THE UPTAKE AND DEPURATION OF 2,3,7,8-TETRACHLORODIBENZOFURAN AND OCTACHLORODIBENZO-p-DIOXIN BY Hydropsyche bidens (Ross) IN MINIATURE LAB STREAMS

BY

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

ABSTRACT

The bioavailability of particle-bound and "freely dissolved" ^{3}H-2,3,7,8-tetrachlorodibenzofuran (TCDF) and ^{14}C-octochlorodibenzo-p-dioxin (OCDD) was investigated using Hydropsyche bidens (Ross), a filter-feeding caddisfly larvae and its non-feeding pupae in miniature labstreams. Uptake (30 d) and elimination (18 d) experiments were carried out with caddisfly larvae exposed to TCDF sorbed to Nutra Fin® at 60 ng g^{-1} concentrations at two velocities: 16 and 24 cm s^{-1}. Dual-labelled experiments were also conducted at 16 cm s^{-1} by feeding algae to larvae at two exposure levels: i) 72 ng g^{-1} (TCDF) and 5,600 ng g^{-1} (OCDD) and ii) 200 ng g^{-1} (TCDF) and 25,800 ng g^{-1} (OCDD). Non-feeding pupae were exposed to a dual-label algae exposure of TCDF (72 ng g^{-1}) and OCDD (26,100 ng g^{-1}).

Steady state concentrations of TCDF and OCDD in the caddisfly larvae were reached within 16 d at both the concentrations that were studied. Non-feeding pupae reached steady state concentrations for TCDF, but not for OCDD, in a 10 d exposure to algae-sorbed TCDF (72 ng g^{-1}) and OCDD (5,600 ng g^{-1}). Body burdens for TCDF and OCDD in the filter-feeding larvae exceeded the levels measured in non-feeding pupae by 50 - 100 fold. Assuming similar respiratory rates for larvae and pupae, the results of the study suggest that food chain transfer of TCDF and OCDD is the main uptake route, and the contribution due to bioconcentration and cuticle adsorption is relatively minor.

TCDF followed a first-order decay model at the highest dietary concentration of 200 ng g^{-1}. At lower TCDF concentrations in food (60 to 72 ng g^{-1}), the elimination curves had a biphasic appearance. The clearance of OCDD consistently resulted in biphasic elimination curves.

First-order half-lives (d) of TCDF (10.0 ± 1.2 to 27.5 ± 6.9) were rapid and helped to explain the fast approach to steady state. Biological half-lives of TCDF and OCDD were not

significantly affected by varying the stream velocities (from 16 to 24 cm s^{-1}), changing the food quality (from biotic to abiotic particles) or the contaminant concentration (from 60 to 200 ng g^{-1} for TCDF and 5,600 to 25,800 ng g^{-1} for OCDD).

Assimilation efficiencies (α) of TCDF and OCDD were low; < 9.2 ± 1.3% and 4.9 ± 0.33% respectively, assuming a feeding rate of 0.89 g g^{-1} d^{-1} (dry wt). Rapid uptake and elimination rates, lower steady state body burdens for the penultimate instar larvae, and low α values suggest feeding rate is one of the most important dietary parameters for predicting and modelling food chain uptake of TCDF and OCDD in aquatic macroinvertebrates.

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A Thesis Experience:

Act 1: Landing on Dr. D. Muir's foodchain pathway, a stream of ideas from Dr. W. Fairchild, many strong coffees, and long walks lead to:

Act 2: Interesting hypothesis formulation sessions with Dr. D. Rosenberg and Dr. B. Hann.

Dr. J. Flannigan and D. Cobb introduced me to my first Assiniboine River riffle experience; hip-waders, sampling bags, a pair of fine tweezers, rocks gleaming with aquatic insects, and the hydropsychid larvae.

With the help of R. McNicol, Dr. E. Scherer, D. Haughan, B. Danell, and Abe, six fine miniature lab streams were built. A. Yarechewski and D. Metner helped with the contamination of food particles. Dr. L. Henzel, H. Kling, and S. Gilbert gave the guidance necessary to grow huge vats of algae: enough to feed 300 hungry hydropsychid larvae per day. Extra consultation with Dr. W. Fairchild, Dr. T. Galloway, S. Brown, Dr. D. Giberson, W. Schefter, and Dr. R. Mackay helped with the experimental design and interpretation of the results.

Act 3: A day off was only possible when larvae-sitter, A. Bordeleau, was able to come and feed the hydropsychids. A healthy balance of grounding came from my friends: J. Rusak, B. Kramarchuk, L. Ankenman, M. Wiens, Riffle (my sampling dog), and partner M. Jull.

Concluding Act: The success of this project, reflected in this document, and its scientific merit could have only been possible through the input of all of these people and the positive support and words from the committee members: Dr. B. Webster, Dr. L. Lockhart, and foremost, Dr. D. Muir.

LIST OF ABBREVIATIONS

^{3}H	Tritium
^{14}C	Radio-isotope
α	Assimilation Efficiency
°C	Degrees Celsius
Å	Angstroms (1×10^{-8} cm)
ADD	Accumulation of Diatoms and Detritus
Ah	Aryl Hydrocarbon
amp	Ampere
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BMF	Biomagnification Factor
BKM	Bleach Kraft Mill
Bq	Becquerels
C_B	Concentration in Biota
C_F	Concentration in Food
C_O	Initial Concentration in Organism
C_s	Water Solubility
C_W	Concentration in Water
cm	Centimeter
cm^3	Cubic Centimeter
d	Day
DOM	Dissolved Organic Matter
dpm	Disintegrations per Minute

DRE	Dioxin Response Element
dry wt	Dry Weight
EROD	Ethoxyresorufin *O*-deethylase
f_{oc}	Fraction of Organic Carbon
f_d	"Freely Dissolved" Concentration
FR	Feeding Rate
g_c	Growth Rate Constant
g	Gram
H_C	Henry's Law Constant
h	Hour
HP	Horse Power
HPLC	High Pressure Liquid Chromatography
Hz	Hertz
id	Internal Diameter
k_1	Uptake Coefficient
k_2	Elimination Rate Constant
kg	Kilogram
km	Kilometer
K_{oc}	Organic Carbon-Water Partition Coefficient
K_{ow}	Octanol-Water Partition Coefficient
L	Liter
LD_{50}	Concentration of Contaminant Necessary to Generate a 50% Lethality
LOEL	Lowest Observable Effects Level
LSC	Liquid Scintillation Counter

m	Meter
m^2	Meter Squared
mg	Milligram
min	Minutes
mL	Milliliter
mm	Millimeter
MO	Monooxygnease
mol	Mole
MBq	Mega Becquerels
mRNA	Messenger RNA
MW	Molecular Weight
ND	Non Detectable
ng	Nanogram
NOEC	No Observable Effect Concentration
NRCC	National Research Council of Canada
OC	Organic Carbon Content
OCDD	1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin
OME	Ontario Ministry of Environment
Pa	Pascal
PCP	Pentachlorophenol
pg	Picogram
POM	Particulate Organic Matter
PCDFs	Polychlorinated Dibenzofurans
PCDDs	Polychlorinated Dibenzo-p-dioxins

Rep #1	Treated Tank #1
Rep #2	Treated Tank #2
RPM	Revolutions Per Minute
s	Second
SETAC	Society of Environmental Toxicity and Chemistry
t	Time
t½	Half-life
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCDF	2,3,7,8-Tetrachlorodibenzofuran
TEF	Toxic Equivalency Factors
TEQ	Toxic Equivalents
µCi	Micro-Curie
µg	Micro-Grams
µm	Microns
µPa	Micro-Pascal
USEPA	United States Environmental Protection Agency
VP	Vapor Pressure
W/L	Width to Length Ratio
wet wt	Wet Weights
WHO	World Health Organization

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1. INTRODUCTION

Food chain transfer has been identified as the most important pathway for accumulation of persistent, hydrophobic, chlorinated aromatic hydrocarbons in aquatic ecosystems (Bruggeman et al. 1981, Thomann et al. 1984, Alasdair et al. 1991, Thomann et al. 1992). In freshwater and marine ecosystems these pollutants are usually found in association with the organic matter of drifting micro-seston (e.g., algae, phytoplankton, plant material, detritus) and dissolved organic matter (Sondergren 1968, Eadie 1982a, McCarthy and Jimenez 1985, Autenreith et al. 1991, Broman et al. 1992). Caddisfly larvae spend the majority of their life span in the aquatic environment and their filter-feeding behavior brings them into intimate contact with particle-bound contaminants (Borgmann 1985). In riverine food webs, a biomagnification pathway for hydrophobic organic contaminants could be from contaminant-bound suspended matter to particulate consumers such as filter-feeding caddisfly larvae (Freeden et al. 1975) to insectivorous fish (Wahl et al. 1988), and conceivably wildlife and humans incorporating fish into their diet.

