (1978) for 2,3,7,8- T_4 CDD which indicate that no ^{14}C -labelled metabolites were present in the soil-associated label. In pool 2, the maximum concentration of ^{14}C occurred at day 4 ($6.6 \pm 0.6 \text{ ng g}^{-1}$) and was maintained until day 15, after which it declined to 3.6 ng g^{-1} (2.8 ng g^{-1} extractable). The ^{14}C -labelled material dissipated at a rate of $0.04 \text{ ng g}^{-1} \text{ d}^{-1}$ until day 57 which resulted in an estimated $t_{\frac{1}{2}}$ of 16.9 d from the maximum accumulation which occurred by day 4. Pool 5, similarly, reached a maximum of 3.1 ng g^{-1} (2.7 ng g^{-1} extractable) on day 2 followed by a decrease at $0.08 \text{ ng g}^{-1} \text{ d}^{-1}$ until a $t_{\frac{1}{2}}$ estimated to be 9.13 d. As seen in the water data (Table 17.0), the rate of dissipation is effected by the amount of the original treatment and the point which the deposition rate decreases in the hydrosol, correlates with the point at which 1,3,6,8- T_4 CDD concentrations in the

Table 14.0 Hydrosol Levels of 1,3,6,8-T₄CDD - Replicate I
(ng/g freeze dried wt.)

Time	Pool 1	Pool 2	Pool 5	Control
0	ND	ND	ND	ND
12 h	2.7 (0.3)	1.8 (0.1)	0.7 (0.2)	
24 h	7.1 (0.4)	4.0 (0.2)	1.1 (0.3)	
48 h	3.2 (0.3)	3.7 (0.2)	3.5 (0.3)	
4 d	9.2 (1.9)	6.1 (0.6)	3.3 (0.8)	
8 d	10.1 (0.8)	5.7 (0.3)	2.8 (1.2)	
15 d	9.0 (0.8)	6.5 (1.1)	2.6 (0.8)	ND
24 d	10.7 (2.1)	2.8 (0.8)	2.3 (0.5)	ND
34 d	12.1 (1.3)	5.0 (1.2)	2.1 (0.8)	
57 d	2.9 (1.5)	3.1 (1.3)	4.0 (1.4)	ND
90 d	4.4 (2.1)	2.5 (2.0)	5.4 (1.2)	
426 d	1.7 (1.1)	0.4 (0.4)	0.4 (0.1)	ND

ND: none detected

(): residue (unextractable compound)

expressed as ng equivalents of 1,3,6,8-T₄CDD

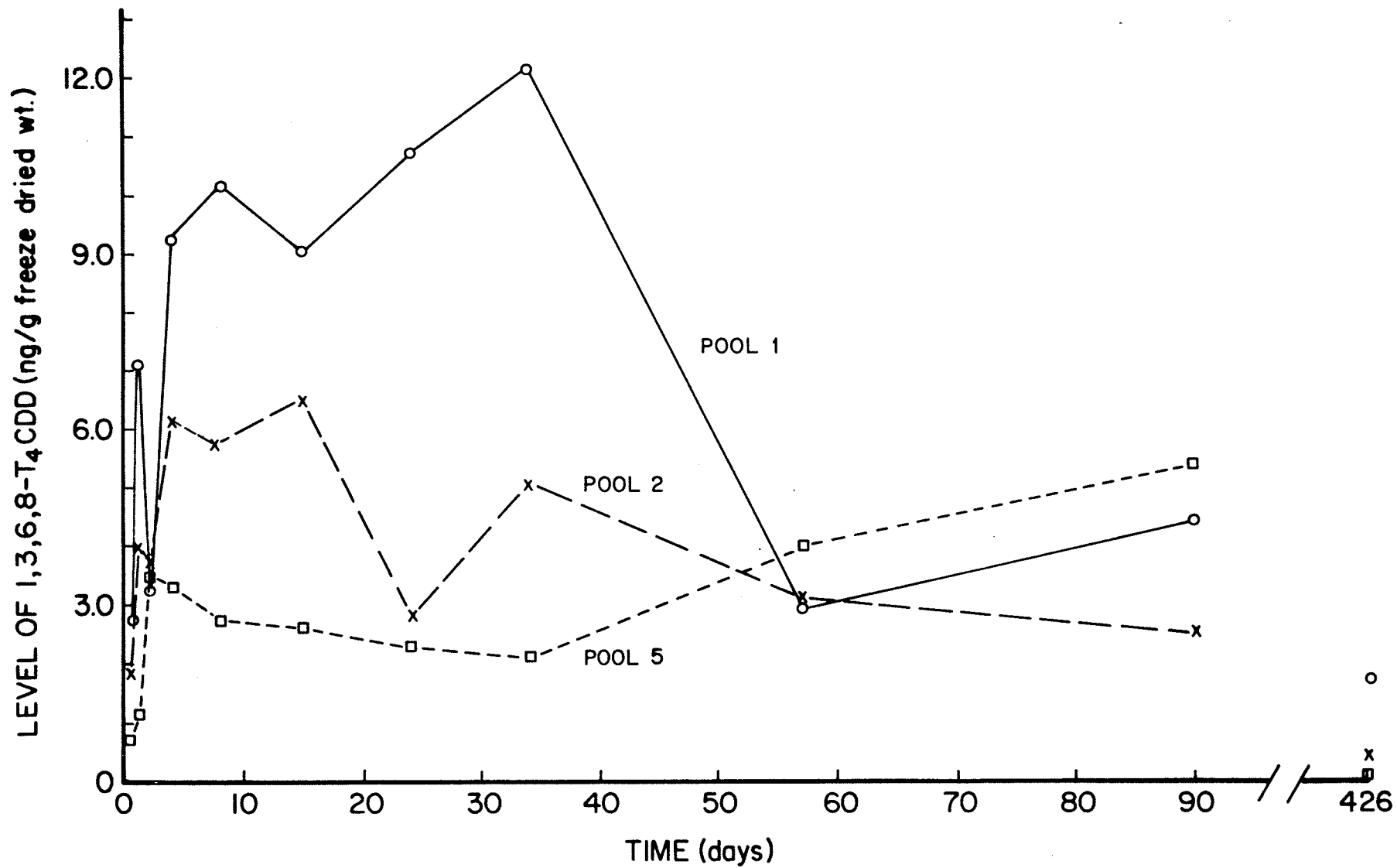


Figure 20.0 Hydrosol Levels of 1,3,6,8-T₄CDD-Replicate I

water column reach equilibrium (Figure 20.0 and 21.0). By day 34, losses slowed and levels of ^{14}C labelled material began increasing in the hydrosol.

For replicate II (Table 15.0) the initial rates of deposition are comparable to replicate I with pool 1 = 0.06, pool 2 = 0.23, and pool 5 = 0.06 $\text{ng g}^{-1} \text{h}^{-1}$. Between day 2 and day 34, the trends vary (Figure 21.0). After day 34 for pools 1 and 5 and day 57 for pool 2, substantial increases in total ^{14}C of 2 to 7 x of the minimum levels were observed. The occurrence of observed increases correlate with the onset of early fall and degradation of the rooted vegetation population. A similar observation was made by Tsushimoto *et al.*, (1982). He believed that much of the variability in his data was due to the biological cycle of the pond weeds (sudden population crash). To further confirm this factor, I used data on the amounts of 1,3,6,8- T_4CDD present in the vegetation and, using estimates of vegetal cover in pool 1, calculated that it would take 94 to 101 g wet weight of rotting vegetal matter to maintain the rate of ^{14}C deposition observed. This is, approximately, 0.92% of the total bulk present, dying on a daily basis after day 34. The possible association of ^{14}C with the fibrous vegetal material may explain the inability to extract all the label, as seen by the results of post extraction oxidation done on the vegetal matter (Table 22.0). Similarly, variability may have occurred during the mixing and subsampling of the lyophilized hydrosol samples.

In all three treatments, the carry over of 1,3,6,8- T_4CDD to the next year was 11.8%, 7.4% and 12.9% for pools 1, 2 and 5, respectively, in replicate II. In replicate I, the carry over, as calculated as a proportion of the maximum amount of compound deposited,

Table 15.0 Hydrosol Levels of 1,3,6,8-T₄CDD - Replicate II
(ng equivalents of 1,3,6,8-T₄CDD per g freeze dried wt.)

Time(d)	Pool 1	Pool 2	Pool 5	Control
0	ND	ND	ND	ND
12 h	1.1±0.2	2.1±0.4	0.5±0.4	
24 h	5.2±1.0	4.9±0.5	2.0±0.4	
48 h	6.3±1.4	4.0±0.4	2.7±0.4	
4 d	7.5±0.6	3.5±0.3	1.8±0.4	
8 d	8.8±0.9	4.2±0.8	2.1±0.1	
15 d	11.9±0.8	2.5±0.6	2.6±0.7	ND
24 d	7.8±0.2	4.4±0.1	5.3±0.1	ND
34 d	5.4±0.1	4.4±0.0	2.7±0.04	
57 d	10.0±1.2	1.0±0.1	3.4±0.1	ND
90 d	14.8±0.6	8.6±0.3	7.2±0.3	
426 d	1.2±0.3	0.3±0.1	0.4±0.04	ND

ND: none detected

n = 3

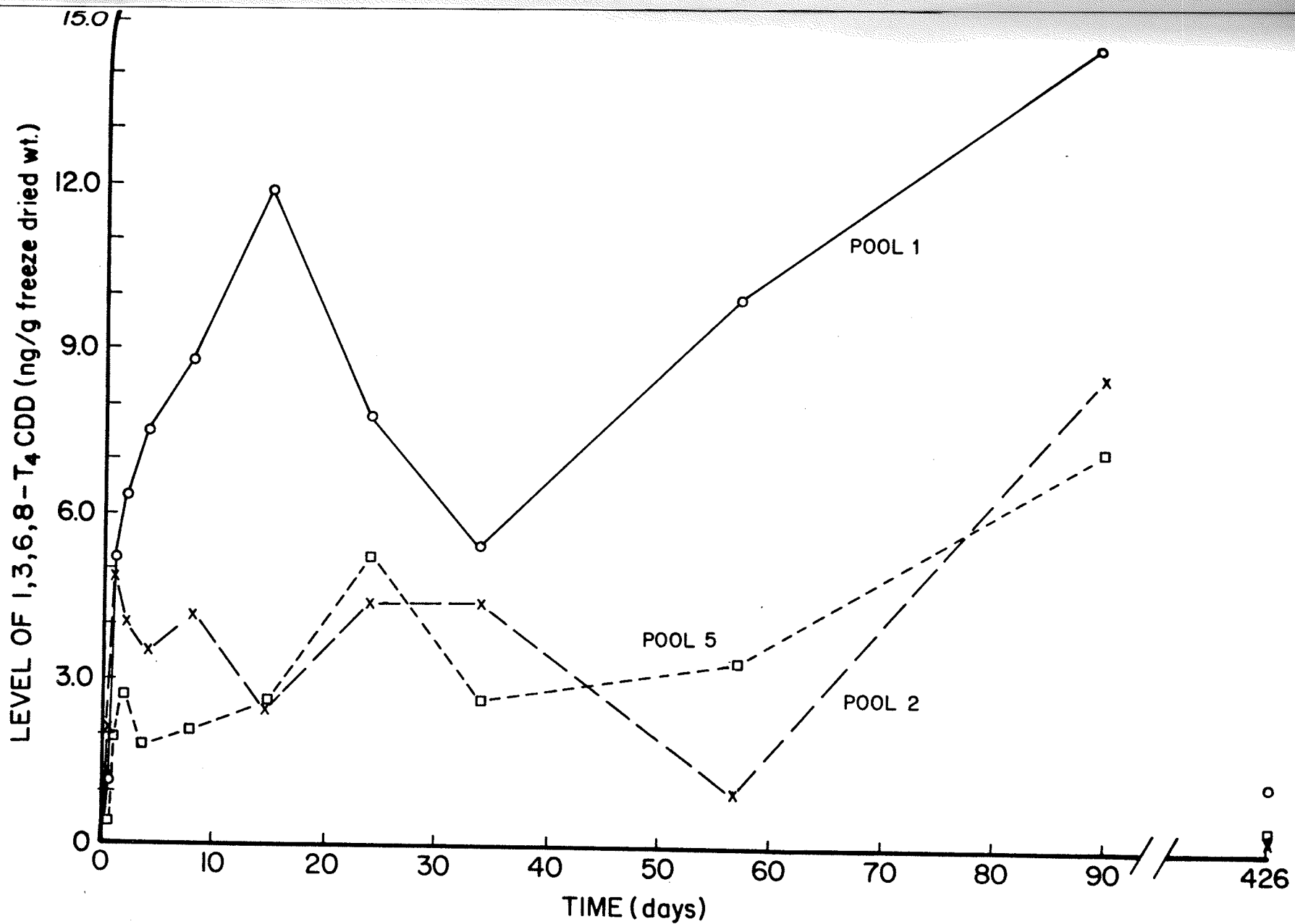


Figure 21.0 Hydrosoil Levels of 1,3,6,8-T₄CDD-Replicate II

was 20.8%, 10.5% and 10.8% in each pond.

The $t_{1/2}$ values indicate a reduced persistence in hydrosol compared to estimates for 2,3,7,8- T_4 CDD determined by Ward and Matsumura (1978) and Tsushimoto *et al.*, (1982). Variability in the presence of 1,3,6,8- T_4 CDD in the sediment over time was observed to occur as was the case in Tsushimoto's 2,3,7,8- T_4 CDD study (1982). Similarly the amount of carry over noted previously is less than that estimated for 2,3,7,8- T_4 CDD by Tsushimoto *et al.*, (1982).

HPLC analysis of the extracts (replicate I) indicated that 100% of the ^{14}C detected was 1,3,6,8- T_4 CDD until day 57 in all but pool 1. On day 57, 6.3% of the pool 1 extract was a polar, compound eluting at 4 min 16 s (1,3,6,8- T_4 CDD retention = 8 min 20 s). By 90 d the level of non-parent label was 1.2% with a retention time of 6 min 46 s. This metabolite increased to 7.6% by day 426.

By day 426, pond 5 extracts contained 16.0% of non-parent compound with a retention time of 4 min 15 s. These results are contrary to observations by Isensee and Jones (1975) and Ward (1976). The identification of the metabolite has not been completed to date.

3.0 FISH (Pimephales promelas)

The uptake of 1,3,6,8- T_4 CDD by fathead minnows (pool 1) was rapid (Table 16.0, Figure 22.0) and the overall uptake and clearance was described as a function of time with the quadratic equation.

$$\text{Conc} = 40.41 + 3.42 t - 0.011 t^2 \quad (1)$$

where

Conc = the total concentration of material per g of freeze dried whole body weight

t = time (h)

Table 16.0 Accumulation of 1,3,6,8-T₄CDD in Fathead Minnows (Pimephales promelas) (ng/g freeze dried wt.)

Time	Pool 1	Residue	Pool 2	Residue	Pool 5	Residue	Control
0	ND	ND	ND	ND	ND	ND	ND
2 h	39.0± 2.5	2.8±0.3	N/A		N/A		ND
12	50.9± 5.1	24.1±6.3	133.5± 4.1	10.7±3.2	N/A		
24	191.6± 3.2	24.9±2.1	N/A		N/A		ND
48	139.4± 3.6	59.4±7.2	277.9±11.4	51.0±5.3	76.6±7.4	14.4±2.3	
103	244.9± 4.1	13.5± .3	N/A		14.7±2.0	12.5±3.1	ND
246	164.9±13.1	23.0±0.8	N/A		N/A		N/A

ND = non detected

N/A = not available (none trapped)

all residues are expressed as ng of 1,3,6,8-T₄CDD equivalents

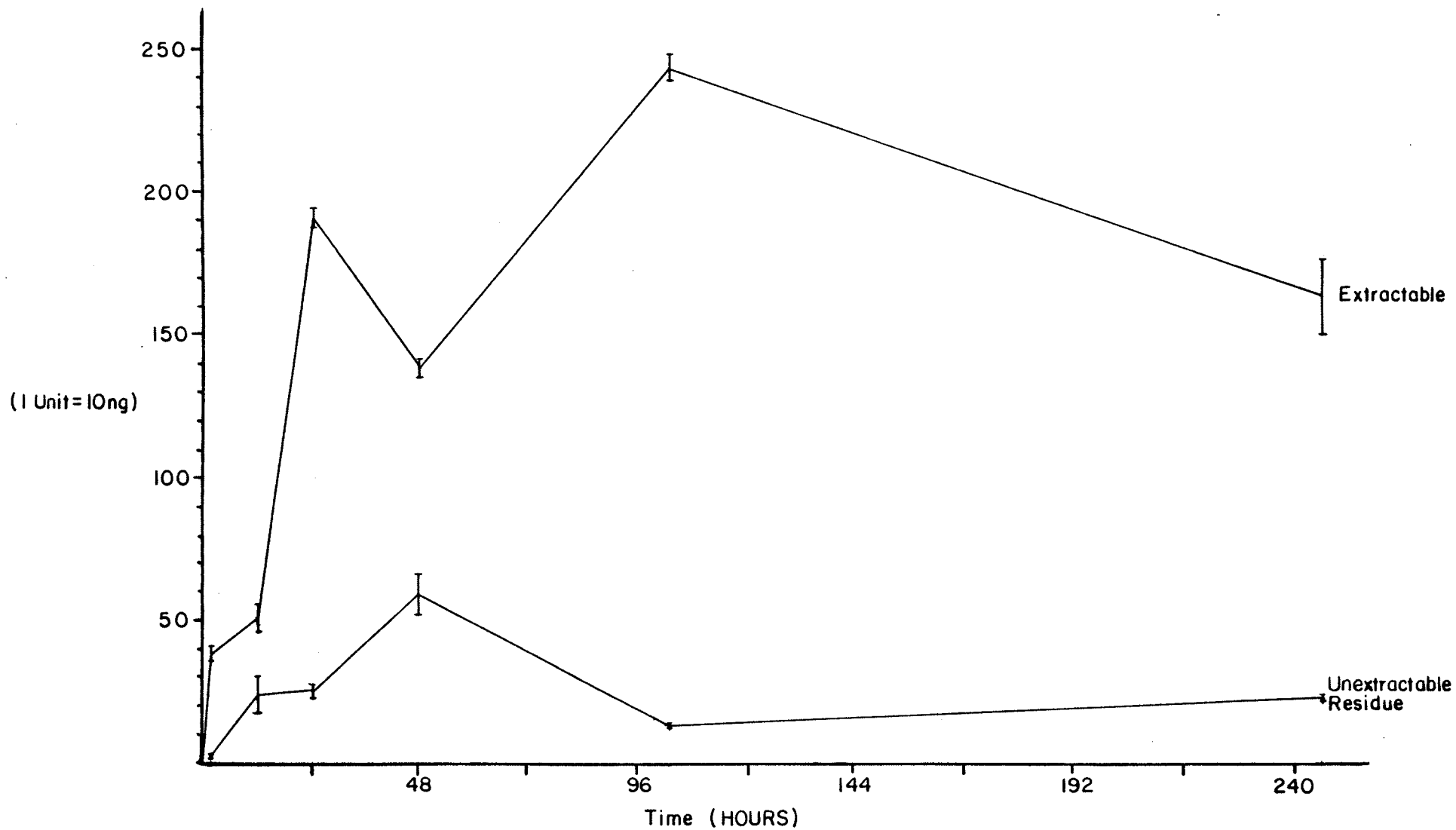


FIGURE 22.0 Accumulation of 1,3,6,8- T_4 CDD in Fathead Minnows (ng/gm Freeze Dried Wt.)

The expression was generated using a computer assisted quadratic regression statistical package (BMDP) with the concentration (in the fish) versus time data. The resulting expression has a correlation coefficient of 0.98. Differentiation of equation (1) gave

$$\frac{d[\text{Conc}]}{dc} = 3.42 - 0.022 t \quad (2)$$

From this expression, an estimate of the time of maximum accumulation was possible.

$$\begin{aligned} t_{\text{max}} &= \frac{3.42}{0.022} = 155.50 \text{ h} \\ &= 6.47 \text{ d} \end{aligned}$$

At 6.47 d the water concentration was 10.5 ng L^{-1} and the projected concentration in the fish was 306 ng g^{-1} resulting in an estimated BCF of 29.1×10^3 (Neely, 1979). Attempts to predict the BCF using Kenaga and Goring's (1980) expression were made. The equation was:

$$\log \text{BCF} = 3.995 - 0.3891 \log [S]$$

where $[S]$ = solubility of 1,3,6,8- T_4 CDD (317 ppt) ($.000317 \text{ mg L}^{-1}$) (in water)

(Webster *et al.*, 1983a).

The BCF value was 2.27×10^5 which is comparable to the field data. Over the sampling period, the BCF increased from 266 to 2.89×10^3 at 246 h. In all calculations, the total amount of label present was used, even though the residue remaining in the tissue was not identified. Recent work by Muir (1983) indicated that the fathead minnow was able to metabolize 1,3,6,8- T_4 CDD. As no metabolites were found in the HPLC analysis of the extractable label, it may be that the procedures for extraction were ineffective in removing non-parent compounds, that the concentration was too low to detect, or that metabolism did

not occur to a measurable extent in the outdoor pools.

Workers had attributed the extreme levels of accumulation to the fish lipid content, solubility of the compound in the water, K_{ow} of the compound and the feeding behaviour of the organism (Kenaga and Goring, 1980). The concentrating mechanism may be a function of the water concentration as noted by the results of a linear regression analysis of water concentration data versus the amount of 1,3,6,8- T_4 CDD found in the minnows. The correlation coefficient was 0.82 indicating possible interaction of these two parameters. This is supported by studies by Isensee and Jones (1975) and Kenaga and Goring (1980). Isensee and Jones (1975) and Yockim et al., (1979) found that the BCF of 2,3,7,8- T_4 CDD ranged from $2 - 2.6 \times 10^4$ and $2 - 6 \times 10^3$ x respectively which compares ^{well} with my study results.

Tsushimoto et al. (1982) found that 2,3,7,8- T_4 CDD peaked at 10.0 d at 8500 ppt with equilibration occurring by day 40 at 2500 ppt. Though his results differ from this study, the treatment level (53.7 ppt) used in Tsushimoto's study was much lower which may affect the rate of uptake of the label.

Yockim et al. (1979) found that the BCF increased with time from 676 x at day 1 to 4.88×10^3 by day 7. Mortality occurred after day 15 and he observed nasal hemorrhage in the carcasses indicating a possible toxic effect. In the 1,3,6,8- T_4 CDD study I recovered dead fish with extensive cellular necrosis in the gills accompanied by extensive capillary hemorrhage. Whether this is a function of anoxia, which is unlikely in this pool design, or a result of 1,3,6,8- T_4 CDD toxicity is unknown. Predation of pool 2 and 5 limited sampling to only 2 times.

4.0 POND WATER

The 1,3,6,8- T_4 CDD moved rapidly through the water column (Table 17.0). The labelled material in pool 1 sharply increased within 2 h while the levels in ponds 2 and 5 increased to 12 h following which the both pools decreased sharply. It is believed that variability observed initially in pool 1 was due to incomplete mixing of the label at treatment (Figure 23.0).

Initially, 1,3,6,8- T_4 CDD was found to increase at the point of sampling at 5.25, 1.13 $\text{ng h}^{-1} \text{L}^{-1}$ for ponds 1, 2, and 5 respectively. This was followed by a logarithmic (\ln) dissipation rate of $0.7 \text{ ng h}^{-1} \text{L}^{-1}$ for pools 2 and 5 resulting in a $t_{\frac{1}{2}}$ of 20.8 h and 17.8 h respectively. At 12 h, pond 1 which decreased at $0.12 \text{ ng h}^{-1} \text{L}^{-1}$ resulting in a $t_{\frac{1}{2}}$ of 134.5 h (over 12 h to 34 d). It should be noted that in calculating the rate of dissipation and $t_{\frac{1}{2}}$, a specific range of time-concentration data was used which encompasses 95-98% of the movement of 1,3,6,8- T_4 CDD. For pools 2, 5 and 1, this meant data between 12 h and 8 d, 12 h and 4 d and 12 h to 34 d.

Beyond 34 d, the extractable label increased 69 to 161% (0.5 to 2.3 ng L^{-1}). In the following year (426 d) the amount of label (1,3,6,8- T_4 CDD equivalents) reached 7.7 ng L^{-1} , 3.1 and 1.6 ng L^{-1} in ponds 1, 2 and 5, all of which was only extractable in an acid polar solvent. As my objective was to describe trends in physical movement of 1,3,6,8- T_4 CDD, it was not possible beyond 34 d to differentiate between mechanisms occurring (i.e., deposition, metabolism or resuspension).

In Isensee's (1975) study with 2,3,7,8- T_4 CDD, the high treatment (3.17 ppm) reached equilibrium in 4 d and in 15 d for the 10 to 100 ppb treatments. This is comparable with the estimated equilibrium

Table 17.0 Presence of 1,3,6,8-T₄CDD in Pond Water (ng of 1,3,6,8-T₄CDD per L. Water)

Time	Pool 1	Pool 2	Pool 5	Control
0 h	ND	ND	ND	ND
2 h	157.1±5.2	7.2± 0.4	7.5±1.0	
6 h	42.3±2.5	43.9±3.2	16.8±3.0	
12 h	78.2±8.1	61.7±4.0	19.4±1.1	
24 h	73.5±5.1	24.3±4.1	13.9±6.2	
48 h	37.5±3.2	7.6±1.5	4.9±1.6	
72 h	15.1±1.3	3.4±1.2	1.9± 0.7	
96 h	13.5±1.5	3.8± 0.5	0.8±0.2	
8 d	7.2±0.9	0.1±0.1	1.7±0.6	ND
15 d	3.5±0.8	0.8±0.2	0.4±0.1	
24 d	2.3±0.1	0.3±0.2	1.0±0.8	ND
34 d	0.8±0.5	0.5±0.4	0.8±0.3	
57 d	2.8±0.6	1.6±0.4	0.9±0.5	ND
90 d	1.6±0.7	1.1±0.5	0.7±0.4	ND
426 d	* 7.7	* 3.1	* 1.6	ND

* - none detected in acid or base dichloromethane extract with label being in ethyl acetate extract

ND- none detected.

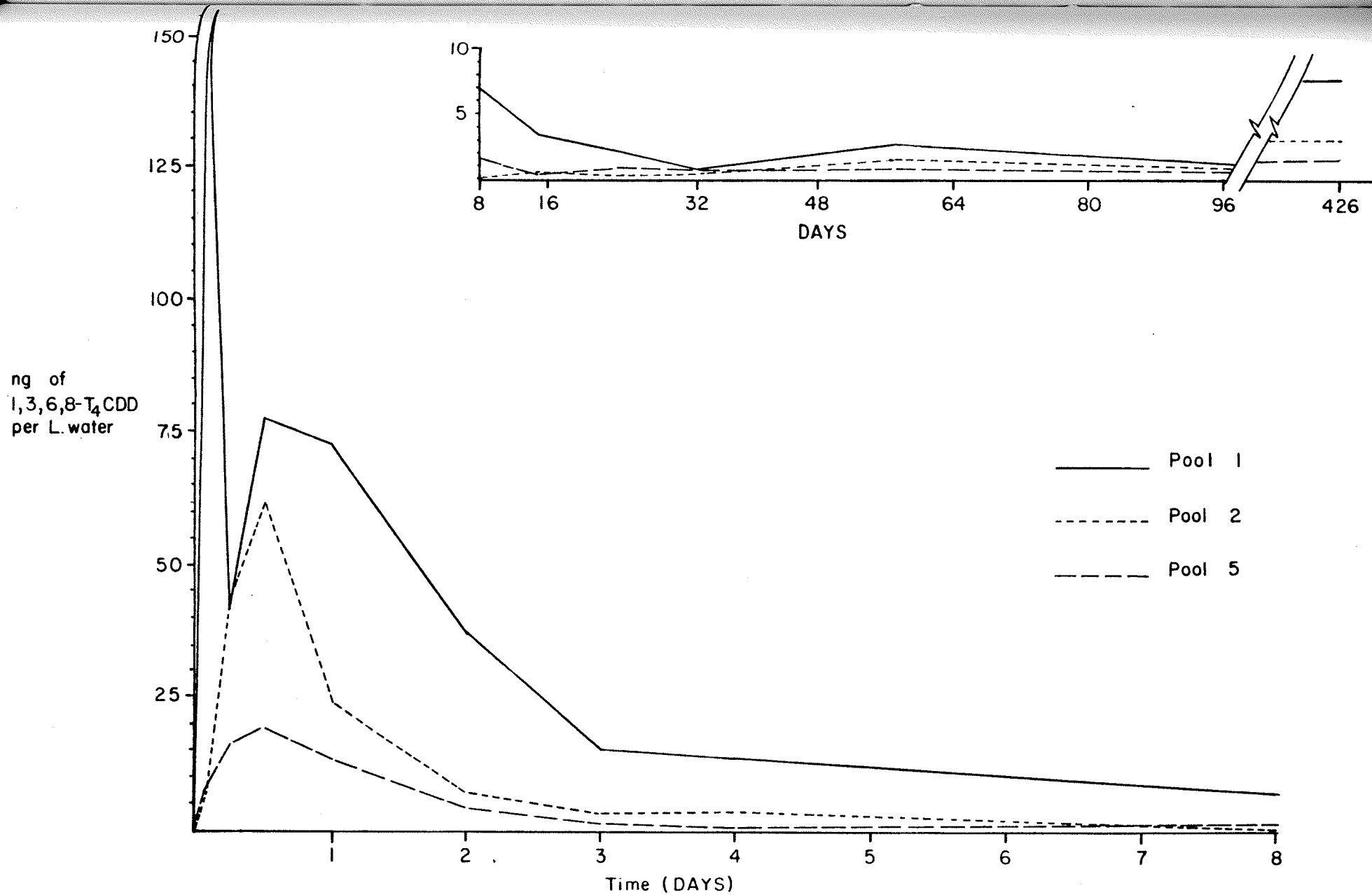


FIGURE 23.0 Presence of 1,3,6,8-T₄CDD in Pond Water

in my study of 15 d, 8 d, and 3 d for pools 1, 2, and 5 respectively. The equilibrium levels were $1.9 \pm 0.5 \text{ ng L}^{-1}$, 0.9 ± 0.3 and $0.9 \pm 0.5 \text{ ng L}^{-1}$ for each treatment. This does not agree with Tsushimoto's (1982) estimate of equilibrium in the water at 30 d. Yockim et al., (1978) determined that water solubility and the rate of soil desorption controlled equilibrium time and levels. To this he estimated that equilibrium was achieved in 24 h for a 2 - 4 ppt treatment.