It has been estimated that stationary filter-feeders can capture and consume between 0.005 to 0.011 % of the seston flowing over them daily in a 1 m^2 area (McCullough 1979, Georgian et al. 1981), thus potentially removing significant quantities of contaminant-bound particulate matter in relatively short stretches of the river. Food chain accumulation of two families of chlorinated hydrocarbons, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) may help to explain the levels found in fish eating a diet consisting predominately of insects downstream from kraft pulp and paper mills (Rodgers et al. 1989, Muir et al. 1992a).

There is limited information on the pharmacokinetics of hydrophobic organics for river

back by the lack of good experimental design, and the difficulty in sampling flow conditions (Muirhead-Thomson 1987). Predicting environmental pathways, risks, and fate for pollutants of concern has forced modellers to extrapolate or estimate toxicokinetic parameters for riverine insects. This is a difficult task when considering that "closely related genera sharing the same habitat may show consistent differences in reaction to one and the same chemical" (Muirhead-Thomson 1987).

Filter-feeding caddisfly larvae, specifically hydropsychids, are a dominate food source for many river fish species (Bond 1979a, Bond 1979b, Bond et al. 1980a, Bond et al. 1980b), macroinvertebrates, and terrestrial animals (Gray 1989). They have a large role in energy and nutrient cycling (Oswood 1979), and constitute the majority of the macroinvertebrates in productive riffle (fast flowing) areas (Hickin 1952, Hynes 1970, Williams and Hynes 1973, Gordon et al. 1975, Mackay 1979, Bush et al. 1985, Cibrowski 1988). Their sedentary lifestyle has led to their use as "sentinel organisms", i.e., indicators of the toxic conditions at a specific site (over time). Hydropsychids are adapted to a wide range of eco-conditions (Gordon et al. 1975, McKay 1978). They have been successfully used for laboratory uptake and elimination experiments (Metcalf et al. 1984).

This study was undertaken with the general goal of enhancing the understanding of the bioaccumulation of particle-bound PCDD/Fs by benthic river invertebrates in order to accurately model food chain transfer in rivers and to improve the ecological understanding of Hydropsyche bidens (Ross). The specific objectives of this study were to

1. Trace the movement of microseston-bound ^{3}H-2,3,7,8-tetrachlorodibenzofuran (TCDF) and ^{14}C-1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (OCDD) in miniature lab streams with the filter-feeding caddisfly larvae, Hydropsyche bidens (Ross).

2. Derive pharmacokinetic parameters such as depuration half-lifes (t½) and

assimilation efficiencies (α).

3. Determine the applicability of Laciousiérè and Craig's (1990) "continuous and static" miniature laboratory stream for bioaccumulation experiments with aquatic invertebrates.

2. REVIEW OF THE LITERATURE

2.0 Filter-feeding Caddisflies

Caddisflies (Trichoptera) are found in a wide range of habitats and trophic categories; i.e., predators, collectors, grazers, and filter feeders. Their unique success and diversity is attributed to their use of silk secreted from a specialized silk gland at the tip of the labium (Hickin 1952, Wiggins 1978, Wallace et al. 1980). "Nearly every type of freshwater habitat harbours its own characteristic species, and as they very often occur in extremely large numbers and as most of them could be called 'medium to large' in size their importance in the freshwater web of life is paramount" (Hickin 1952).

The success of the filter-feeding Hydropsychidae (a Family of Trichoptera) lies in their ability to tap into a microworld of nutrients and seston spiralling dowstream. Certain benthic invertebrates, e.g., blackflies and caddisflies, have adapted passive filter feeding mechanisms to both collect and consume fine organic particulate matter 50 μm - 1 mm and ultra-fine organic particulate matter 0.5 - 50 μm carried by river currents (Merritt et al. 1982, Georgian et al. 1981, Alstad 1987a). They accomplish this by producing efficient filtering nets which are positioned perpendicular to the current (Wallace et al. 1980). Early instars construct smaller mesh sizes as a result of their smaller anatomical mouthpart structures (Wallace et al. 1980, Thorp et al. 1986). Mesh sizes for some species of hydropsychids can vary with

different flow conditions (Fuller et al. 1980a).

Hydropsychidae larvae are omnivorous feeders. Their diet consists of abiotic and biotic particles: detritus, pollen grains, and vascular plant fragments, diatoms, moss, algae, and animal remains (Benke et al. 1990). "Almost without exception, Trichoptera are fortuitous feeders as the food ingested corresponds to that organic matter which is available at a particular time in a given lake or stream" (Mecom 1972). The food particles readily available in the spring and summer consist of algae, detritus, and plant fragments. Some Hydropsyche species resort to grazing in the winter when diatom mats become a more important food source (Wallace et al. 1980). Variation between hydropsychid species and instars has facilitated larvae to consume different sizes and types of particles (Wallace et al. 1977).

As a rule, hydropyschids have between five and seven aquatic larval instars (Wiggins 1978). More than six species have been recorded to coexist together on the surfaces of boulders, rocks, and twigs associated with the stream bed (Alstad 1987b). In these locations all but coarse substrates are washed downstream under fast flowing conditions (Cummins et al. 1974).

Hydropyschids are adapted for fast current and have been found in velocities approaching 51 cm s^{-1} (Wallace 1975). Besides delivering micron-sized food particles, fast flowing water is essential for respiration. Larvae undulate their bodies within their cases to generate a current over their tracheal gills permitting efficient oxygen exchange (Wiggins 1978).

Overall very little is known about the life histories of the 70 plus species (Mackay 1984). Generally, the life span of the adult phase ranges from 10 to 15 d, eggs hatch within eight to ten days at 20 °C, and the larval and pupal period are both between two to three weeks (Badcock 1953). At the onset of the pupation period, the front and back of their

"hibernaculums" are closed-off, to form a cocoon. Tiny openings permits aqueous flow through for ventilation. The larvae undergo a quiescent stage prior to metamorphosis into active pupae. The decticious (functional) toothed mandibles and dramatic body undulations of the newly transformed pupae aid its ability to rupture through the cocoon in order to emerge. Specialized second legs which resemble hairy oars, propel the pupae in the aqueous medium to the surface. The pupae shed their exuviae at either the neuston or in the littoral zone to emerge as winged adults (Hickin 1952).

Most species are univoltine and emerge in either late spring or summer (Hilsenhoff 1975). Mackay's (1979) sampling regime was sensitive enough to demonstrates that both univoltine and bivoltine life strategies could exist for the same species of hydropsychid (e.g., H. sparna) in the same river. The adults lead a nocturnal existence for up to one month and generally are non-feeding (Wiggins 1978).

2.1 Suspended Solids in Streams

Chlorinated hydrocarbons readily associates with the organic carbon (OC) of suspended particulate organic matter (POM), dissolved organic matter (DOM), and sediment particles (Eadie et al. 1982b, McCarthy et al. 1985) and partitions to the lipids of aquatic organisms (Thomann et al. 1992). The size spectrum, quality, and flux of particles affects both their availability as food, and their contaminant binding characteristics (Lush and Hynes 1973, Benke et al. 1990). In natural lake mesocosm experiments, waterborne TCDF concentrations were highest on the smallest particles sizes (0.22 to 1.0 μm) with the highest OC (Muir et al. 1992b). River characteristics such as stream order can affect food quality; for example, the fraction of organic carbon (f_{oc}) increases with stream order (Naiman 1983, Alstad 1987a).

The main source of energy and nutrients in streams is allochthonous detritus (Wallace et al. 1977, Wahl et al. 1988). The concentration and quality of seston depends on many river attributes: stream order, geo-physical properties, spatial patchiness, temporal changes, diurnal fluxes, allochthonous inputs, anthropogenic inputs, flooding, autochthonous production changes, biological utilization, and the storage of organic matter along the length of the river (Vannote et al. 1980, Naiman 1983). Maciolek and Tunzi (1968) conclude that microseston losses in streams were primarily caused by filter-feeding activities of invertebrates, followed by sedimentation and then decimination.

Particle concentrations measured in rivers ranged from 1.8 to 4.8 X 10^6 particles mL^{-1} (Freeden 1964, Wallace et al. 1977). Weights of suspended aquatic matter in the Tallulah River, CA (USA) tend to have seasonal lows (0.54 mg L^{-1}) in November and highs (2.1 mg L^{-1}) in July (Georgian et al. 1981). Lows occur as a result of decreasing flows and increasing sedimentation (Maciolek and Tanzi 1968). Higher weights for suspended particles were found in the Assiniboine River, MB (Canada) during the summer of 1992, ranging from 70 mg L^{-1} in June to 298 mg L^{-1} in August. Total DOC concentrations reported by Ciario et al. (1990) in the Chillisquaque Creek, PA (USA) and by Merritt et al. (1982) in Mud Creek, MI (USA) vary from 4.92 mg L^{-1} to 13.66 mg L^{-1}.

A study undertaken by Egglishaw and Shackley (1971) demonstrated that suspended sediments in River Almond (Scotland) were made up of mainly abiotic particles; 45 to 83% of the total suspended particulate matter was fine noncellular detritus. However, river systems can also have a characteristic biotic component. Freeden (1964) characterized the macroplanktonic cells in eight prairie streams and found between 5,000 to 224,000 protozoa and algae cells mL^{-1} and 3.1 x 10^6 bacterial cells mL^{-1}.

Approximately 90% of the suspended particle (by weight) transported by rivers fall

into the smallest particle class, < 106 μm (Hynes 1970, Cudney et al. 1980, Ross et al. 1981, Merritt et al. 1982, Taylor et al. 1982, Naiman 1983, Alstad 1986). Alstad (1987a) found that 84% of the river seston had particle sizes between 5 and 50 μm. Taylor et al. (1982) showed there were two concentration peaks, one with particles sizes < 10 μm and one for the class between 30 and 110 μm.

2.3 Physicochemical Parameters of Chlorinated Dioxins and Furans

OCDD and 2,3,7,8-TCDF are important members of the large families of PCDDs and PCDFs. There are 75 PCDD and 135 PCDF congeners (Figure 1). The core molecules consist of tricyclic aromatic structures: two benzene rings connected by a third ring containing a single oxygen atom for the furans and two oxygen atoms for the dioxins.

Knowledge of physical and chemical properties is essential to understanding modelling environmental transport and fate as well as pharmacokinetic and toxicological behaviours of organic contaminants (USEPA 1992). The physicochemical properties of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), TCDF, and OCDD are summarized below in Table 1: Water Solubility (C_s), Vapor Pressure (VP), Henry's Law Constant (H_c), Octanol-Water Partition Coefficient (K_{ow}), Organic Carbon-Water Partition Coefficient (K_{oc}), and Molecular Weight (MW). Increase in chlorine substitution of PCDD/Fs usually indicates greater hydrophobicity, lipophilicity, and environmental persistence.

2.3.0 Solubility (C_s)

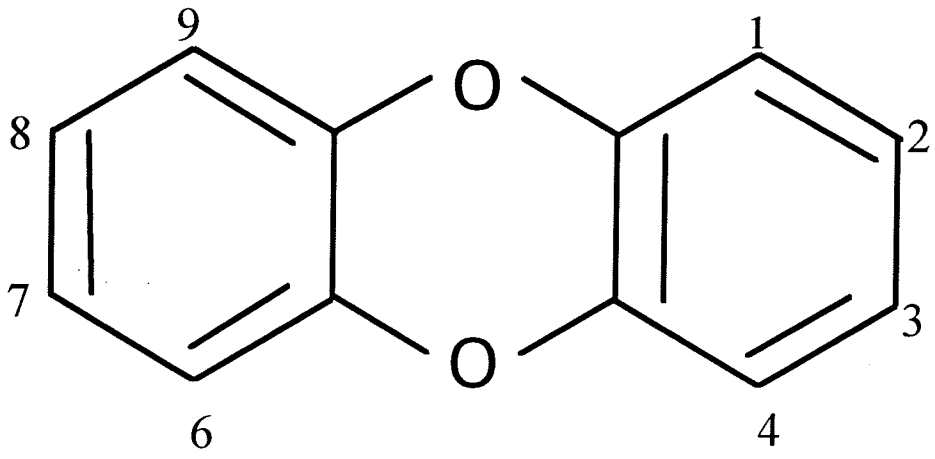
TCDD, TCDF and OCDD are extremely low solubility in water. This low solubility is often attributed to their large molecular size, volume, and overall low polarity which yields little interaction with the polar water molecules. Although TCDD and TCDF have similar

Table 1. Physicochemical properties for 2,3,7,8-TCDD, 2,3,7,8-TCDF, and OCDD: C_s (Water Solubility), VP (Vapor Pressure), H_c (Henry's Law Constant), K_{ow} (Octanol-Water Partition Coefficient), K_{oc} (Organic Carbon-Water Partition Coefficent), MW (Molecular Weight)

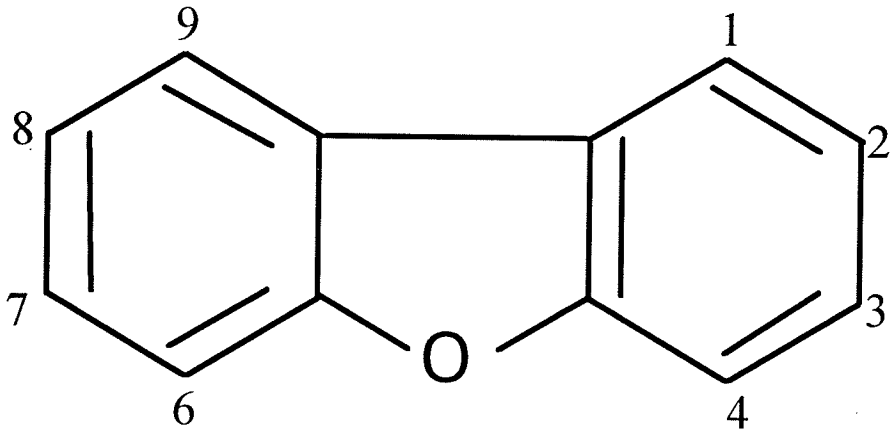
Properties	TCDD	TCDF	OCDD
C_s (ng L^{-1} @ 25° C)	8-200[1]	*419[2]	0.074[1]
VP (μPa @ 25°C)	0.15-0.62[1]	123[3]	0.00011[1]
H_c (Pa m^3 mol^{-1})[4]	1.62	1.46	0.683
Log K_{ow}	6.80[1]	6.53[5]	8.20[1]
Log K_{oc}[6]	6.8	7.5	7.9
MW	321.98	305.98	460.76

* @ 23 °C

1. Shiu et al. (1988)
2. Friesen et al. (1990)
3. Eitzer and Hites (1988)
4. USEPA (1992) - calculated values
5. Burkhard and Keuhl (1986)
6. Broman et al. (1991)



PCDDs
(75 congeners)



PCDFs
(135 congeners)

Figure 1. Positioning and numbering of chlorine atoms of PCDDs and PCDFs

molecular, physical, and chemical properties, TCDF (C_s = 419 ng L^{-1}) is more soluble than TCDD (C_s = 8 to 200 ng L^{-1}). Friesen et al. (1990) identified a decrease in C_s with greater chlorination for PCDDs/Fs. OCDD is the most insoluble of the PCDD/Fs with a reported C_s of 0.074 ng L^{-1} (Shiu et al. 1988).

2.3.1 Vapor Pressure (VP) and Henry's Law Constant (H_C)

Despite low VPs for 2,3,7,8-TCDD, 2,3,7,8-TCDF, and OCDD (Table 1), volatilization can be an important transport mechanism (USEPA 1992). Henry's Law constant (H_c) can be defined as the ratio of thecompound's VP to C_s denoting the transfer between water and air. The H_c values for TCDF (1.46 Pa m^3 mol^{-1}) and OCDD (0.683 Pa m^3 mol^{-1}) denotes a significant net-transfer from the aqueous medium to air. These values also suggest that atmospheric transport could contribute to the global redistribution of these contaminants.

Volatilization half-lives in flowing river water have been estimated to be 14 d for TCDF and 16 d for TCDD based on two-film theory (Podoll et al. 1986, Versar 1989).

2.3.2 Octanol-Water Partition Coefficient (K_{ow})

The octanol-water partition coefficient (K_{ow}) is defined as the aqueous concentration of a chemical in octanol (water-saturated) in equilibrium with that found in water (octanol-saturated). Octanol is regarded as a surrogate for lipids (Connell 1990). The tendency for the contaminant to partition from water into octanol is taken to be analogous to the net exchange from an aqueous medium to the final storage in the lipids of organisms. Literature values for log K_{ow} for 2,3,7,8-TCDD range from 6.15 to 8.93 (Kenaga 1975, Sarna et al. 1985), from 5.80 to 5.82 for 2,3,7,8-TCDF (Burkhard and Keuhl 1986, Lupp and McCarthy 1989), and from 7.53 to 12.7 for OCDD (Doucette 1985, Sarna et al. 1985).