HPLC analysis of the water extracts indicated that no metabolic forms were present during the first 90 d. Characterization of the pool 1 (426 d) extract indicated the presence of a polar metabolite with a retention time of 4 min 35 s which elutes within 0.2 mL of the metabolite noted in the hydrosol.

5.0 INVERTEBRATES

5.1 Gyraulus parvus (the pond snail)

As the snail was analyzed by oxidation for total ^{14}C , the discussion refers only to 1,3,6,8- T_4CDD equivalents. The data of Table 18.0 describes a well defined relationship between treatment, time and the significant levels of accumulation reached by G. parvus.

The loss of ^{14}C is well described by a logarithmic (\ln) relationship with time (Figure 24.0). Regression analysis of the \ln of 1,3,6,8- T_4CDD equivalents versus time noted a linear relationship with an r^2 of 0.91 for pool 1 from which the $t_{\frac{1}{2}}$ was calculated to be 35.1 d ($t_{\frac{1}{2}} = \frac{0.693}{\text{slope}}$). Pools 2 and 5 had calculated $t_{\frac{1}{2}}$ of 32.7 d ($r^2 = 0.79$) and 32.6 d ($r^2 = 0.78$) respectively. Using a regression analysis package, analysis between the three treatments indicated a significant treatment effect ($F_{.05} \ll 27$) between the pools. Isensee (1975) estimated the bioconcentration factor (BCF) in snails to be

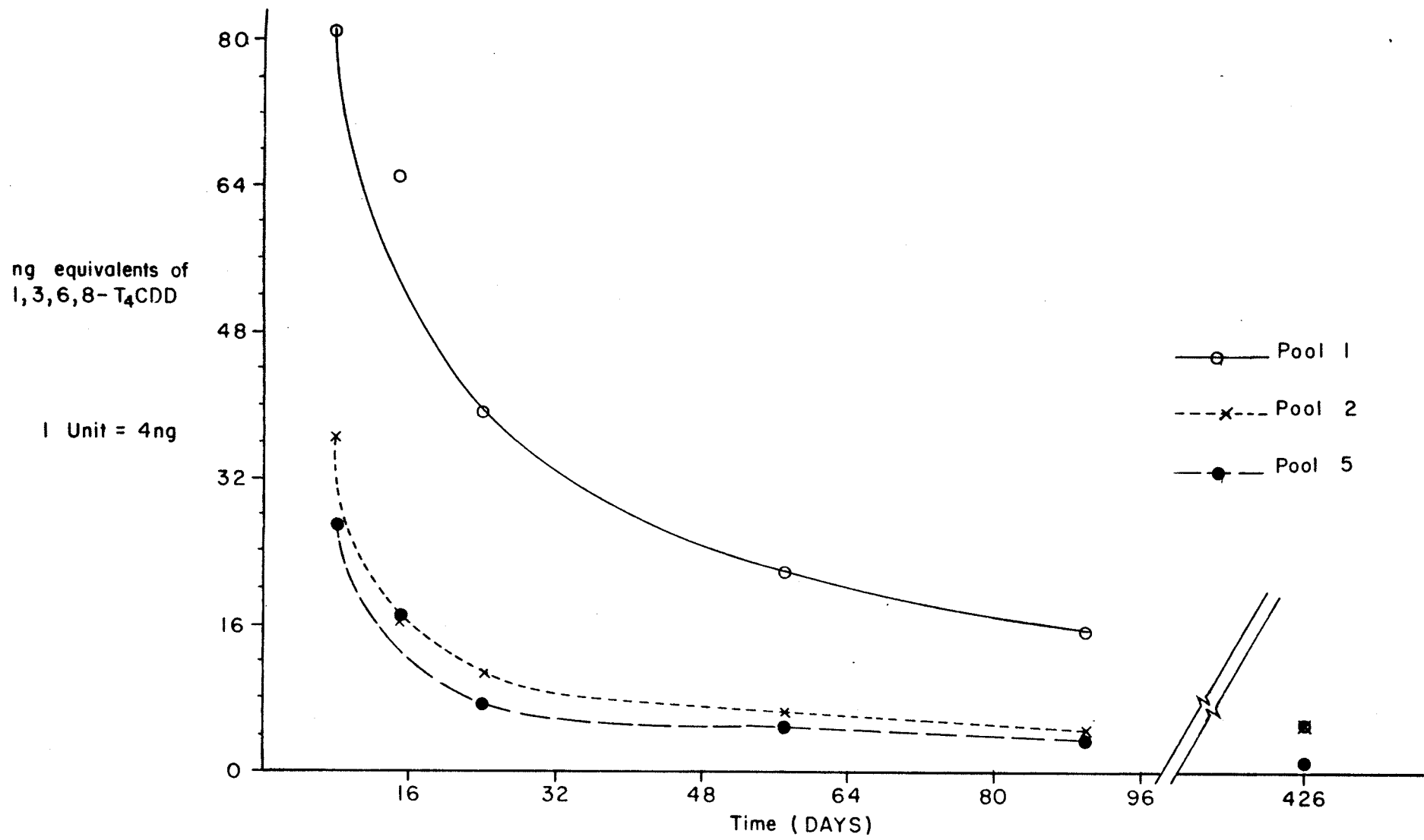


FIGURE 24.0

Pond Snail (*Gyraulus parvus*) Accumulation of 1,3,6,8-T₄CDD Equivalents

Table 18.0 Levels of 1,3,6,8- T_4 CDD equivalents present in
Pond Snail (G. parvus) (ng per g)

Time (d)	Pool 1	Pool 2	Pool 5	Control
0	ND	ND	ND	ND
8	**8261.1 ± 48.6 *(80.96 ± .47)	3726.9 ± 563.8 (36.52 ± 5.53)	2753.4 (26.98)	
15	6653.2 ± 10.2 (65.20 ± 0.10)	1659.1 ± 98.1 (16.26 ± 0.96)	1706.9 (16.72)	ND
24	3981.0 ± 1133.8 (39.01 ± 11.11)	1062.7 ± 35.5 (10.41 ± 0.34)	707.6 ± 141.8 (6.93 ± 1.39)	
57	2258.3 ± 323.9 (22.13 ± 3.17)	670.1 ± 20.6 (6.57 ± 0.20)	610.2 ± 76.3 (5.98 ± 0.75)	ND
90	1596.2 ± 82.8 (15.64 ± 0.81)	477.6 ± 32.3 (4.68 ± 0.32)	372.9 (3.65)	ND
426	566.5 ± 95.04 (5.55 ± 0.93)	566.5 ± 95.0 (5.55 ± 0.93)	107.5 ± 14.9 (1.05 ± 0.14)	

* () = ng equivalents of 1,3,6,8-Tetrachlorodibenzo-p-dioxin
per gram of freeze dried material.

** = ^{14}C activity

ND = none detected

$2 - 2.6 \times 10^4$ while Yockim et al. (1971) noted an increasing BCF from 735 at day 1 to 3731 at day 15, followed by a decline. From the data of Table 18.0 and the water concentrations (Table 17.0), the BCFs were calculated on an air dry wt. basis and found to increase with time to a maximum by day 15 in pool 1, day 24 in pool 2 and day 15 in pool 5. The BCF* s were 18.6×10^3 , 34.7×10^3 and 41.8×10^3 in pools 1, 2 and 5 respectively. These values are comparable to values determined for 2,3,7,8,-T₄CDD (Isensee and Jones, 1975). In the following year (426 d), the amount of material (¹⁴C) present was similar for all treatments with the estimated equilibrium levels being $5.6 \pm 0.9 \text{ ng g}^{-1}$ for pool 1 (day 57), $5.5 \pm 1.4 \text{ ng g}^{-1}$ for pools 2 and 5 (day 24) respectively. The label found in the next year may not be due to 1,3,6,8-T₄CDD as trace metabolite forms have been found in the soil and water after 90 d.

5.2 Snail Clearance

Attempts were made to assess the clearance rate of 1,3,6,8-T₄CDD equivalents from Gyraulus parvus. The study was performed as described in section III. 7.1 and temperature and photoperiod (12:12) were maintained to limit the shock of translocation. From Table 26.0, several trends were evident. The observed concentrations of 1,3,6,8-T₄CDD equivalents increased with the rate of increase being $0.041 \text{ ng g}^{-1} \text{ h}^{-1}$ (benthic) to $0.045 \text{ ng g}^{-1} \text{ h}^{-1}$ (pelagic) for pool 1 and 0.022 (benthic) to $0.027 \text{ ng g}^{-1} \text{ h}^{-1}$ (pelagic) for snails from pool 2. The mean concentrations of label over the 96 h test were:

Pool 1 benthic	Pool 1 pelagic	Pool 2 benthic	Pool 2 pelagic
$13.2 \pm 2.9 \text{ ng g}^{-1}$	7.4 ± 1.5	2.5 ± 0.9	2.4 ± 1.3

* $\text{BCF} = \frac{\text{concentration per g snail at time } x_i}{\text{concentration per mL water at time } x_i}$

Table 26.0 Pond Snail (Gyraulus parvus) Clearance Experiment

Time	Location	Pool #	*ng/gm wet wt.	Replicates
0 h	Benthic	1	14.1±3.2	2
	Benthic	2	1.2±0.4	2
	Pelagic	1	5.1±1.2	2
	Pelagic	2	0.6±0.2	2
6	Benthic	1	13.3±1.9	2
	Benthic	2	1.4±0.3	2
	Pelagic	1	6.0±1.2	2
	Pelagic	2	1.3±0.2	2
12	Benthic	1	10.1±1.1*	2
	Benthic	2	2.4±0.7	2
	Pelagic	1	7.9±0.5	2
	Pelagic	2	2.8±0.6	2
24	Benthic	1	11.4±7.7	2
	Benthic	2	2.6±0.8	2
	Pelagic	1	6.3±2.1	2
	Pelagic	2	1.7±0.5	2
48	Benthic	1	14.3±3.3	2
	Benthic	2	3.2±0.2	2
	Pelagic	1	7.5±0.8	2
	Pelagic	2	4.2±0.4	2
72	Benthic	1	14.8±2.1	3
	Benthic	2	3.7±1.5	3
	Pelagic	1	9.2±1.5	3
	Pelagic	2	3.2±1.0	3
96	Benthic	1	17.0±4.4	3
	Benthic	2	3.2±1.3	3
	Pelagic	1	9.6±1.9	3
	Pelagic	2	3.6±0.8	3

* = expressed as ng equivalents of 1,3,6,8-T₄CDD

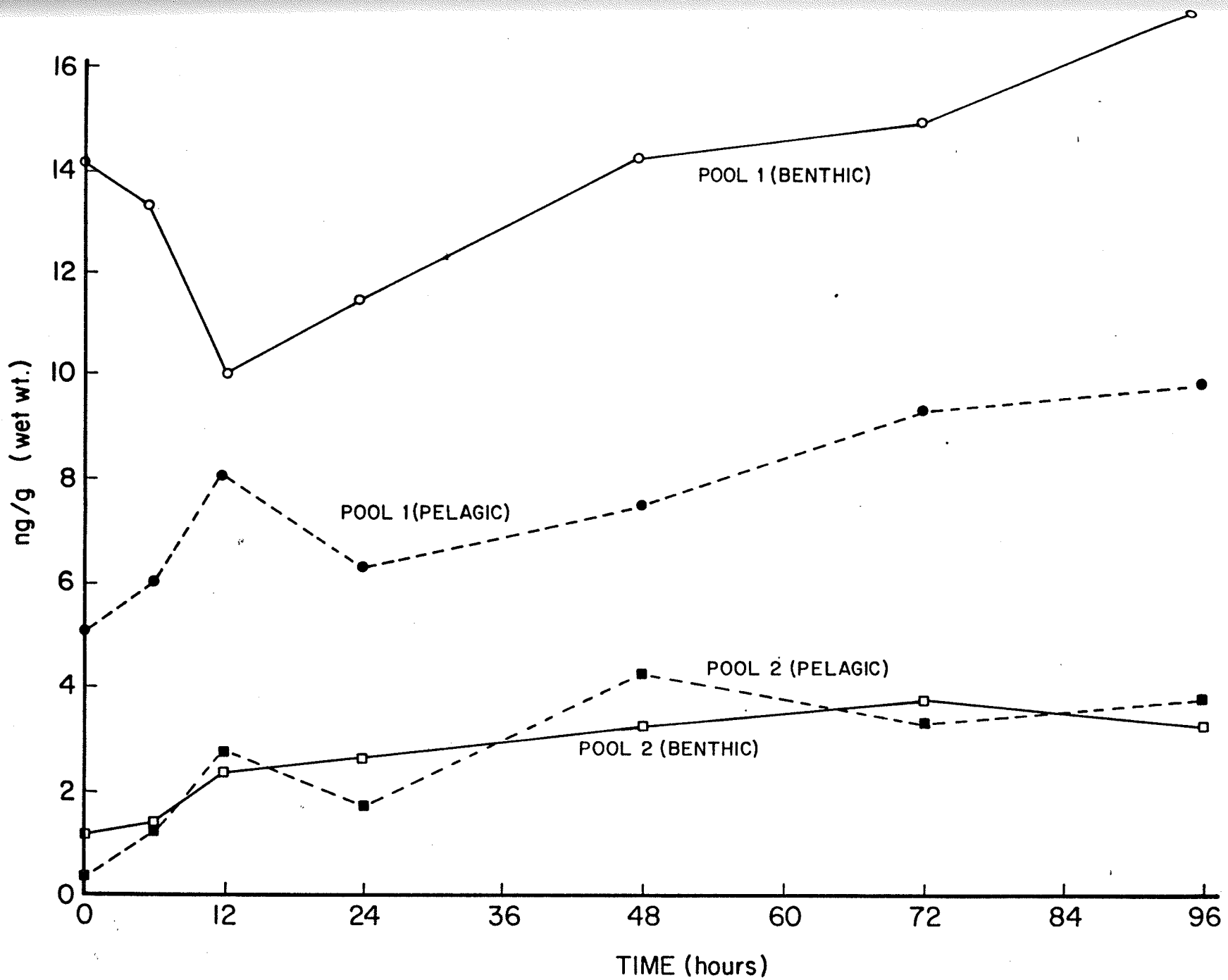


Figure 29.0 Pond Snail Clearance of Equivalents of 1,3,6,8-T₄CDD

The data describes differences between levels of accumulation between pools 1 and 2 and between the benthic and pelagic samples. The differences in pool 1 indicate the presence of a spatially (location of invertebrate) dependant mechanism of uptake and retention as the amount of label is greater in the hydrosol than in the water column. As this is not evident in pool 2, other factors may be at work. This experiment indicates that clearance is not readily occurring and, in fact, the trend is slightly upward. This may be explained in two ways as follows:

1.) The variations in trend may be due to handling shock which results in uncontrolled expulsion of gut contents.

Sudden changes in light, temperature or water quality could cause this.

2.) As subsampling of the tanks progressed a noticeable decrease in the average weight per individual occurred. The weights decreased from 4.5 to 6.0 mg to 1.0 to 3.5 mg. The use of different size ranges may signify the sampling of differing instars and developmental stages, and, therefore, result in individuals with varying metabolic rates which could effect the presence or absence of clearance.

5.3 Other Invertebrates

Over the study, opportunities to sample other invertebrate groups occurred which allowed determination of the relative magnitudes of uptake in species of divergent habitats and behavior. As all the data are from oxidation for total ^{14}C , the amounts present are expressed in 1,3,6,8- T_4CDD equivalents.