K_{ow} values can express the potential for an organism to accumulate or depurate a compound. Halogenated hydrocarbons with high K_{ow} values (e.g., larger than 10^6), such as TCDF and OCDD, are members of a class of chemicals which are extremely slowly metabolized and eliminated by fish (Gobas 1988). It has been determined that bioaccumulation factors (BAFs) are maximum for compounds with a log K_{ow} of 6.7 (Connell 1990). The log K_{ow} value for TCDF of 6.53 suggests a high potential for bioaccumulation. With a log K_{ow} value of 8.6, OCDD is expected to have lower accumulation. OCDD belongs to a group of persistent "superlipophilic" chemicals that do not cross biological membranes efficiently (Geyer et al. 1992).

2.3.3 Organic Carbon-Water Partition Coefficient (K_{oc})

The fate and transport of TCDF and OCDD in aquatic environments is influenced by their ability to bind to POM and DOM. The organic carbon-water partition coefficient (K_{oc}) is the ratio of the contaminant bound to POM to the concentration found freely dissolved in water (f_d) (Equation 1).

$$\mathbf{K_{oc} = [pollutant\ on\ particulate\ organic\ carbon] \div [f_d]} \qquad (1)$$

Log K_{oc} values for marine particulates are 6.8, 7.5, and 7.9 for TCDD, TCDF and OCDD respectively (Broman 1991). Broman's marine log K_{oc} value for TCDF is similar to those obtained by Muir et al. (1992b) from a natural lake mesocosm study where the log K_{oc} varied from 7.5 to 8.0. These values are similar in magnitude to K_{ow} values (see Table 1).

2.3.4 Persistence

PCDD/Fs are environmentally stable and persistent compounds. The different environmental degradation processes are summarized in Table 2 for dibenzofurans. PCDD/Fs do not react with either weak acids/bases or most redox agents (USEPA 1990a). The only significant environmental pathway for the degradation of ^{3}H-TCDF was shown to be photodechlorination by sunlight in natural waters with a net degradative rate constant of 0.50 ± 0.05 d^{-1} (Foga 1991). This process, however, requires the presence of another organic material to donate hydrogen atoms, as well as being affected by the degree of chlorination of the pollutant of interest (USEPA 1990a). OCDD is vulnerable to photolysis and is resistant to oxidation, hydrolysis, and biotic and abiotic degradation (Geyer et al. 1993). The metabolic transformation of other PCDD/F congeners have been measured in fish (Gobas and Schrap 1988, Muir et al. 1992c).

Even though the major global release of PCDD/Fs appears to be atmospheric, the final deposition for these compounds is largely bottom sediments (Fiedler et al. 1990). The background levels of PCDD/Fs are higher in aquatic than terrestrial environments. Knowledge about aquatic transfer pathways are therefore important in understanding the mobility of these hazardous compounds (Rappe et al. 1987a).

2.4 Sources and Environmental Concentrations of PCDDs and PCDFs

2.4.0 Bleached Kraft Mills (BKMs)

Currently in Canada there are 47 chlorine bleached kraft pulp and paper mills (BKMs) which generate a wide range of toxic chemicals including PCDD/Fs (Environment Canada 1993). PCDDs/Fs have been detected in the pulp, effluent, and sludge from BKMs (Keuhl et

Table 2. Environmental fate of chlorinated dibenzofurans[1].

Environmental process	Summary statement	Confidence in data
Photolysis	May be the only natural mechanism leading to destruction of dibenzofurans as seen for TCDF (2).	High
Oxidation	No information found.	Low
Hydrolysis	Dibenzofurans are stable to hydrolysis.	High
Volatilization	No information found.	Low
Sorption	Dibenzofurans strongly sorbed by solids, especially with high organic content.	Medium
Bioaccumulation	High potential for bioaccumulation in aquatic food chains (3,4).	High
Biodegradation	Probably nondegradable in sediments. Some congeners rapidly degraded in fish.	Low to High

(1) USEPA (1990a)
(2) Foga (1991)
(3) Merhle et al. (1988)
(4) Muir et al. (1992c)

al. 1987, Merriman 1988, Swanson et al. 1988, Amendola et al. 1989, Clement et al. 1989, Safe 1991) and in many pulp products such as paper, coffee filters, and diapers (Safe 1991). The predominant PCDD/Fs identified in the effluent are 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 1,2,7,8-TCDF (Muller and Halliburton 1990). The results of the "104 Mill Study", USA, identify mean 2,3,7,8-TCDF concentrations as 94.9, 1.033, and 749.6 ng kg^{-1} for pulp, effluent, and sludge, respectively (USEPA 1990a).

The site of dioxin or furan formation has been narrowed to either the chlorination or extraction stages during paper production (USEPA 1990). During these stages, a complex series of reactions takes place, chlorination, oxidation, and demethylation, ultimately producing the chlorinated phenolic precursors for PCDD/Fs (Alasdaire et al. 1990, Fiedler et al. 1990, USEPA 1990b, Safe 1991). Sources for dioxin/furan precursors have been identified in treated wood (i.e., anti-sapstain fungicide pentachlorophenol), natural wood constituents (i.e., lignin), paper additives, and contaminated pipes (Muller and Halliburton 1990, USEPA 1990b). The production of PCDD/F is the result of chlorination of the precursor molecules. The substitution of the strong chlorination agent, molecular chlorine, with other oxidants such as ClO_2 and hydrogen peroxide has reduced environmental inputs of the chlorinated PCDD/Fs (Swanson et al. 1988, Craig et al. 1990).

2.4.1 Other Sources

PCDD/Fs are also generated during the production or combustion of industrial, agricultural, and commercial chemicals such as pentachlorophenol and the herbicide 2,4,5-T (Ree et al. 1988, Christmann et al. 1989a, Fiedler et al. 1990, Rappe et al. 1990). They are detected in the emissions from both municipal and industrial incinerators (Meyerson et al. 1981) as well as in the chemical and biological wastes from municipalities and wood treatment

plants (Weerasinghe et al. 1985, Christmann et al. 1989b).

Other anthropogenic sources include exhaust from automobile combustion of diesel and leaded fuels, cigarette smoke, and forest fires (OME 1985, Buchert and Ballschmiter 1986, Marklund et al. 1990). PCDD and PCDF congeners have also been found associated with other chlorinated hydrocarbons at scrap metal refineries and copper smelters (Cooper 1989).

2.4.2 Environmental Concentrations of TCDF and OCDD

Table 3 presents a selection of representative concentrations of PCDD/Fs in various environmental compartments: air, water, soils, sediments, and biota. The major input sources into river ecosystems include direct effluent discharge, land drainage, and aerial deposition (Muirhead-Thompson 1987).

2.5 Factors Affecting Dietary and Water Accumulation of PCDD/Fs

Dietary transfer has been accepted to be the most important bioaccumulation pathway for chlorinated hydrocarbons with log K_{ow} approaching 6.0 (Thomann 1989, Connell 1990). Bioconcentration and cuticle adsorption are believed to be secondary uptake routes. Bioconcentration is the process by which contaminants enter the organism through the gills and skin. Cuticle adsorption is the direct adherence of the contaminant to the body surface of the organism. Contaminants that enter the organism are subject to processes such as storage in lipids; elimination via gills, urine, or feces; and metabolic biotransformations (Connell 1990, USEPA 1993). The uptake and depuration pathways have been summarized in Figure 2.

There is a tendency for chlorinated hydrocarbons with a molecular weight (MW) of up to 350 to accumulate in the lipids of organisms (Connell 1990). TCDF has a MW of 305.98

Table 3. Mean 2,3,7,8-TCDF and OCDD concentrations measured in environmental samples.

Compartment	Compd.	Mean Concentration	Location	Sources
Soils	TCDF	2.38×10^{2} ng kg^{-1}	MI, USA	USEPA (1989)
	OCDD	9.98×10^{3} ng kg^{-1}	England	Creaser et al. 1990
Sediments	TCDF	4.50×10^{3} ng kg^{-1}	NJ, USA	Bopp et al. 1991
	OCDD	1.41×10^{5} ng kg^{-1}	ON, Canada	McKee et al. 1990
Water	TCDF	2.40×10^{-5} ng L^{-1}	Sweden	Rappe et al. 1989
	OCDD	8.45×10^{-3} ng L^{-1}	ON, Canada	Jobb et al. 1990
River (Sediments)	TCDF	7.26×10^{-1} ng kg^{-1}	BC, Canada	Trudel et al. 1991
	OCDD	4.06 ng kg^{-1}		
Air	TCDF	7.8×10^{-4} ng m^{-3}	NY, USA	Smith et al. 1990
	OCDD	13.5×10^{-2} ng m^{-3}	Hamburg	Rappe et al. 1987
Trichoptera	TCDF	1.30×10^{-2} ng kg^{-1}[1]	NRBS[3], AB	Anderson 1993[4]
	OCDD	ND[2]		

1. dry wt
2. non detectable
3. Northern River Basin Study, Athabasca River, AB
4. A.M. Anderson, 1994, pers. comm.

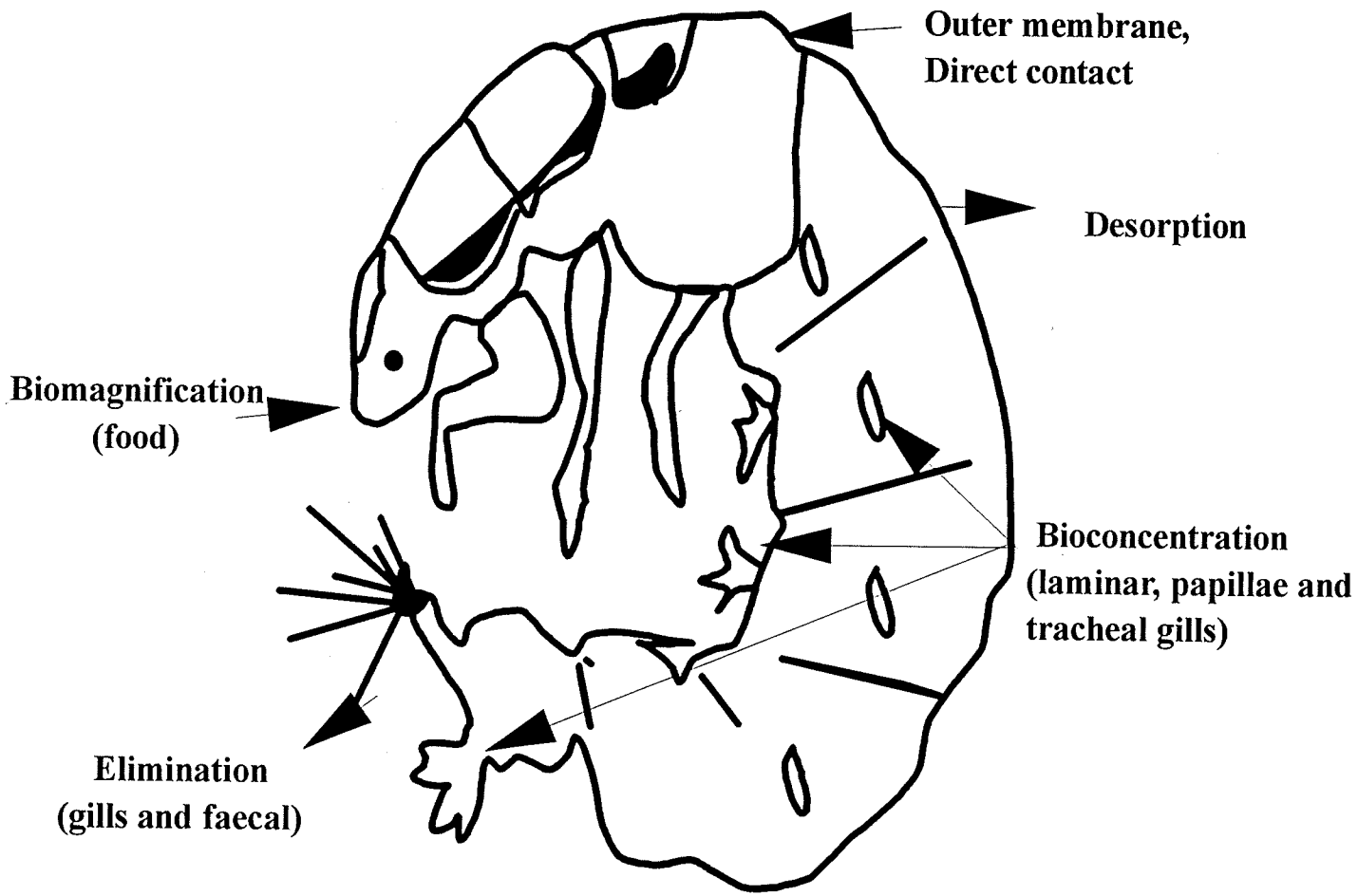


Figure 2. Uptake and elimination pathways for xenobiotics

and has been shown to bioaccumulate in benthic macroinvertebrates (i.e., Chironomidae larvae and Hexagenia nymphs) in lake mesocosms experiments; (Fairchild et al. 1992, Muir et al. 1992c), and to biomagnify up the foodchain (Keuhl et al. 1987). The highest accumulation of OCDD was seen in the lower trophic levels (Broman et al. 1992), unlike the 2,3,7,8-substituted congeners. This reverse trend for bioaccumulation could be explained by OCDD's larger molecular size ()9.5 Å cross-section) and MW (460.76), and limited ability to move through membrane pores. In addition, OCDD is found associated with the high OC and the large surface area of small particles (Broman et al. 1992).

Chlorinated hydrocarbons associated with DOM or particles are considered to be relatively unavailable for uptake by the gills in fish species (Landrum et al. 1984, McCarthy et al. 1985, Servos 1988). It is believed that an increase of contaminant-sorption to DOM and POM reduces BCFs in fish and invertebrates by reducing the amount of PCDD/Fs in true solution and therefore the its bioavailabilty (Muir et al. 1985, and Servos et al. 1989a).

Accurate foodchain modelling requires an understanding of the factors that affect the accumulation and depuration of xenobiotics, such as the age, size, habitat, and mode of life your organism; its feeding rates (FR), growth, and assimilation efficiencies (α); membrane permeability, bioavailability and physicochemical properties of the contaminant; uptake and elimination rates; biotransformation capacity; and the duration of the experiment (Opperhuizen et al. 1988, Thomann 1989, Muir et al. 1990, Broman et al. 1992, Thomann et al. 1992). Modellers also have to take into account the uptake route and the factors that affect them as summarized in Table 4.

Pharmacokinetic models have been used to explain the uptake and clearance of chemical pollutants (Spacie and Hamelink 1982, Thomman et al. 1992). Most models for accumulation and depuration of hydrophobic organics into the lipids of aquatic organisms

Table 4. Factors that affect biomagnification and bioconcentration of PCDD/Fs in aquatic organisms.

Biomagnification	Bioconcentration
Concentration of PCDD/F in food	Concentration in water
Composition of organism and food	Composition of organism and water
Feeding Rate	Water volume passing gills (i.e., ventilation rate)
Absorption efficiency from food	Absorption efficiency from water
Elimination rate	Elimination rate
Metabolic and biotransformation capabilities of intestine and liver	Metabolic or biotransformation capabilities of intestine and liver
Lipid content of food and organism	Lipid content of organism
Molecular size and shape of chemical	Molecular size and shape of chemical

generally fit first-order kinetics or first-order two compartment kinetics (biphasic). Biphasic elimination is characterized by two linear components: an initial fast phase followed by a slower rate of elimination. Occasionally a biphasic or multiphasic approach has been necessary to adequately describe PCDD/F uptake and elimination results (Opperhuizen et al. 1990).

2.6 Toxicity of PCDD/Fs

More toxicity information is available on PCDD/Fs for fish than for aquatic invertebrates (Cooper et al. 1989). Most studies support the hypothesis that mammals and aquatic macroinvertebrates are less sensitive to TCDD than fish (Yockim 1978, Adams et al. 1986, USEPA 1993) For example, the levels of TCDD necessary to generate 50% lethality in carp and rainbow trout range from one to two ng g^{-1}; whereas, in hamsters the LD_{50} for TCDD is 1500 ng g^{-1} (Cook et al. 1990).

2.6.0 Mode of Action

It is believed that the toxicity of halogenated aryl hydrocarbons is initiated by a receptor-mediated mechanism of action in fish and mammals (Safe et al. 1990). Hydrocarbons upon entering a cell form complexes with intracellular cytosolic proteins. The specific binding site on the protein is called the aryl hydrocarbon (Ah) receptor (Safe and Phil 1990). This "newly" activated or transformed complex, upon entering the nucleus of a cell has a direct affect on nuclear receptors. The altered nuclear receptor interacts with precise DNA positions or "dioxin response elements" (DRE) which induce mRNA that code for specific gene expressions and enhance production of "induced" proteins and enzymes. Induced enzymes (i.e., cytochrome P-450 and monooxygenases (MO))

are dominant catalysts for oxidative transformation of xenobiotics (Stegeman et al. 1987). Some other toxic responses measured are body weight loss, immune system impairment, and thymic atrophy (Safe et al. 1990).