Table 19.0 indicates the substantial accumulation of label in the snail Lymnea stagnalis Say. At one point the levels reached

Table 19.0 Equivalents of 1,3,6,8-T₄CDD in Lymnea stagnalis

Time	Pool #	Shell Activity/gm	Body Activity/gm	Total* ng/gm	% Shell by wt.
8 d	1	** 43801.4 *(429.3)	80242.5 ± 9996 (786.4 ± 98)	1215.7	39.5
15	1	25472.9 (249.6)	64397.2 ± 7270 (631.1 ± 71.2)	880.7	65.5
24	1	53249.8 (521.8)	60137.5 ± 5900 (589.4 ± 57.8)	1111.2	61.5
57	1	2207.8 (21.6)	9887.3 ± 779.6 (96.9 ± 7.6)	118.5	57.0
426	1	490.7 (4.8)	1163.2 ± 153 (11.4 ± 1.5)	16.2	62.7
4	2	20305.4 (199.0)	32822.9 (321.7)	520.7	62.7
24	2	674.3 (6.6)	56.36 ± 348 (55.2 ± 3.4)	61.8	60.3
426	2	214.7±48.4 (2.1±0.5)	386.8 ± 36.5 (3.8 ±0.4)	5.9	63.6

* () = ng equivalents of 1,3,6,8-T₄CDD per g of freeze dried wt.

** = ¹⁴C activity- dpm

1.22 ppm (1.22 mg kg^{-1}) in pool 1. The BCF ranged from 1.68×10^5 to 4.83×10^5 in relation to the water concentration and up to 1.38×10^5 in relation to the 1,3,6,8- T_4 CDD levels in the hydrosol. L. stagnalis is an omnivorous predator which is known to feed on rotting vegetal matter and other organisms including small fish (Clark, 1973). It accumulated label to levels 15 x greater than the pond snail (G. parvus) which is primarily a herbivore and detritus feeder.

In pool 2, similar trends were seen with the BCF reaching 6.5×10^5 on day 4. Unlike pool 1, where the amount of accumulation remained elevated for 24 d, pool 2 accumulation levels dropped to 11.6% of the maximum level (day 4), by day 24. The obvious differences in losses and movement of 1,3,6,8- T_4 CDD between treatments is seen here also.

Table 20.0 describes oxidation data of the family Notonectidae. The BCF in this pelagic species reached 9.8×10^4 on day 15 in pool 1 and as high as 21.6×10^4 in pool 5. Once again the level of bio-magnification is significantly higher in the lower treatment level. This family of invertebrates are swimming, piercing, carnivores usually found in the lentic-littoral habitats (Merritt and Cummins, 1978). As a predator, rather than exclusively a scavenger, it remains in the water column where the availability of 1,3,6,8- T_4 CDD quickly decreases in comparison to the benthic, detritus substrate dwellers.

In tests done one year after treatment (426 d) labelled material was still present in Notonectidae at levels comparable to those noted on day 90. Even so, no equilibrium or stable concentration was noted at that time nor was an estimate made of equilibrium.

The importance of such levels of bioconcentration may prove

Table 20.0 Equivalents of 1,3,6,8- T_4 CDD in the Invertebrate Family Notonectidae (ng per g freeze dried) wt.

Time	Pool 1	Pool 2	Pool 5
0	0.0	0.0	0.0
8 d	N/A	5835.5 \pm 1077.7 (57.19 \pm 10.56)	7700.2 (75.46)
15	** 35293.9 (345.89)	N/A	8839.5 (86.63)
24	N/A	N/A	2335.4 (22.88)
57	10660.9 (104.48)	3597.5 (34.98)	2767.5 (27.11)
90	5221.4	N/A	N/A
426	2005.5 (1.96)	90.0 (0.88)	213.8 \pm 117.5 *(2.09 \pm 1.15)

* = composite sample of mature and early instar individuals
(130.7, 296.9 dpm/gm)

() = ng (per g freeze dried) equivalents of 1,3,6,8- T_4 CDD

N/A = not available

** = ^{14}C activity in dpm

to be important in environmental assessment of 1,3,6,8-T₄CDD in its impact on the food chain and toxic effects to members of the aquatic system.

6.0 VEGETATION

6.1 Lemna trisulca L. (Duckweed)

From the data (Table 21.0), L. trisulca appears to have a strong ability to bioconcentrate 1,3,6,8-T₄CDD. Between 15 d and 90 d (pool 1) the mean BCF (bioconcentration factor) was 8756 x in relation to water concentration. Initially the BCF reached 3.38×10^4 shortly after water concentrations of 1,3,6,8-T₄CDD were elevated (day 15). The label dissipation by duckweed (pool 1) was neither linear nor ln linear (Figure 25.0) while pool 2 and 5 were linear allowing the calculations ($t_{\frac{1}{2}} = \frac{0.693}{\text{slope}}$) of the $t_{\frac{1}{2}}$ values of 11.0 and 9.9 d respectively. Pool 1 reached an equilibrium level of $21.2 \pm 1.7 \text{ ng g}^{-1}$ at 24 d. This corresponded to a BCF value of 1.13×10^4 (water concentration = $1.80 \pm .80 \text{ ng L}^{-1}$). The corresponding values for pool 5 was 3.15×10^4 declining to 1.28×10^4 after 24 d. Pool 2 duckweed levels increased and maintained an estimated BCF of 2.55×10^4 . These values are higher than those described by Isensee and Jones (1975), $4 \text{ to } 9 \times 10^3$ x for 2,3,7,8-T₄CDD. Part of the variance may be due to decreased extraction efficiencies of duckweed averaging 75.4, 56.8, and 45.4% for pool 1, 2, and 5, also during certain sampling periods insufficient sample was available <<3-5 g.

6.2 Potamogeton berchtoldii Fieber var. acuminatus Fieber (Rooted Vegetation)

From the data, Table 22.0, rooted vegetation does not accumulate

Table 21.0 Accumulation of 1,3,6,8-T₄CDD in Lemna trisulea L.

Time	(Duckweed)						Control
	Pool 1		Pool 2		Pool 5		
	ng/g dry wt.	residue	ng/g dry wt.	residue	ng/g dry wt.	residue	
0	ND		ND		N/A	N/A	ND
8 d	231.5±65.2	11.9	17.1±1.9	6.3	N/A	N/A	ND
15	25.6± 4.1	10.0	N/A	N/A	19.2±2.7	8.5	
24	14.4± 2.0	7.1	19.4±4.5	5.4	15.1±1.5	16.4	ND
57	16.1± 2.2	2.8	11.9±3.8	4.7	12.3±1.4	4.3	ND
90	20.6± 3.0	2.5	14.6±1.8	10.0	10.5±3.2	3.2	ND
426	N/A	N/A	N/A	N/A	N/A	N/A	N/A

ND = none detected

N/A = not available

all residues are expressed as ng equivalents of 1,3,6,8-T₄CDD

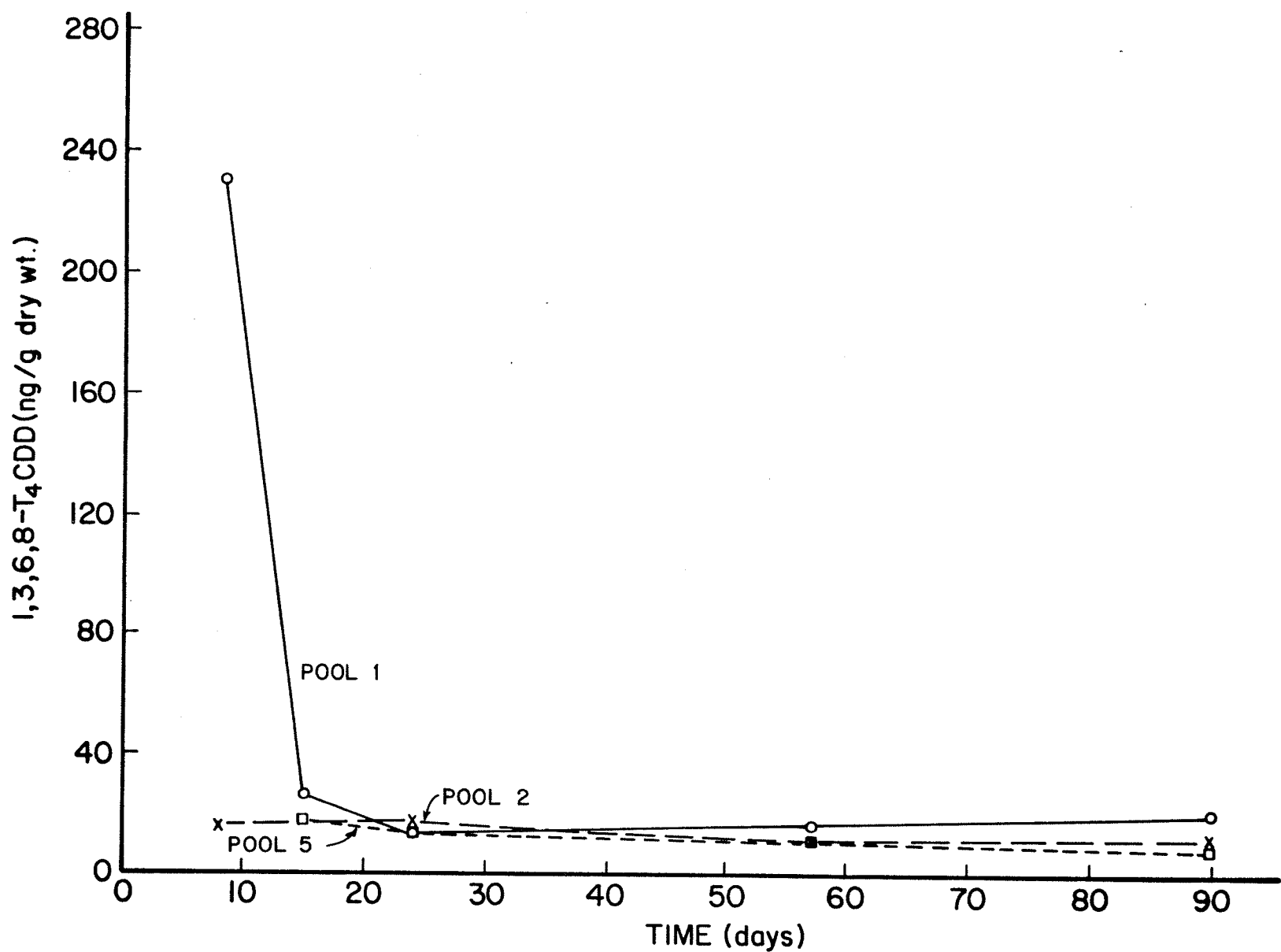


Figure 25.0 Accumulation of 1,3,6,8-T₄CDD in Duckweed

Table 22.0 Accumulation of 1,3,6,8-T₄CDD in Potamogeton berchtoldii

Fieber var. acuminatus Fieber (rooted vegetation)

Time	Pool 1		Pool 2		Pool 5		Control
	ng/g dry wt.	residue	ng/g dry wt.	residue	ng/g dry wt.	residue	
0	ND		ND		ND		ND
8 d	172.4± .8	40.4	75.3±5.2	17.7	43.4±2.1	10.9	ND
15 d	90.1±23.8	8.8	68.7±5.6	20.1	39.2±5.5	5.1	
24 d	52.9± 3.4	1.9	51.3±3.9	21.1	29.5±3.6	9.9	ND
57 d	43.6± 1.9	29.8	42.2±2.5	6.5	19.0±5.4	8.1	ND
90 d	50.8± 3.7	10.3	18.5±5.6	3.7	N/A	N/A	N/A
426 d	6.8± 2.4	5.5	7.3±1.0	5.6	3.7±1.9	1.5	ND

ND = none detected

N/A= not available

all residues are expressed as ng equivalents of 1,3,6,8-T₄CDD

1,3,6,8- T_4 CDD to the magnitude found in duckweed, but retains the label far longer. Using linear regression analysis, slopes of the dissipation rates of 1,3,6,8- T_4 CDD were determined, (\ln concentration versus time) and from that, $t_{\frac{1}{2}}$ values derived. The values were 112.6, 40.7, and 28.2 d using the total amount of ^{14}C found in the samples for pools 1, 2, and 5. By day 24, the dioxin had reached an equilibrium in pool 1 at 63.1 ng g^{-1} dry wt, but levels in the samples from pools 2 and 5 continued to decrease at 1.15 and 1.84 ng g^{-1} dry wt d^{-1} (Figure 26.0). The significance of the concentrations and extended retention of 1,3,6,8- T_4 CDD in the vegetation may be a function direct movement from the water, slow clearance or hydrosol based uptake (roots). Tsushimoto *et al.* (1982) observed that 2,3,7,8- T_4 CDD, accumulating in pond weeds, reached a maximum (7000 ppt) after 5 d and equilibrium by 1 month at a level of 2500 ppt. He assumed that all the extracted label was 2,3,7,8- T_4 CDD. For the 1,3,6,8- T_4 CDD, the times at which equilibrium was evident were comparable to Tsushimoto's results. From my data and earlier discussions regarding the hydrosol data (Section IV. 2.0) the importance of the vegetal compartment is evident as it was in Tsushimoto's study (1982). There, he found that the biological cycle of the vegetation could significantly affect the translocation of ^{14}C in an aquatic system.

The bulk of vegetal matter in pools 1 and 2 was *P. berchtoldii* and at its peak (mid August) reached an estimated $1.4 \text{ kg wet wt m}^{-3}$ and $1.1 \text{ kg wet wt m}^{-3}$ respectively providing a significant reservoir for residues.