The activation of a number of enzyme systems has been documented for aquatic biota. Both TCDF and 2,3,4,7,8-P_5CDF induced an hepatic MO enzyme in rainbow trout which was measured by an increase in ethoxyresorufin *O*-deethylase (EROD) activity (Muir et al. 1990, Muir et al. 1992d). An increase in P450 protein and EROD activity was measured in livers of carp and sculpin ingesting particle-bound PCDFs (Hahn 1989, Van der Weiden 1989). Cytochrome "P450" was extracted from Daphnia (Fiorucci et al. 1988) indicating a potential for biotransformation capacity of PCDD/Fs in aquatic invertebrates. There are over 100 specific P450 enzymes from approximately 20 families and more research is needed to identify the P450 enzyme associated with invertebrates.

2.6.1 Studies on TCDD and Fish.

There is limited information on the toxicity of TCDF. Toxic effects can however be inferred from studies conducted on TCDD (USEPA 1990b). Available toxicological information on TCDD/F for fish (Table 5) illustrates that exposure to pg g^{-1} quantities has species specific and multiple effects. These effects include enzyme induction, carcinogenicity, reproductive toxicity, behavioral (e.g., swim-ups), immunotoxicity, developmental effects (e.g., wasting syndrome), hepatoxicity (e.g., liver lesions), and teratogenicity (Cooper 1989, USEPA 1993).

Muir et al. (1992d) showed an increase in mortality and EROD activity for rainbow trout fed TCDF concentrations ranging from 0.36 to 42.88 ng g^{-1}. A decrease in growth was also seen at TCDF food concentrations of 42.8 ng g^{-1}.

Table 5. Toxic effects of 2,3,7,8-TCDD and 2,3,7,8-TCDF observed in fish species

Organism	d	Compd.	Conc. ng L^{-1}	Adverse Effect	Source
Rainbow trout	56	TCDD	0.176 *	survival	(1)
Rainbow trout	56	TCDD	0.038 *	growth	(1)
Rainbow trout	56	TCDF	3.93 * (1.79 **)	survival	(1)
Rainbow trout	56	TCDF	0.9 * (0.41 **)	growth	(1)
Rainbow trout	28	TCDF	1.79 **	mortality	(1)
Rainbow trout	28	TCDF	0.41 **	growth	(1)
Rainbow trout eggs	3	TCDD	10	survival	(2)
Rainbow trout eggs	3	TCDD	1	hatching, teratologic	(2)
Rainbow trout eggs	2	TCDD	83-500	survival, swim behavior	(3)
Rainbow trout eggs	2	TCDD	21	mortality	(3)
Lake trout eggs/fry	2	TCDD	10	mortality	(4)
Juvenile Coho Salmon	60	TCDD	5.6 *	mortality, growth	(5)
Fathead minnow	2-3	TCDD	7.1 0.71 **	mortality	(6)
Fathead minnow	3	TCDD	63	mortality	(6)
Guppy	1	TCDD	0.1	fin necrosis	(7)
Mosquito fish	15	TCDD	2.8	100 % mortality	(8)
Northern pike	3	TCDD	0.1	survival body lengths	(9)
Northern pike	3	TCDD	1.0 - 10	survival	(9)
Northern pike	3	TCDD	10	histopathological	(9)
Japanese medaka	11	TCDD	0.4-13.2	lesions	(10)
Japanese medaka	11	TCDD	14	life-threatening	(10)

* Lowest observable effects level (LOEL)
** No observable effect concentration (NOEC)

Sources:

1. Mehrle et al. (1988)
2. Helder et al. (1981)
3. Walker et al. (1991)
4. Spitsburgen et al. (1991)
5. Miller et al. (1973)
6. Adams et al. (1986)
7. Miller et al. (1979)
8. Yockim et al. (1978)
9. Helder et al. (1980)
10. Wisk and Cooper (1990)

The most sensitive life stage for fish exposed to chlorinated hydrocarbons appears to be their earliest stage (fry), followed by their oldest stage (USEPA 1990). Increases in mortality normally occurs in fish fry when body burdens exceed 0.05 ng g^{-1} (species specific) and ranges from 1 to 1.5 ng g^{-1} in older fish (USEPA 1993).

2.6.2 Tissue Distribution

The 2,3,7,8-substituted PCDD/Fs have been shown to be selectively accumulated in fish species in comparison to other tetrachloro to heptachloro-CDD/Fs (Keuhl et al. 1986, Safe 1991, Walker et al. 1991). Greater than 90% of accumulated 2,3,7,8-TCDD in rainbow trout was found in the visceral fat, carcass, skin, and pyloric caeca. While the remaining amount is distributed to the skeletal muscle, gills, gastrointestinal tract, liver, kidney, heart and spleen (Kleeman et al. 1986).

2.6.3 Food chain transfer of PCDD/Fs downstream from BKMs

Characteristic isometric patterns, i.e., high tetra-and-penta CDD/Fs (especially 2,3,7,8-congeners) have been detected in environmental samples (crabs, sediments) close to kraft pulp and paper plants (Swanson et al. 1988). A parallel study on the Strait of Georgia, B.C. (Canada) found similar results (Norstrom et al. presented at SETAC 1991, Abstract No. 133). High levels of the 2,3,7,8-congeners in crustaceans in the vicinity of some BKM required the closure of some Canadian crustacean fisheries (Muller and Hallibuton 1990).

Dietary transfer may help to explain the significant levels of TCDD and TCDF detected in insect-eating fish sampled downstream from BKMs (Birkholz et al. 1991, Presented at Dioxin '92, Ecotoxicology Session 5). One study conducted on the Fraser River in the winter revealed that chinook salmon (Oncorhynchus tshawytscha) eating a diet of

predominantly insects downstream from BKMs contained body burdens that approached 68 ng kg^{-1} of TCDD and 370 ng kg^{-1} of TCDF (Rodgers et al. 1989). In another study levels of TCDD and TCDF in mountain whitefish (Prosopium willamsoni) exceeded those in suckers (Catostomus sp.) by two to five fold at five out of six western Canadian kraft mills investigated (Muir et al. 1992c). Evidence for a trophic transfer is supported by the fact that mountain whitefish are predominantly insectivorous (Davies et al. 1976, Thompson et al. 1976) and suckers feed mainly on detritus (Marshall 1965, McPhail et al. 1970). A more recent study of the gut contents of mountain whitefish (n=35) consisted of 71.5% trichoptera and contained TCDD (TEQs) body burdens 15 x greater than longnosed suckers (n=32) consuming a diet of 43.5% sediment and 39.3% chironomids from fish sampled downstream from a BKM (Swanson et al. 1992).

2.6.4 Studies on TCDD and Aquatic Invertebrates.

Aquatic macroinvertebrates may be less sensitive to TCDD than fish although experimental data are limited. Yockim et al. (1978) found adverse effects on reproduction, and an increase in mortality, hemorrhaging, and fin necrosis for catfish (Ictalurus punctuts) and mosquito fish (Gambusia affinis) exposed to TCDD sediment concentration of 100 μg g^{-1} and no adverse effects on reproduction, feeding, or growth for Daphina magna, algae (Oedogonlum cardiacum) and snails (Physia sp.). Exposure to water-borne TCDD concentrations of 3.1 ng L^{-1} for 32 d also did not influence the growth or reproduction of water fleas, Daphnia magna or snails (Helosoma sp.). Reduced reproductive success was reported for snails (Physa sp.) and for oligochaete worms (Paranais sp.) exposed to aqueous TCDD concentrations at 200 ng L^{-1} for 36 and 55 d. At this concentration no impairment in the macroinvertebrate pupation process was seen for the mosquito larvae (Aedes aegypti) (Miller et al. 1973). These

concentrations exceed C_s of TCDD. Reported exposure concentrations probably reflects TCDD associated with DOC.

2.6.5 Toxic Equivalency Factors (TEFs)

TEFs are employed in risk assessment to characterize the toxicity of an assemblage of PCDD/Fs in terms of a single TCDD-equivalent value or TEQ. TEF values have been assigned "based on the limited data base of in vivo and in vitro toxicity testing" (USEPA 1990). The TEF for OCDD (0.001) is considered to be relatively low when compared to the extremely toxic TCDF (0.1) and reference TCDD (1) congener (Cooper 1989, USEPA 1992, Environment Canada 1993). Although 2,3,7,8-TCDD is evaluated by the EPA to be the most potent chemical carcinogen, 2,3,7,8-TCDF warrants concern because it is often found at ten-fold higher environmental concentrations (Travis et al. 1989).

TCDD and TCDF can account for greater than 90% of the dioxin toxic equivalents TEQ (Swanson et al. 1988, USEPA 1989, USEPA 1990, Safe 1991). OCDD can account for up to 40% of the total TEQs in sewage sludge (Darskus and Schlesing 1989) and up to 70% of the total PCDD load entering the environment from all sources (NRCC 1981). OCDD is the most prevalent PCDD congener found in "pentachlorophenol (PCP), sludge, sediment fly ash, fresh water and marine biota (e.g., mussels, fish) and in human blood, milk and adipose tissue" (Geyer et al. 1992).

2.6.6 Environmental Quality Guidelines for PCDD/Fs

In Canada, Environmental Quality Guidelines for TCDD have been drafted to protect water uses, sediment quality, biotic tissue residues for humans, wildlife, as well as aquatic life (Environment Canada 1993). The draft Canadian Environmental Quality Guideline protection

limits for aqueous and sediment bound TCDD are set at 3.8 pg L^{-1} and 79 ng kg^{-1} respectively. These values were established on the basis of available toxicity and persistence data, bioaccumulation potentials, and analytical detection technology. Protection limits of 0.6 ng kg^{-1} and 4.7 ng kg^{-1}) were set for wildlife and aquatic life species, respectively. Under the Canadian Environment Protection Act, the permissible human consumption level for TCDD (TEQs) was set at 10 pg kg^{-1} (body wt) d^{-1}.

2.7 Literature Review Summary

PCDD/Fs are environmentally persistent compounds found in aquatic ecosystems and contain congeners known to be the most potent chemical carcinogens in the world. Low quantities in fish species are known to have toxic effects ranging from immunotoxicity, neurotoxicity, carcinogenicity, and endocrine, developmental, reproductive and behavioral toxicity. Food chain transfer is believed to be the most important pathway of transfer for these compounds. Few studies have been conducted on filter-feeders aquatic invertebrates (important fish food). As a result these are gaps in our understanding and ability to model food chain transfer of PCDD/Fs. The aim of this study was to determine the uptake and depuration characteristics of particle-bound PCDD/Fs for an aquatic filter-feeder species, Hydropsyche bidens (Ross) in miniature lab streams.

3 MATERIALS AND METHODS

3.0 Environmental Setting

Final instar Hydropsyche bidens (Ross) were collected on June 30 1991, July 27 1991, June 15 1992, July 8 1992, and August 15 1992 from the Assiniboine River, MB. The Assiniboine River is an 8th order stream (two 7th order streams merging) on a 1:20,000 scale map (Neil Harden, pers. comm., Manitoba Water Resource Branch, Winnipeg, MB). The sampling area was located at Beaudry Provincial Park 21 km west of the fork of the Red and Assiniboine Rivers (Figure 3). The field site, a small riffle area, is located immediately downstream from the Beaudry Park canoe launch. Beaudry Park is situated in the Manitoba Red River Plain Lowlands. A population of deciduous trees and shrubs dominate the area: trembling aspen, cottonwood, American elm, basswood, green ash, Manitoba maple, bur oak, hazelnut, hawthorn, red osier dogwood, and willow (Dorber 1978). The vegetation associated with the river shoreline consists of arrowhead, common burdock, swamp smartweed, water plantain, horsetail, wild mint, ostrich-fern, poison ivy, moonseed, riverbank grape, wood nettle, wild sarsaparilla, wild black currant, rose, and snowberry (Dorber 1978).

The hydrodynamics of Assiniboine River has been reviewed by Dorber (1978). The Assiniboine is a tributary of the Red River with a drainage area of 162,652 km^2. The width of the river ranges from 60 to 75 m, bordered by a six to eight meter bank. The riffle sampling zone, approximately 30 m x 3 m, contained glacially deposited rocks and was surrounded by deeper sandy-to-silty clay-lined pools. These glacially deposited rocks provided an essential substrate for benthic invertebrates to remain stationary in the fast current.

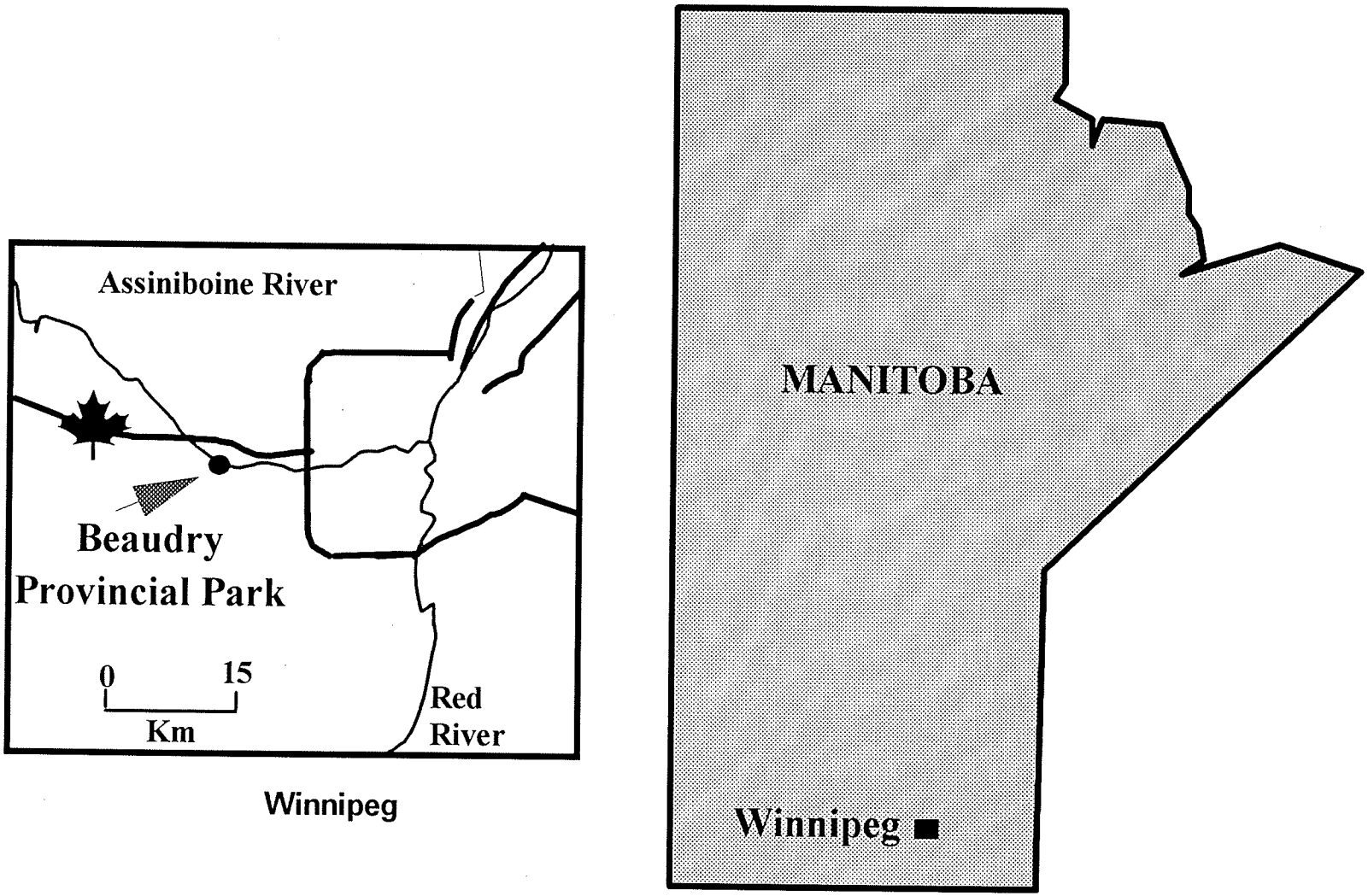


Figure 3. Location of Beaudry Provincial Park and sampling site

3.1 Sampling

H. bidens (Ross) were found abundantly in riffle areas attached to "basketball-size" boulders (Wentworth Classification phi scale rating of -8 (Hynes 1970)) in 1 to 2 m of water. A Type C "10.150" No. 48520 current meter (A. Ott Kempten, Germany) was used to determine current velocities at the sampling site. The measurements were taken approximately 3 cm above the sampling rocks. The white-crowned caps on the heads of H. bidens larvae allowed quick differentiation from similar hydropsychid species, without magnification, producing 25 final instar H. bidens (Ross) h^{-1} $person^{-1}$. The hydropsychids were hand-picked with fine tweezers from their retreats and placed in permeable sampling bags filled with Assiniboine River water. In order to reduce injury, water logged sticks were introduced for substrate attachment. The sampling bags were placed in coolers of ice-water and transported to the laboratory. H. bidens (Ross) were detached from the water-logged sticks, placed on a paper towel for 3 s, and weighed on a Sartorious 1207 MPZ electrobalance (0.0001 g). The weighing took place prior to introducing them into a lotic microenvironment provided by a compact laboratory flume, as described below. A minimum weight of 10 mg was selected for final instars. No attempt was made to sex the larvae.