In pool 1, at equilibrium (24 d), the BCF was estimated at $2.69 \pm 0.22 \times 10^4$ while pool 2 never stabilized decreasing from $2.4 \times$

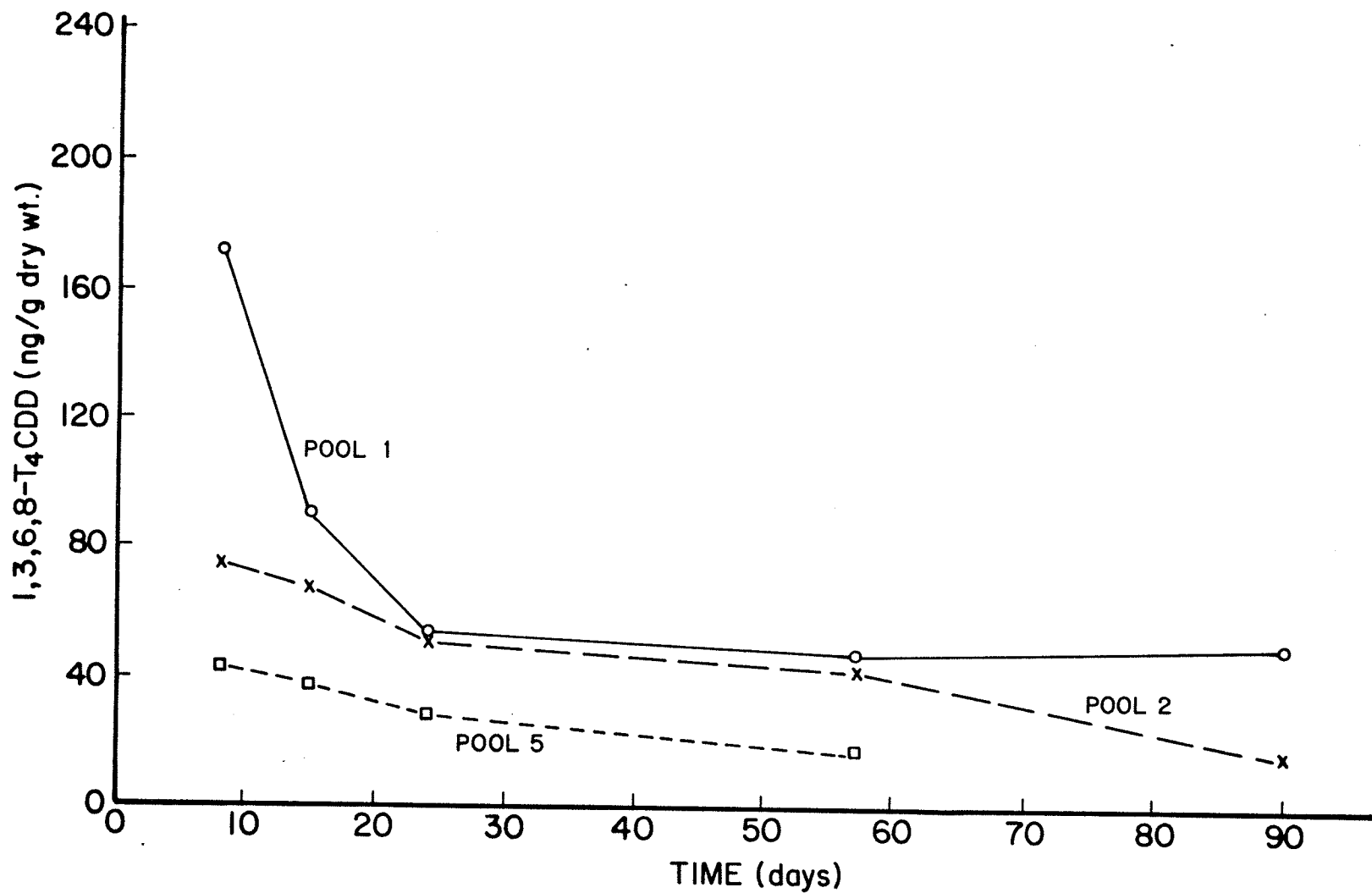


Figure 26.0 Accumulation of 1,3,6,8-T₄CDD in Rooted Vegetation

10^5 to 2.0×10^5 . Pool 5 decreased from 1.1×10^5 to 3.0×10^4 .

The efficiency of extraction for the rooted vegetation was $72.9 \pm 13.6\%$. As with the duckweed, these lower recoveries may have been a function of the procedure's inability to totally reduce the vegetal fibers, and exposure to the extracting solvents.

HPLC analysis of the extracts indicated that all the ^{14}C -labelled material eluted at the Rf value for 1,3,6,8- T_4CDD . If metabolites were being generated, they were rapidly dissipating or were unextractable with these extraction procedures.

7.0 PHOTOLYTIC STUDIES

7.1 Experiment: Year I

This experiment involved the direct exposure of unfiltered, unaltered pond water, containing 1,3,6,8- T_4CDD , to sunlight. As there was no information in this area regarding 1,3,6,8- T_4CDD , this experiment provided the basis for future experiments. There was a uniform dissipation of extractable label (Table 23.0) which may be due to photolytic action or irreversible adsorption to suspended matter (Hasset and Anderson, 1982). As seen in Figure 27.0, the non-extractable form increased over the 30 d test. The extractable form decreased at $0.08\% \text{ h}^{-1}$ (Figure 27.0) and the estimated $t_{1/2}$ was 8.93 d. This test indicated that

- 1.) degradation or transformation was occurring.
- 2.) the rate of change was significant (56.6% in 30 d).
- 3.) the possibility of numerous mechanisms at work would necessitate better design in the next experiment and a more comprehensive extraction procedure.

Table 23.0 Photodegradation - Year I

Time	% Labelled material present	
	% extracted	% unextracted
0 h	94.8	5.2
4 h	64.6	35.4
9 h	62.7	37.3
24 h	50.3	49.7
48 h	40.1	59.9
3.0 d	69.3	30.7
7.0 d	60.4	39.6
14.0 d	49.8	50.2
30.0 d	44.1	55.9
Initial inoculation: 308.7 ng of 1,3,6,8-T ₄ CDD		
Black Bottle		
14 d	79.0	21.0
30 d	95.4	4.6

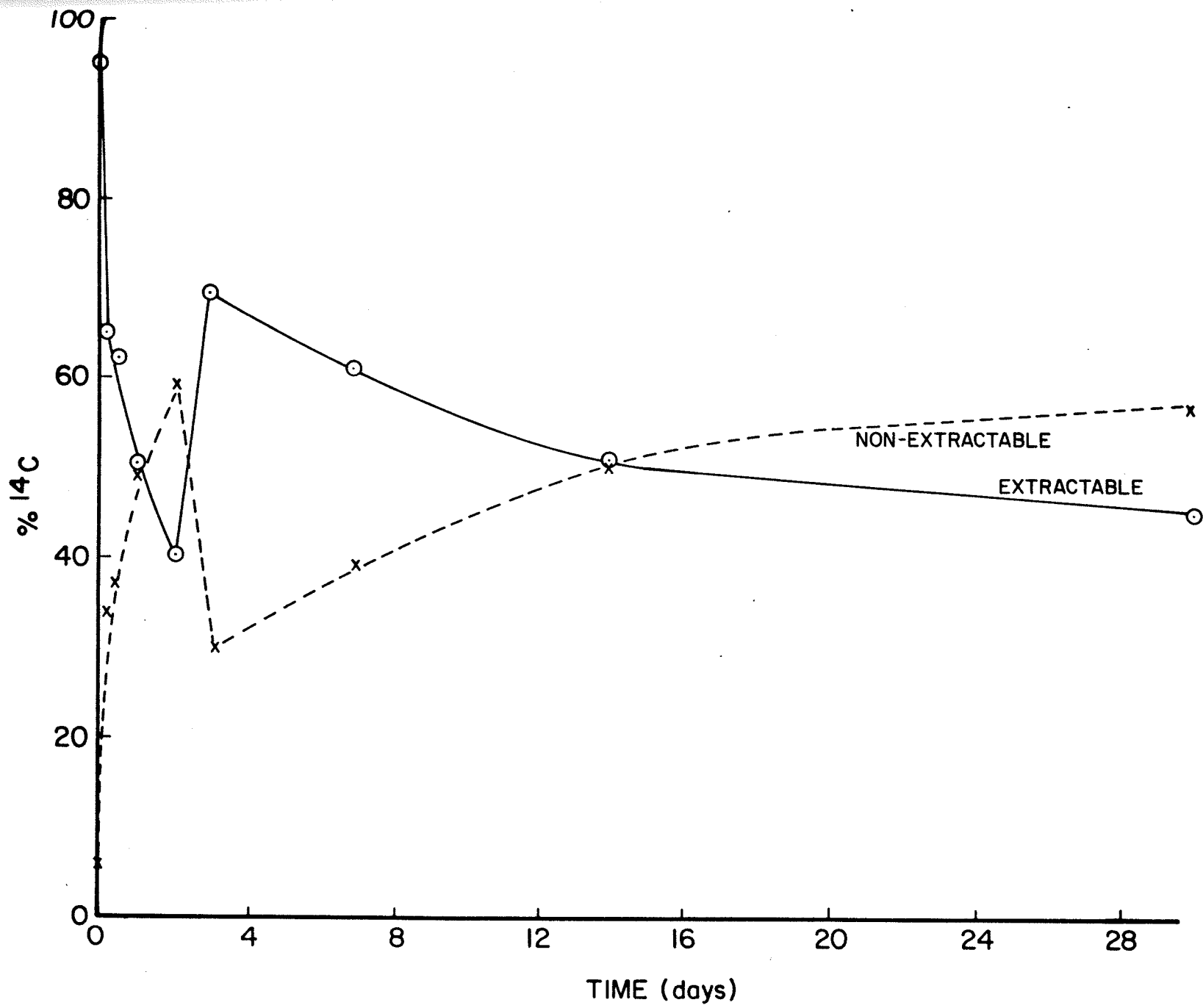


Figure 27.0 Photodegradation Experiment : Year I

- 4.) the unextractable form was highly water soluble which indicates a hydroxylated metabolite or association with soluble compounds (adsorption), unaffected by liquid-liquid extraction.

7.2 Experiment: Year II

The design of this experiment was to gather information regarding the effect of light, suspended solids, microbial activity and thermal input in the degradation of 1,3,6,8-T₄CDD.

As described in Section III. 7.3, treated pond water was exposed to light, without suspended solids, in shielded containers and in cold environments. As noted in Figure 28.0, a sharp decrease in extractable compound (primarily 1,3,6,8-T₄CDD) (Table 24.0 and 25.0) occurred and the rate of this loss was enhanced by the presence of microbes. In the autoclaved-exposed flasks (Auto), the rate of change of fraction I (basic extract) followed a logarithmic (ln) relationship (ln concentration versus time) with a degradation rate of 1.0 ng h⁻¹. This results in an estimated t_½ of 415 h or 17.3 d. When the non-autoclaved flasks (Micro) were analyzed, the rate of loss was 1.0 ng h⁻¹ with an r² of 0.91 and estimated t_½ of 265 h or 11.1 d. The t_½ of 1,3,6,8-T₄CDD was estimated using an equation derived by Nestrick et al. (1980). From laboratory studies they proposed that:

$$t_{\frac{1}{2}} = \frac{t \ln 2}{\ln (a/x)} \quad (\text{Section II. 6.0})$$

Therefore for Auto - FRACTION I, the predicted t_½ over 28 d would be:

$$\text{with } C_0 = 1580.5 \text{ ng L}^{-1}$$

$$C_f = 28 \text{ d} = 495.3 \text{ ng (96.6\% parent compd) L}^{-1}$$

Table 24.0 ¹⁴C Levels in Photodegradation Expt. - Year II

mean inoculation: 164,254.7±10,318 dpm
(1.609±.101 µg)

Time	Fraction I	Fraction II	Fraction III	Fraction IV	(Unextracted Residue)
Treatment: Auto* ¹					
0 h	161272	10545	5339	1475	
25 h	163090	1445		1100	
48 h	12997	4149	6571	5925	
60 h	122860	4204		11375	
72 h	123719	7068		19925	
84 h	84551	10355	13599	19600	
97 h	124280	5487.0	10613	11175	
5.0 d	117216	2110	5707	12075	
7.0 d	88983	3329	8980	25025	
10.0 d	49497	2524	13169	37575	
14.0 d	63873	3851	13056	33875	
28.0 d	56982	3096	7848	31900	
Treatment: Micro* ²					
0 h	155756	2895	3834	1600	
25 h	146809	3188		4650	
48 h	119070	5915	3630	6525	
60 h	125369	6250		22375	
72 h	98105	16771		23475	14175
84 h	10892	7245	7055		
97 h	117655	8101	10952	14550	
5 d	108759	4160	5498	16225	
7 d	87260	6416	11359	23850	
10 d	59563	4961	8590	34400	
14 d	42805	4361	10051	38750	
28 d	29298	2469	9897	36950	
Treatment: Black Bottle* ³					
0 d	175736	2604	1801	1850	
7 d	154789	1669	4618	1075	
14 d	152419	2967	3040	1575	
28 d	120198	570	4599	950	
Treatment: Black Bottle Fridge* ⁴					
0 d	175736	2604	1801	1850	
7 d	156300	2027	1636	300	
14 d	158575	1409	2490	(<100)	
28 d	133467	330	1641	1250	

Auto*¹ - water was filtered, autoclaved

Micro*² - water was filtered

Black Bottle*³ - water was filtered, autoclaved and bottles light proofed

Black Bottle Fridge*⁴ - water was filtered, autoclaved and bottles light proofed and held at 4 °C.

Table 25.0 Percent of ^{14}C Applied Present in Photodegradation
Expt. - Year II

mean treatment: $164,254.7 \pm 10,318$ dpm = 100%
($1.609 \pm .101$ μg of 1,3,6,8- T_4CDD)

Time	Treatment	Fraction I	Fraction II	Fraction III	Residue %	Present %
0 h	1*Auto	98.2	6.4	3.3	0.9	108.8
	2*Micro	94.8	1.7	2.3	1.0	99.8
	3*Black Bottle	106.9	1.6	1.1	1.1	110.7
25 h	Auto	99.3	0.9	0.7		101.2
	Micro	89.4	2.4	2.8		94.5
48 h	Auto	79.1	2.5	4.0	3.6	89.2
	Micro	72.5	3.6	2.2	4.0	82.3
60 h	Auto	74.8	2.6		6.9	84.3
	Micro	76.3	3.8		13.74	93.8
72 h	Auto	75.3	4.3		12.1	91.7
	Micro	59.7	10.2		14.3	84.2
84 h	Auto	51.5	6.3	8.3	12.5	78.6
	Micro	66.3	4.4	4.3	8.6	83.6
97 h	Auto	75.6	3.3	6.5	6.8	92.2
	Micro	71.6	4.9	6.7	8.9	92.1
5.0 d	Auto	71.4	1.3	3.5	7.4	83.6
	Micro	66.2	2.5	3.3	9.9	81.9
7.0 d	Auto	54.2	2.0	5.5	15.2	76.9
	Micro	53.1	3.9	6.9	14.5	78.4
	Black Bottle	94.2	1.0	2.8	0.7	119.5
	4*Black Bottle Fridge	95.2	1.2	1.0	0.2	97.6
10.0 d	Auto	30.1	1.5	8.0	22.9	62.5
	Micro	36.3	3.0	5.2	20.9	65.4
14.0 d	Auto	38.9	2.3	7.9	20.6	69.7
	Micro	26.1	2.7	6.1	23.6	58.5
	Black Bottle	92.8	1.8	1.9	1.0	97.5
	Black Bottle Fridge	96.5	0.9	1.5	0.02	98.9
28.0 d	Auto	34.7	1.9	4.8	19.4	60.7
	Micro	17.8	1.5	6.0	22.5	47.8
	Black Bottle	73.2	0.4	2.8	0.6	76.9
	Black Bottle Fridge	81.3	0.2	1.0	0.8	83.3

$$t_{\frac{1}{2}} = 18.1 \text{ d}$$

for MICRO-FRACTION I,

$$t_{\frac{1}{2}} = 10.8 \text{ d.}$$

These estimates are comparable to estimates from the field and both estimates denote a microbial enhancement of compound degradation by 56.4% and 67.1% (pool study and Nestruck's estimates respectively). Photolysis as the major route of degradation cannot be confirmed; however, it is suggested to be occurring due to the appearance of non-parent compound and the non-comparable results in the shielded treatments. The black bottle and black bottle cold treatments indicated progressive losses of material. HPLC analysis indicated that all fractions of both these treatments were unchanged and that the primary compound was 1,3,6,8-T₄CDD. Therefore metabolic losses are unlikely; however, the amount lost was 23.1% in the black bottles and 16.7% in the cold black bottles by day 28. The losses were 6.4% lower in the 4°C bottles possibly indicating a minor thermal effect; therefore, the major losses can only be attributed to (1) adsorption onto a substrate which prevented analysis (2) irreversible binding to the flask or (3) volatilization losses. Item 2 is unlikely due to precautions taken to lessen adsorption by silanization. This procedure was tested and found to limit losses to < 0.5%. If item 1 was occurring, labelled material would have appeared in the residue or water soluble fractions (III). The proportions of all the fractions remained unchanged; therefore, volatile loss is suggested.

Liberti et al. (1978) suggested temperature may be a factor in photolysis and from my results, there was little thermal effect on 1,3,6,8-T₄CDD photolysis. Losses do occur, but no metabolic forms were found during HPLC analysis. If temperature affects the rate of volatile

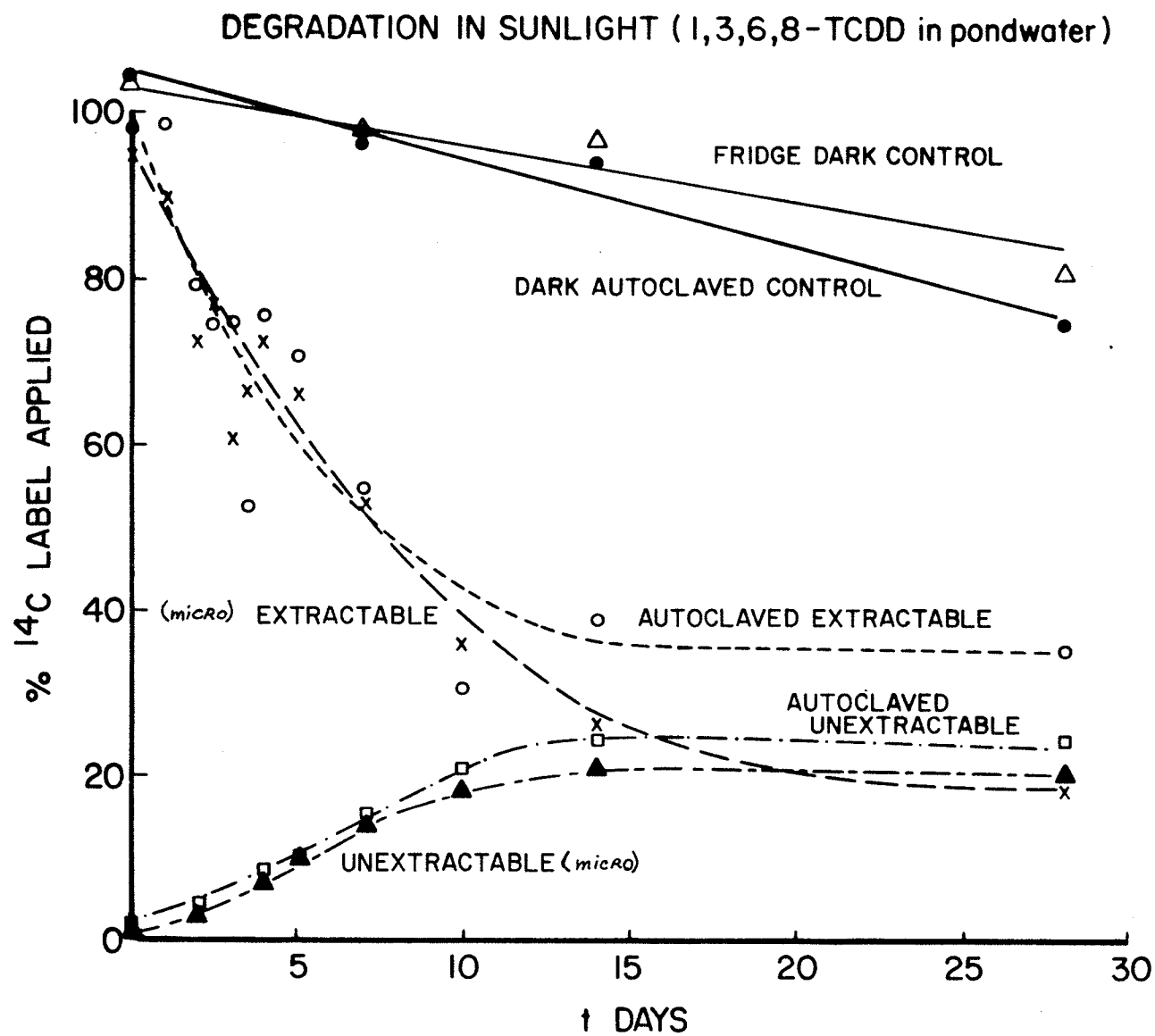


Figure 28.0 Photodegradation Experiment : Year II

movement, then exposure to daily 10°C to 26°C temperature shifts in the field may allow the label to be released.

As photolysis progressed in the exposed flasks, significant amounts of material appeared as an unextractable residue, soluble in water. Attempts to further extract the water failed to remove this fraction. During the experiment, material did not increase in the acid extract and the maximum amount of metabolites found in the ethyl acetate (fraction III) was 8.3% of the total treatment. As ethyl acetate is widely used to remove polar compounds, it was unexpected that over 28 d, 22.5 and 19.4% of the micro and auto treatments would remain in solution. The residue in the black bottles ranged from 0.2 to 1.1% of applied 1,3,6,8-T₄CDD.

Photolysis may be a major mechanism in the auto treatment losses, causing dechlorination, as suggested by Crosby *et al.* (1973), giving rise to a soluble form. The pre-treatment of the pond water removed many factors however quantities of natural humic and fulvic acids remained which could allow extensive photochemical oxidation to occur through singlet oxygen generation (Zepp *et al.*, 1977) or radical generation (Choudhry *et al.*, 1980).

The HPLC analysis of the micro and auto fractions indicated that in the micro treatment, fraction III, 79.8% of the label was metabolite, with a retention time of 6 min 48 s, 88.7% of the base extract (fraction I) was parent compound and the remaining material was a metabolite with a retention of 6 min 40 s. Of the acid extracts (fraction II), only 31.8% were present with the remaining being metabolite with a retention time of 6 min 45 s. For the auto treatment, fraction I, was 96.6% 1,3,6,8-T₄CDD with 3.4% being one metabolite (R_f = 6 min 46 s).

Fraction II was 94.0% parent 1,3,6,8-T₄CDD, and fraction III was 100% metabolite (R_f = 6 min 53 s). It appears that though a polar compound was detected in most of the treatments after 28 d, the relative proportions of the total treatment are small with the exception of the non-extractable residue which has yet to be characterized.

V. SUMMARY

Replicated pool systems were treated with ^{14}C -1,3,6,8- T_4CDD and the isomer was found to move into the air above the water and throughout the water column. Characteristically hydrophobic, the non-polar 1,3,6,8- T_4CDD volatilized from the surface at rates as high as 33.7 (pool 1) and 21.4 (pool 2) $\text{ng m}^{-2} \text{d}^{-1}$. In calculations of the estimated flux, according to Claith *et al.*, 1980, and the predicted flux, according to Mackay and Leinonen, 1975, the results were not comparable as seen in Table 12.1 possibly due to other sources of ^{14}C loss. If rates described in Table 12.1 were maintained, removal of trace amounts of the total treatment could have occurred denoting the significance of this mechanism in pollutant (1,3,6,8- T_4CDD) fate. Once in the water column, the labelled material increased to a maximum concentration of 157.1, 61.7 and 19.4 ng L^{-1} in ponds 1, 2 and 5. This was followed by a (ln) logarithmic dissipation of label of 0.12, 0.79, and 0.79 $\text{ng h}^{-1} \text{L}^{-1}$ for each pool (1, 2 and 5) respectively. The estimated $t_{1/2}$ values were 134.5, 20.8 and 17.8 h for 1,3,6,8- T_4CDD in pools 1, 2 and 5 with a stable equilibrium being attained on day 15, 8 and 3. No metabolites were detected in the water until 426 d (refer to Section IV. 4.0). Uptake of 1,3,6,8- T_4CDD by the hydrosol occurred over a period of 4 to 8 d. The rate of deposition decreased at a point corresponding to the achievement of equilibrium in the water column of each pool. Dissipation of compound from the hydrosol resulted in $t_{1/2}$ of 16.9 d (pool 2) and 9.1 d (pool 5) while fluctuations made accurate estimates for pool 1 impractical. The levels of 1,3,6,8- T_4CDD in pool 1 stabilized on day 4 at levels reaching $11.6 \pm 1.3 \text{ ng g}^{-1}$ on approximately

day 4; at $6.63 \pm 0.6 \text{ ng g}^{-1}$ for pool 2; and on day 2 at 3.1 ng g^{-1} for pool 5 (total equivalents of 1,3,6,8- T_4 CDD). All treatments declined between day 34 and 57 after which the levels of total ^{14}C increased somewhat. The concentration increase may be due to the sudden release of labelled compound in rotting material from a population collapse of the rooted vegetation, as the season closed. Similar trends were observed by Tsushimoto et al. (1982).

Still, 7.4 to 20.8% of the maximum concentration in the hydrosoil was present by day 426. These values are less than the estimated persistence of similar isomers such as 2,3,7,8- T_4 CDD (Isensee and Jones, 1975; Ward and Matsumura, 1978).

The presence of 1,3,6,8- T_4 CDD in both floating and rooted vegetation reached levels within 8 days of treatment as high as 3.37×10^4 times the concentration in the surrounding waters. The $t_{1/2}$ of 1,3,6,8- T_4 CDD in the duckweed was 11.0 and 9.9 d for pools 2 and 5, while 1 decreased dramatically stabilizing at 21.7 ng g^{-1} on day 24. The rooted vegetation did not accumulate 1,3,6,8- T_4 CDD to the levels seen in the duckweed, but the levels persisted longer with the estimated $t_{1/2}$ of 112.6, 40.7 and 28.2 d in pools 1, 2, and 5 respectively. Rooted vegetation predominated and significant the total amount of label in this compartment was considered small. It can be seen in the hydrosoil data (Tables 14.0 and 15.0) that displacement of vegetally bound ^{14}C residues can affect the fate of a compound such as 1,3,6,8- T_4 CDD.

Data collected for other biotic compartments such as the fathead minnow, pond snails and miscellaneous invertebrates indicate a strong capacity for bioaccumulation. In the minnows, 1,3,6,8- T_4 CDD reached 2.9×10^4 x the concentration in the surrounding water on a

freeze dried weight basis. Similarly, the pond snail accumulated ^{14}C to 1.8 to 4.1×10^4 x the water concentration in the three treatments. The $t_{\frac{1}{2}}$ was 32 to 35 d and the label dissipated in a logarithmic (\ln) manner as a function of time. These estimates and the concentration factors are comparable to results of studies by Yockim et al., (1971) and Isensee and Jones (1975). Data regarding the Lymnea stagnalis snail showed high levels of accumulation: 6.5×10^5 x day 4 in pool 2 and 4.8×10^5 x in pool 1. As this species is a consumer of rotting matter and larger organisms, these elevated levels may be a function of its feeding habits or the locality or range within the pool system. A predatory invertebrate (Notonectidae sp.) was also found to accumulate ^{14}C to a level 2.16×10^5 x that in the water. Though these biota comprise a small proportion of the total bulk of the system, the enhanced concentration of 1,3,6,8- T_4CDD in certain compartments may present an environmental or toxicity related problem through the food chain.

Photolytic experiments on 1,3,6,8- T_4CDD determined that a polar product was formed (Section III. 7.2).

Photolytic degradation is also enhanced by the presence of microbial organisms. The estimated $t_{\frac{1}{2}}$ of 1,3,6,8- T_4CDD , when exposed to light (natural) and microbes, was 11.1 d. The $t_{\frac{1}{2}}$ when exposed with a reduced microbial activity was 17.3 d. The predicted $t_{\frac{1}{2}}$ values for the above conditions were 10.8 d, and 18.1 d., respectively, as projected by an equation derived by Nestrick et al. (1980). Over the 30 d experiment (Year II), the losses of 1,3,6,8- T_4CDD formed a logarithmic relationship with time and losses reached 40.0 to 52.2% of the original treatment in the autoclaved and non autoclaved treatments, respectively. Results from the shielded and refrigerated treatments

showed that significant losses occurred (15-18%) but the losses were not seen as degradation losses as no increases were noted in the extracted fractions. Therefore the losses may be due to adsorption to glass or volatile leakage. The unextractable residue fraction increased to 20.6 and 22.5% of the initial treatments which indicated the formation of a complex or compound unextractable by polar and non polar solvents. Characterization of the unextractable form was not achieved in spite of attempts to extract it with several solvent systems.

VI. REFERENCES

- Adams, J. D. and J. H. Caro. 1980. Polyurethane foam as a trapping for airborne pesticides: analytical method development. U.S.E.P.A. Report EPA-600/4-80-008.
- Ahling, A. and Lindskog. 1980. Emission of chlorinated organic substances from combustion. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (ed.). Chlorinated Dioxins and Related Compounds. Effects on the Environment. Pergamon Press, Oxford, New York. pp. 651.
- Ahling, B. and A. Lindskog, B. Jansson and G. Sundstrom. 1977. Formation of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans during combustion of 2,4,5-T formulations. Chemosphere 8:460-468.
- Albro, P. W. and B. J. Corbett. 1977. Extraction and cleanup of animal tissues for subsequent determination of mixture of chlorinated dibenzo-p-dioxins and dibenzofurans. Chemosphere 7:381-385.
- Aniline, O. 1973. Preparation of chlorodibenzo-p-dioxins for toxicological evaluation. IN: E. H. Blair (ed.). Chlorodioxins - Origin and Fate. Am. Chem. Soc., Washington, D. C. pp. 126-135.
- Arsenault, R. D. 1976. Pentachlorophenol and contained chlorinated dibenzodioxins in the environment. Am. Wood Preservers Assoc. Rept.
- Baker, P. G., R. A. Hoodless and J. F. C. Tyler. 1981. A review of methods for the determination of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in phenoxyalkanoic

- acid herbicides. *Pestic. Sci.* 12:297-304.
- Baughman, R. and M. Meselson. 1973. An analytical method for detecting tetrachlorodibenzo-p-dioxin: levels of TCDD in samples from Vietnam. *Environ. Health Perspect.* 5:27-35.
- Bickel, M. H., and S. Muhlebach. 1980. Persistent and unmetabolizable lipophilic compounds: from pharmacokinetics to ecotoxicology. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, N. Y. pp. 303-307.
- Birnbaum, L. S., G. M. Decad and H. B. Matthews. 1980. Deposition and excretion of 2,3,7,8-tetrachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* 55:342-352.
- Bradlaw, J. A., L. H. Garthoff and N. E. Hurley. 1980. Comparative induction of AHH activity in vitro by analogues of dibenzo-p-dioxin. *Food Cosmet. Toxicol.* 18:627-635.
- Bumb, R. R., W. B. Crummett, S. S. Cutie, J. R. Gledhill, R. H. Hummel, R. O. Kagel, L. L. Lamparski, E. V. Luoma, D. L. Miller, T. J. Nestruck, L. A. Shadoff, R. H. Stehl, and J. S. Woods. 1980. Trace Chemistries of fire: A source of chlorinated dioxins. *Sciences* 210:385-390.
- Buser, H. 1975. Polychlorinated dibenzo-p-dioxin: Separation and identification of isomers by gas chromatography - mass spectrometry. *J. Chromatogr.* 114:95-108.
- Buser, H. 1976. Preparation of qualitative standard mixture of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by ultraviolet and irradiation of the octachloro compounds. *J. Chromatogr.* 129:303-307.

- Buser, H. R., and H. P. Bossehardt. 1976. Determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in commercial pentachlorophenols by combined GC-MS. *J. Assoc. Off. Anal. Chem.* 59(3):562-569.
- Buser, H. R., H. Bossenhardt and C. Rappe. 1978. Identification of polychlorinated dibenzo-p-dioxin isomers found in fly ash. *Chemosphere* 2:165-172.
- Buser, H. R. 1979a. Formation and identification of tetra- and pentachlorodibenzo-p-dioxins from photolysis of two isomeric hexachlorodibenzo-p-dioxins. *Chemosphere* 4:251-257.
- Buser, H. R. 1979b. Formation of polychlorinated dibenzofurans and polychlorinated dibenzo-p-dioxins from the pyrolysis of chlorobenzenes. *Chemosphere* 8:415-424.
- Buser, H. R., and C. Rappe. 1980. High resolution gas chromatography of the 22 tetrachlorodibenzo-p-dioxins isomers. *Anal. Chem.* 52:2257-2262.
- Buu-Hoi, N. P. 1971. Preparation, properties and identification of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in the pyrolyzates of defoliants based on 2,4,5-T and its esters and in contaminated plants. *C. R. Acad. Sci., Ser. D*:273-708.
- Cantrell, J. S., N. C. Webb and A. J. Mabis. 1967. The identification and crystal structure of a hydropericardial producing factor: 1,2,3,7,8,9 hexachlorodibenzo-p-dioxins. *Acta Crystallogr. Sec. B.* 25:150-155.
- Chittum, B. G., B. S. Clegg, S. H. Safe and O. Hutzinger. 1982. Chlorinated dibenzofurans and dibenzo-p-dioxins: detection and quantification in electrical equipment and the formation during incineration of PCBs (IN PRESS).

- Choudhry, C. C., K. Olie, and O. Hutzinger. 1980. Mechanisms in the thermal formation of chlorinated compounds including polychlorinated dibenzo-p-dioxins. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (eds.). Chlorinated dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, N. Y. pp. 651.
- Claith, M. M., W. F. Spencer, W. J. Farmer, T. D. Shoup and R. Grover. 1980. Volatilization of s-ethyl N, N-dipropylthiocarbamate from water and wet soil during and after flood irrigation of an alfalfa field. *J. Agric. Food Chem.* 28:610-613.
- Clark, A. H. 1973. The freshwater molluscs of the Canadian interior basin. *Malacologia* 13(1-2):1-509.
- Cochrane, W. P., J. Singh, W. Miles, B. Wakeford, and J. Scott. 1980. Analysis of technical and formulated products of 2,4-dichlorophenoxy acetic acid for the presence of chlorinated dibenzo-p-dioxins. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, N. Y. pp. 209-215.
- Courtney, K. D., D. W. Gaylor, D. W. Hogan, M. D. Falk, H. L. Bates, and R. R. Mitchell. 1970. Teratogenic evaluation of 2,4,5-T. *Sciences* 168:864.
- Crosby, D. G., A. S. Wong, J. R. Plimmer, and E. A. Woolson. 1971. Photodecomposition of chlorinated dibenzo-p-dioxins. *Science* 173:748-749.
- Crummett, W. B., and R. N. Stehl. 1973. Determination of chlorinated dibenzo-p-dioxins and dibenzofurans in various materials. *Environ. Health Perspect.* 5:15-25.

- Crummett, W. B. 1980. Environmental chlorinated dioxins from combustion- the trace chemistries of fire hypothesis. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 651.
- Desideri, D., A. Di Domenico, R. Vanzati, P. Tancioni and A. Di Muccio. 1979. Photolysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin in iso-octane, hexane and cychohexane. *Boll. Chim. Farm.* 118(5):274-281.
- Di Domenico, A., V. Silano, G. Viviano and G. Zapponi. 1980. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin at Seveso, Italy: I. Sensitivity and specificity of analytical procedures adopted for TCDD assay. *Ecotoxicol. Environ. Safety* 4:283-297.
- Dobbs, A. J., and C. Grant. 1979. Photolysis of highly chlorinated dibenzo-p-dioxins by sunlight. *Nature* 278:163-165.
- Dobbs, A. J., and C. Grant. 1981. Octachlorodibenzo-p-dioxin in wood treatment materials and treated wood. *Chemosphere* 10(10):1185-11983.
- Dow. 1978. "The True Chemistries of Fire-A Source of and Routes for the entry of Chlorinated Dioxins into the Environment;" The Chlorinated Dioxin Task Force, Michigan Div. Dow Chemical Co., Midlands, Il. 1978.
- Edmunds, J. W., D. F. Lee and C. M. L. Nickels. 1973. Determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 2,4,5 acetic acid and 2,4,5-T alkyl ester herbicides. *Pestic. Sci.* 4:101-105.

- Eiceman, G. A., R. E. Clement and F. W. Karasek. 1981. Variations in concentration of organic compounds including polychlorinated dioxins and polynuclear aromatic hydrocarbons in fly ash from a municipal incinerator. *Anal. Chem.* 53:955-959.
- Eiceman, G. A., A. C. Viau and F. W. Karasek. 1980. Ultrasonic extraction of polychlorinated dibenzo-p-dioxin and other organic compounds from fly ash from municipal incinerators. *Anal. Chem.* 52:1492-1496.
- Eiceman, G. A., R. E. Clement and F. W. Karasek, 1979. Analysis of fly ash from municipal incinerators for trace organic compounds. *Anal. Chem.* 51:2343-2350.
- Esposito, M. P., T. O. Tiernan and F. E. Dryden. 1980. Dioxins. EPA Rept. EPA-600/2-80-197. pp. 351.
- Federal Register. 1979. Emergency suspension orders for 2,4,5-T and silvex. *Federal Register* 15874-15920.
- Fishbien, L. 1980. Potential carcinogenic and mutagenic industrial chemicals 1. Alkylating agents. *Risk Branches in Industry* 1133:213-257.
- Fishbien, L. 1973. Mutagens and potential mutagens in the biosphere. I. DDT and its metabolites, polychlorinated biphenyls, chlorodioxins, polycyclic aromatic hydrocarbons, haloethers. *Sci. Total Environ.* 4:305.
- Firestone, D. 1977. The determination of polychlorodibenzo-p-dioxin and polychlorodibenzofurans in commercial gelatins by gas-liquid chromatography. *J. Agric. Food Chem.* 25(6):1275-1280.
- Firestone, D., J. Ress, N. L. Brown, R. P. Barron and J. N. Dominico. 1972. Determination of polychlorinated dibenzo-p-dioxins and related compounds in commercial chlorophenols. *J. Assoc.*

- Off. Anal. Chem. 55(1):85-92.
- Gasiewicz, T. A., and R. A. Neal. 1979. 2,3,7,8-tetrachlorodibenzo-p-dioxin tissue distribution, extraction and effects on clinical chemical parameters in guinea pigs. Toxicol. Appl. Pharmacol. 51:329-339.
- Gesser, H. D., A. Chow, F. C. Davis, J. F. Uthe and J. Reinke. 1971. The extraction and recovery of polychlorinated biphenyls (PCB) using porous polyurethane foam. Anal. Lett. 4(12): 883-886.
- Goldstein, J. A., J. R. Hass, P. Lindo and D. Haravan. 1978. 2,3,7,8-tetrachlorodibenzofuran in commercially available 99% pure. PCB isomer identified as the inducer of hepatic cytochrome P-448 and aryl hydrocarbon hydroxylase in the rat. Drug Metab. Dispos. 6:258-264.
- Gray, A. P., S. P. Cepa and J. S. Cantrelli. 1975. Intervention of the Smile's rearrangement in the synthesis of dibenzo-p-dioxin. 1,2,3,6,7,8 and 1,2,3,7,8,9 hexachloro-dioxin. Tetrahedron Lett. 33:2875-2876.
- Greenlee, W. F. and A. Poland. 1979. Nuclear uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6J and DBA/2J mice. J. Biol. Chem. 254:9814-9821.
- Grover, R., and L. A. Kerr. 1981. Evaluation of polyurethane foam as a trapping medium for herbicide vapor in air monitoring and worker inhalation studies. J. Environ. Sci. Health B16(1):59-66.

- Grussendorf, O. W., A. J. McGinnis and J. Solomon. 1970. Rapid sample preparation for gas liquid chromatography of pesticide residues by ball-grinding extraction, freezeout and semimicro column cleanup. *J. Assoc. Off. Anal. Chem.* 53:1048-1054.
- Hardell, L. and A. Sandstrom. 1978. Malignant mesenchymal soft tissue tumors and exposure to phenoxy acid or chlorophenols. A case control study. *Lakartidningen* 75(49):3535-3536.
- Harless, R. L., and R. G. Lewis. 1980. Quantitative determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin residues by gas chromatography/mass spectrometry. Workshop on the Impact of Chlorinated Dioxins and Related Compounds on the Environment. Rome, Italy. Oct./1980.
- Hass, J. R., M. D. Freisen, and M. K. Hoffman. 1979. The mass spectrometry of polychlorinated dibenzo-p-dioxins. *Organic Mass Spectrom.* 14(1):9-16.
- Hassett, J. P., and M. A. Anderson. 1982. Effects of dissolved organic matter on adsorption of hydrophobic organic compounds by river and sewage borne particles. *Water Res.* 16:681-686.
- Hay, A. 1981. New pathways for chlorinated dioxins. *Nature* 294: 514-515.
- Helder, T. L. 1980. Effects of 2,3,7,8-T₄CDD on early life stages of two freshwater fish species. IN: O. Hutzinger, R. W. Frei, E. Merian, F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 455-462.
- Helling, C. S., A. R. Isensee, E. A. Woolson, P. D. J. Ensor, G. E. Jones, J. R. Plimmer, and P. C. Kearney. 1973. Chlorodioxins

- in pesticides, soils and plants. *J. Environ. Qual.* 2(2): 171-178.
- Huff, J. E., J. A. Moore, R. Saracci and L. Tomatis. 1980. Long term hazards of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. *Environ. Health Perspect.* 36:221-240.
- Hummel, R. A. 1977. Cleanup techniques for the determination of ppt residue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. Agric. Food Chem.* 25(5):1049-1053.
- Iarc. 1978. Polychlorinated and polybrominated biphenyls. Monographs on the evaluation of the carcinogenic risk of chemicals to humans. *Int. Agency Res. Cancer, Lyon.* 18:6365.
- Isensee, A. R., and G. E. Jones. 1975. Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in an aquatic model ecosystem. *Environ. Sci. Technol.* 9(7):668-672.
- Isensee, A. R., and G. E. Jones. 1971. Absorption and translocation of root and foliage applied, 2,4-dichlorophenol; 2,7-dichlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J. Agric. Food Chem.* 19(6):1210-1214.
- Jansson, B., G. Sundstrom and B. Ahling. 1978. Formation of polychlorinated dibenzo-p-dioxins during combustion of chlorophenol formulations. *Sci. Total Environ.* 10:209-217.
- Jensen, S., and L. Renberg. 1972. Contaminants in pentachlorophenol: chlorinated dioxins and pre-dioxins. *Ambio* 1(2):62-65.
- Jirasek, L., J. Kalensky and K. Kubec. 1976. Chloracne, porphyria cutanea tarda, and other poisonings due to the herbicides. *Hautarzt* 27(7):328-333.

- Jones, P. A. 1981. Chlorophenols and their Impurities in the Canadian Environment (EPS 3-EC-81-2) Environmental Protection Service, Ottawa. pp. 434.
- Junk, G. A., and J. J. Richards. 1981. Dioxins not detected in effluents from coal-refuse combustion. *Chemosphere* 10(11/12): 1237-1241.
- Karickhoff, S. W., D. S. Brown, and T. A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res.* 13(2):241-248.
- Kearney, P. C., E. A. Woolson, and C. P. Ellington, Jr. 1972. Persistence and metabolism of chlorodioxins in soils. *Environ. Sci. Technol.* 6(12):1017-1019.
- Kenaga, E. E., and C. A. I. Goring. 1978. Relationship between water solubility, soil sorption octanol/water partition and bioconcentration of chemicals in biota. *Aquatic Toxicology Symp.*, New Orleans, 1978 (October).
- Kimble, B. J., and M. L. Gross. 1980. Tetrachlorodibenzo-p-dioxin quantitation in stack collected coal fly ash. *Science* 207: 59-61.
- Kimbrough, R. D. 1980. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier/North Holland Biomedical Press, N. Y. pp. 422.
- Kitchen, K. T., and J. S. Wood. 1979. 2,3,7,8-tetrachlorodibenzo-p-dioxin effects on hepatic microsomal cytochrome P-448 mediated enzyme activities. *Toxicol. Appl. Pharmacol.* 47:536-546.

- Kociba, R. J., D. J. Keyes, J. E. Beyer, R. M. Carreon, C. E. Wade, D. A. Dittenber, R. P. Kalnins, L. E. Frauson and C. N. Park. 1978. Results of a two year chronic toxicity and oncogenicity study of 2,3,7,8-T₄CDD in rats. *Toxicol. Appl. Pharmacol.* 46(2):279-303.
- Kociba, R. J., and B. A. Schwetz. 1982. Toxicity of 2,3,7,8-tetrachloro-dibenzo-p-dioxin. *Drug Metabl. Rev.* 13(3)L387-406.
- Kriebel, D. 1981. The dioxins: toxic and still troublesome. *Environ.* 23(1):6-13.
- Lamberton, J., D. Griffin, B. Arbogast, R. Inman, and M. Deinzer. 1979. The determination of polychlorinated dibenzo-p-dioxins in penta-chlorophenol and wood treatment solutions. *J. Am. Ind. Hyg. Assoc.* 40:816-822.
- Lamparski, L. L., R. H. Stehl, and R. L. Johnson. 1980. Photolysis of pentachlorophenol-treated wood. Chlorinated dibenzo-p-dioxin formation. *Environ. Sci. Technol.* 14:196-200.
- Lamparski, L. L., and T. J. Nestruck. 1980. Determination of tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxin isomers in particulate samples at parts per trillion. *Anal. Chem.* 52:2045-2054.
- Lamparski, L. L., and T. J. Nestruck. 1981. Synthesis and identification of the 10 hexachlorodibenzo-p-dioxin isomers by HPLC and packed column gas chromatography. *Chemosphere* 10:3-18.
- Langhorst, M. L., and L. A. Shadoff. 1980. Determination of parts-per-trillion concentrations of tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxin in human milk samples. *Anal. Chem.* 52:2037-2044.

- Liberti, A., and D. Brocco. 1980. Formation of polychlorodibenzo-dioxins and polychlorodibenzofurans in urban incinerators emissions. IN: O. Hutzinger, R. W. Frei, E. Merian, F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 145-253.
- Liberti, A., and B. Brocco, I. Allegrini, A. Cecinato, and M. Possanzini. 1978. Solar and U. V. photodecomposition of 2,3,7,8-tetra-chlorodibenzo-p-dioxin in the environment. *Sci. Tot. Environ.* 10:97-104.
- Liss, P. S., and P. G. Slater. 1974. Flux of gases across the air-sea interface. *Nature* 247:181-183.
- Lustenhouwer, J. W. A., K. Olie, and O. Hutzinger. 1980. Chlorinated dibenzo-p-dioxin and related compounds in incinerator effluents. A review of measurements, mechanisms of formation. *Chemosphere* 9:501-522.
- Mackay, D., and W. B. Neely. 1981. An evaluative model for estimating environmental concentration. Workshop on Modeling the Fate of Chemicals in the Aquatic Environment. Pelleston, Michigan.
- Mackay, D., and P. J. Leinonen. 1975. Rate of evaporation of low solubility contaminants from water bodies to atmosphere. *Environ. Sci. Technol.* 9:1178-1180.
- Madder, D. J. 1978. The disappearance from, efficacy in and effect on non target organisms of diflubenzuron, methoprene and chloropyrfos in a lentic ecosystem. M.Sc. Thesis. University of Manitoba. pp. 139.

- Matsumura, F., and H. J. Benezet. 1973. Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Environ. Health Perspect.* 5:253-258.
- Matsumura, F., and E. G. Essac. 1979. Degradation of pesticides by algae and aquatic organisms. IN: Pesticide and Xenobiotic Metabolism in Aquatic Organisms. (eds.). M. A. Q. Khan, J. J. Zech and J. J. Menn. ACS Symposium Series 99. pp. 436.
- McCall, P. J., D. A. Laskowski, R. L. Swann, and H. J. Dishburger. 1982. Estimation of environmental partitioning of organic chemicals in model ecosystems. Dow Chemical, Agricultural Products Dept., Midlands, MI. pp. 26.
- McConnell, E. E., and J. A. Moore. 1979. Toxicological pathology characteristics of the halogenated aromatics. *Ann. N. Y. Acad. Sci.* 320:138-150.
- McConnell, E., J. A. Moore, J. K. Haseman, and M. W. Harris. 1978. The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. *Toxicol. Appl. Pharmacol.* 44:335.
- McKinney, J. D. 1978. Analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin in environmental samples. IN: C. Ramel (ed.). Chlorinated Phenoxy Acids and Their Dioxins Mode of Action, Health Risks and Environmental Effects. *Ecol. Bull. (Stockholm)* 27:53-66.
- McKinney, G., and E. McConnell. 1980. Structural Specificity and the Dioxin Receptor. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 279.

- Merritt, R. W., and K. W. Cummins. 1978. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa. pp. 441.
- Miller, G. C., and R. G. Zepp. 1979. Photoreactivity of aquatic pollutants sorbed on suspended sediments. Environ. Sci. Technol. 13(7):860-863.
- Mitchum, R. K., G. F. Moler, and W. A. Korfmacher. 1980. Combined capillary gas chromatography/atmospheric pressure negative chemical ionization mass spectrometry for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in tissue. Anal. Chem. 52:2278-2282.
- Moore, J. A. 1978. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. IN: C. Ramel (ed.). Chlorinated Phenoxy Acids and Their Dioxins Mode of Action, Health Risks and Environmental Effects. Ecol. Bull. 27:134-144.
- Muir, D. C. G., H. P. Grift, A. P. Blouw, and W. L. Lockhart. 1980. Fate of fluridone in small ponds. J. Environ. Qual. 9:151-156.
- Muir, D. C. G. 1983. Personal communication.
- Nash, R. G., and M. L. Beall. 1977. Environmental distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin applied with silvex to turf in microagroecosystem. Final Report U.S.E.P.A.-USDA interagency agreement. EPA-1AG-D6-0054, ARS173, EPA#1001-704.
- Nash, R. G., and M. L. Beall. 1980. Distribution of silvex, 2,4-D, and TCDD applied to turf in chambers and field plots. J. Agric. Food Chem. 28(3):614-623.

- Nau, H., R. Bass, and Neubert. 1980. Transfer of 2,3,7,8-tetrachloro-dibenzo-p-dioxin to the mouse embryo, fetus and neonate. IN: O. Hutzinger, R. W. Frei, E. Merian, and F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 651.
- Neal, R. A., P. Beatty, and t. A. Gasiewicz. 1979. Studies of the mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Ann. N. Y. Acad. Sci. 320:204-214.
- Neely, W. B., D. R. Branson, and G. E. Blau. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. Technol. 8:1113-1115.
- Neely, W. B. 1979. A preliminary assessment of the environmental exposure to be expected from the addition of a chemical to a simulated aquatic ecosystem. Intern. J. Environ. Studies 13:101-108.
- Nestrick, T. J., and L. L. Lamparski. 1982. Isomer specific determination of chlorinated dioxins for assessment of formation and potential environmental emission from wood combustion. Anal. Chem. 54:2292-2299.
- Nestrick, T. J. 1981. Personnel communication.
- Nestrick, T. J., L. L. Lamparski, and D. I. Townsend. 1980. Identification of tetrachlorodibenzo-p-dioxin isomers at the one nanogram level using photolytic degradation and pattern recognition techniques. Anal. Chem. 52(12):1865-1874.
- Nestrick, T. J., L. L. Lamparski, and R. H. Stehl. 1979. Synthesis and identification of the 22 tetrachlorodibenzo-p-dioxin (TCDD) isomers by high performance liquid chromatography and gas chromatography. Anal. Chem. 51(13):2273-2281.

- Nilsson, C. A. 1977. Studies of Polychlorinated 2-phenoxyphenols precursors of chlorinated dibenzo-p-dioxins. Doctoral Thesis. University of Umea. pp. 124.
- Nilsson, C. A., and L. Renberg. 1974. Impurities in chlorophenols. *J. Chromatogr.* 89(2):325-333.
- NRCC. 1981a. Polychlorinated Dibenzo-p-dioxins: limitations to the current Analytic Techniques. Publ. No. 18576, Environmental Secretariat, Ottawa. pp. 183.
- NRCC. 1981b. Polychlorinated Dibenzo-p-dioxins: Criteria for their Effects on Man and his Environment. Publ. No. 18574, Environmental Secretariat, Ottawa. pp. 251.
- Olie, K., J. W. A. Lustenhouwer, and O. Hutzinger. 1980. Polychlorinated dibenzo-p-dioxins and related compounds in incinerated effluents. IN: O. Hutzinger, R. W. Frei, E. Merian, F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 651.
- Olie, K., P. L. Vermeulen, and O. Hutzinger. 1977. Chlorodibenzo-p-dioxins and chlorodibenzofurans are trace components of fly ash and flue gas of some municipal incinerators in the Netherlands. *Chemosphere* 6(8):455-459.
- Oliver, R. J. 1975. Toxic effects of 2,3,7,8-tetrachlorodibenzo 1,4 dioxin in laboratory workers. *British J. Ind. Med.* 3:49-53.
- Paris, D. F., W. C. Steen, and G. L. Baughman. 1978. Role of physiochemical properties of Arochlors 1016 and 12142 in determining their fate and transport in aquatic environments. *Chemosphere* 7:319-325.

- Pfeiffer, C. D., T. J. Nestruck, and C. W. Kocher. 1978. Determination of purified pentachlorophenol by liquid chromatography. *Anal. Chem.* 50:800-804.
- Plimmer, J. R. 1978. Photolysis of TCDD and trifluralin on silica and soil. *Bull. Environ. Contam. Toxicol.* 20:87-92.
- Pocchiari, F. 1978. 2,3,7,8-tetrachlorodibenzo-p-dioxin decontamination. IN: C. Ramel (ed.). Chlorinated Phenoxy Acids and their Dioxins Mode of Action, Health Risks and Environmental Effects. *Ecol. Bull. (Stockholm)* 27:67-70.
- Pohland, A. E., and G. C. Yang. 1972. Preparation and characterization of chlorinated dibenzo-p-dioxins. *J. Ag. Food Chem.* 20:1093.
- Poiger, H., H. Weber, and C. Schlatter. 1980. Special aspects of metabolism and kinetics of TCDD in dogs and rats. Assessment of toxicity of TCDD-metabolites in guinea pigs. IN: O. Hutzinger, R. W. Frei, E. Merian, F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 317-325.
- Poland, A., W. F. Greenlee, A. S. Kende. 1979. Studies on the mechanism of action of the chlorinated dibenzo-p-dioxin and related compounds. *Ann. N. Y. Acad. Sci.* 320:214-230.
- Poland, A., E. Glover, and A. S. Kende. 1976. Stereospecific high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytol. *J. Biol. Chem.* 251:4936-4946.
- Poland, A., and A. Kende. 1976. 2,3,7,8-tetrachlorodibenzo-p-dioxin: Environmental contaminant and molecular probe. *Federal Proceedings.* 35(12):2404-2411.

- Rappe, C., and C. A. Nilsson. 1972. An artifact in the gas chromatographic determination of impurities in pentachlorophenol. *J. Chromatogr.* 67:247-253.
- Rappe, C. 1978. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) Introduction. IN: F. Cattabeni, A Cavallaro, and G. Galli (eds.). Dioxin - Toxicological and Chemical Aspects. SP Medical and Scientific Books, N. Y. pp. 9-11.
- Rappe, C., S. Marklund, H. R. Buser, and H. P. Bosshardt. 1978. Formation of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by burning or heating chlorophenates. *Chemosphere* 7:269-281.
- Rappe, C. 1979. Polychlorinated dibenzodioxins and dibenzofuran compounds of current interest. *Lakartidningen* 76(1-2):21-29.
- Rawn, G. P. 1981. Fate and Degradation of Permethrin in a Model Aquatic Ecosystem. Phd. Thesis. University of Manitoba, Winnipeg, Manitoba, Canada. pp. 114.
- Reggiani, G. 1980. Toxicology of TCDD and related compounds observations in man. IN: O. Hutzinger, R. W. Frei, E. Marian, F. Pocchiari (eds.). Chlorinated Dioxins and Related Compounds. Impact on the Environment. Pergamon Press, Oxford, New York. pp. 463-495.
- Rose, J. Q., J. C. Ramsay, T. H. Wentzler, R. A. Hummel, and P. J. Gehring. 1976. The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral dose to the rat. *Toxicol. Appl. Pharmacol.* 36:209-226.

- Safe, S. 1980. Metabolism, uptake, storage and bioaccumulation. IN: R. D. Kimbrough (ed.). Halogenated biphenyls, terphenyls, naphthalenes, dibenzo-dioxins and related products. Elsevier/N. Holland Biomedical Press, N. Y. pp. 81-109.
- Shadoff, L. A., and R. A. Hummel. 1978. The determination of tetrachlorodibenzo-p-dioxin in biological extracts by GC-MS. *Bio-med. Mass Spect.* 5(1):7-13.
- Smith, J. H., D. C. Bomberger and D. L. Haynes. 1981. Volatilization rates of intermediate and low volatility chemicals from water. *Chemosphere* 10(3):281-289.
- Smith, R. M., P. W. O'Keefe, D. R. Hilker, B. L. Jelus-Tyror and K. M. Aldous. 1982. Analysis for 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin in a soot sample from a transformer explosion in Binghamton, N. Y. *Chemosphere* 11(8):715-720.
- Southworth, G. R. 1979. The role of volatilization in removing polycyclic aromatic hydrocarbons from aquatic environments. *Bull. Environ. Contam. Toxicol.* 21:507-514.
- Stehl, R. H., R. R. Papenfuss, R. A. Bredweg, and R. W. Roberts. 1973. The stability of pentachlorophenol and chlorinated dioxins to sunlight, heat and combustion. IN: E. H. Blair (ed.). Chlorodioxins - Origin and Fate. Am. Chem. Soc. Washington, D. C. pp. 119-125.
- Stehl, R. H., and L. L. Lamparski. 1977. Combustion of several 2,4,5-T compounds: formation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Science* 197:1008-1009.
- Taylor, J. S. 1979. Environmental chloracne: Update and overview. *Ann. N. Y. Acad. Sci.* 320:295-307.

- Taylor, J. S. 1974. Chloracne - a continuing problem. *Cutis* 13: 585-591.
- Theiss, J. C. 1981. Cancer: Achievements, Challenges, Prospects 1980's Proc. Int. Symp. Cancer 1:281-290.
- Thibodeau, L. J. 1982. Off-site transport of 2,3,7,8-TCDD from a production dispersal facility. Presented at: ACS meeting on Environ. Chemistry, Kansas City, Missouri (Sept. 82). pp. 42.
- Tiernan, T. O., and M. L. Taylor. 1978. Development and application of analytical methodology for determination of hexa-, hepta-, and octachlorodibenzodioxins in beef samples. Final Rept. USDA contract no. 12-64-4-378.
- Tsushimoto, G., F. Matsumura and R. Sago. 1982. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin in an outdoor pond and in model aquatic ecosystems. *Environ. Toxicol. Chem.* 1:61-68.
- Tulp, M. T. M. Rat metabolism of polychlorinated dibenzo-p-dioxins. *Chemosphere* 7:761-768.
- Veterans Administration. 1980. Review of literature on herbicides, including phenoxy herbicides and associated dioxins. Vol. I: analysis of literature. Veteran's Administration, Washington V10(93)P-823. pp. 285.
- Villanueva, E. C., V. A. Burse, R. W. Jennings. 1973. Chlorodibenzo-p-dioxin contamination of two commercially available pentachlorophenol. *J. Agric. Food Chem.* 21(4):739-740.
- Villanueva, E. C., V. A. Burse, R. W. Jennings. 1974. Evidence of chlorinated dioxins and chlorodibenzofuran in hexachlorobenzene. *J. Agric. Food Chem.* 22(5):916-917.

- Vos, J. G. 1977. Immune suppression as related to toxicology. CRC Critical Reviews in Toxicology 67-79.
- Ward, C. 1976. Fate of 2,4,5-T and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in Aquatic Environments. M.Sc. Thesis. Wisconsin University, Michigan. pp. 107.
- Ward, C. T., and F. Matsumura. 1978. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin in a model aquatic environment. Arch. Environ. Contam. Toxicol. 7:349-357.
- Webster, G. R. R., K. Olie, and O. Hutzinger. 1983a. Polychlorinated dibenzo-p-dioxins and Polychlorinated dibenzofurans. IN: O. Hutzinger, S. Safe, and F. W. Karasek. GC-MS of Environmental Chemicals. Pergamon Press, Oxford, New York, in press.
- Webster, G. R. B., L. Sarna and D. Muir. 1983b. K_{ow} of 1,3,6,8-tetrachlorodibenzo-p-dioxin and octachlorodibenzo-p-dioxin by reverse phase high pressure liquid chromatography. ACS Meeting on Chlorodioxins and Chlorofurans. Washington, D. C., August, 1983.
- Wheland, E. 1980. 2,4-D Tests. News Release, Agriculture Canada. October 3, 1980.
- Wipf, K. K., E. Homberger, N. Neuner and F. Schenker. 1978. Field trials of photodegradation of TCDD on vegetation after spraying with vegetable oil. IN: F. Cattabeni and G. Gallia (ed.). Dioxin-Toxicological and Chemical Aspects. S. P. Medical and Scientific Books, New York. pp. 201-216.
- Woolson, E. A., R. F. Thomas, and P. D. J. Ensor. 1972. Survey of polychlorinated dibenzo-p-dioxins in selected pesticides. J. Agric. Food Chem. 20(2):351.

- Yamagishi, T., T. Miyazaki, K. Akiyama, M. Morita, J. Nakagawa, S. Horii, and S. Kaneko. 1981. Polychlorinated dibenzo-p-dioxins and dibenzofurans in commercial diphenyl ether herbicides and in freshwater fish collected from the application area. *Chemosphere* 10(10):1137-1144.
- Yochim, R. S., A. R. Isensee, and G. E. Jones. 1978. Distribution and toxicity of TCDD and 2,4,5-T in a aquatic model ecosystem. *Chemosphere* 93:215-220.
- Zepp, R. G., G. L. Baughman, and P. F. Schlotzhauer. 1981. Comparison of photochemical behavior of various humic substances in water: I Sunlight induced reactions of aquatic pollutants photosensitized by humic substances. *Chemosphere* 10:109-117.
- Zepp, R. G., N. L. Wolfe, G. L. Baughman, and R. C. Hollis. 1977. Singlet oxygen in natural waters. *Nature* 267:421-423.