3.2 Labstreams

A simplified version of the compact flume, described by Lacousiérè et al. (1990), was constructed for this experiment (Figure 4). This micro-hydrodynamic system allowed the recirculation of 9.1 L of dechlorinated tap water. Six miniature streams were housed in an exposure room (Coldsteam Fleming Pedlar Ltd., Winnipeg, MB), with ambient temperatures of 14 °C and a 14 h light : 8 h dark photoperiod. For each experiment, two test streams and one control stream were run simultaneously.

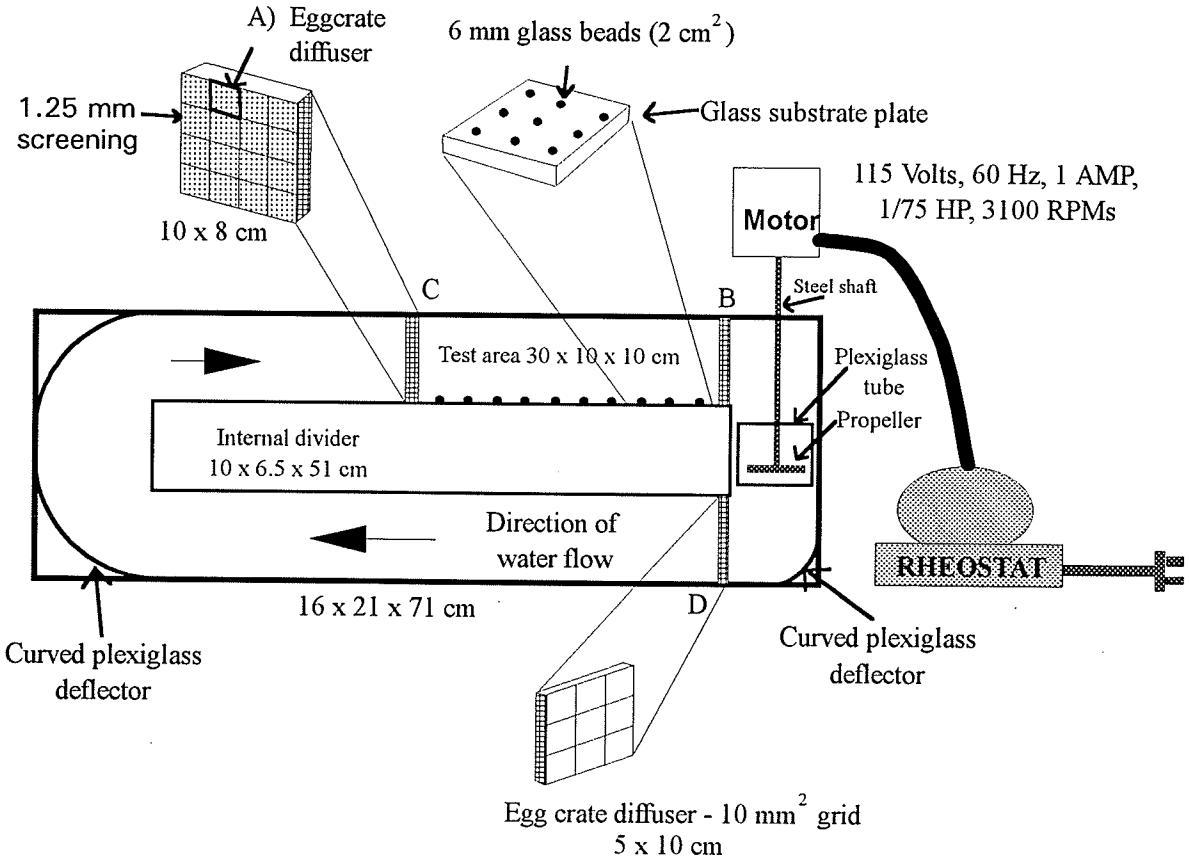


Figure 4. Side view of minature laboratory flume

The compact recirculating stream was constructed principally of 5.8 mm clear, double diamond glass and silicone aquarium glue. The portable streams were 10 x 21 x 71 cm. An internal divider measuring 10 x 6.5 x 51 cm was glued in the center, 5 cm from the bottom of the tank.

The driving module consisted of an external power source: a Type 21 motor, (Fasco Industrial Inc., Rochester, NY, USA): 115 volts, 60 Hz, 1 amp, 1/75 HP, and 3100 RPM. The motors were oiled every 1000 h with "3 in 1®" oil. The motor was positioned on a wooden stand made from 10 x 20 cm lumber. The speed of the motor was regulated by Powerstat Type 3PN116 Rheostat (Superior Electric Co., Bristol, CO, USA). The motor was coupled with a stainless steel shaft (5.0 mm external diameter) with a 7.5 cm diameter plastic model airplane propeller to generate an internal current. The propeller was attached by tightening a brass clip. The rotating propeller created a continuous current by forcing water down away from the propeller.

To minimize turbulence and to ensure uniform flow, diffusers and curved plexiglas deflector plates were placed into the system. To create the test chamber, 1.25 mm^2 screening was sewn with fishing line onto 10 cm^2 eggcrates (a grid material used commercially as diffusers for fluorescent lighting (A)). Weather stripping (4 x 10 mm) was placed on the exterior eggcrate edges to create a tight fit with the aquarium. One screened eggcrate (B) was placed at the entrance of the return chute, furthest downstream from the current source. The second eggcrate (C) was placed 30 cm upstream from this point. A third egg crate (D) was placed at the entrance of the downstream channel to reduce turbulence.

The airplane propeller was housed in a 50 mm length, 9.9 mm id plexiglas tube. The return module consisted of a 19.4 cm id plexiglas tube cut in half with sanded edges and was placed symmetrically at the far end of the downstream channel. An outlet curved deflector

plate was made out of plexiglas.

Additional substrate was made by attaching 6 mm glass beads every 2.0 cm^2, totalling 4 rows by 14 on a thin 30 x 10 x 0.2 cm glass sheet. The artificial substrate was placed on the bottom of the test area.

3.3 Description of Treatments

A series of experiments were design to assess the availability of particle-sorbed TCDF and OCDD to caddisfly larvae and the direct effects of velocity, food quality, and contaminant concentration. In addition to particle-feeding experiments, direct uptake from water, cuticle adsorption, and uptake by an earlier instar were conducted. The exposure parameters for nine separate experiments conducted are outlined in Table 6. For each experiment there were two replicate treated tanks and one control.

3.4 Acclimatization

Each stream was calibrated with the Ott current meter to the experimental velocities of 5, 16 and 24 cm s^{-1}. Fifty preweighed (Mettler AE100, Greifensee, Switzerland) hydropyschids were introduced into each system and were exposed to current speeds of 5 cm s^{-1}. The initial slow current speed allowed the hydropsychids to disperse away from each other, find suitable substrate for attachment, and orient themselves relative to the current. After 3 h, the rheostat settings were set for the experimental velocities; either 16 cm s^{-1} or 24 cm s^{-1}. H. bidens were acclimatized for 3 d prior to the start of the experiment.

3.5 Chemicals

Both 2,3,7,8-tetrachloro[4,6-^{3}H]dibenzofuran (^{3}H-2,3,7,8-TCDF) and uniformly labelled

Table 6. Description of laboratory experiments conducted on H. bidens' larvae and pupae

Exp. #	Phase	Life Stage	#org. /tank	Duration d	Food	Food Concentration TCDF ng g^{-1}	OCDD ng g^{-1}	Velocity cm s^{-1}
1	uptake	final instar	50	30	Nutra Fin	60	--	16
2	uptake	final instar	50	30	Nutra Fin	60	--	24
3	elimination[1]	final instar	50	18	Nutra Fin	60	--	16
4	elimination	final instar	50	18,52	Nutra Fin	60	--	24
5	uptake	final instar	50	30	Algae	72	5,600	16
6a	elimination	final instar	50	18	Algae	72	5,600	16
b	uptake	pupae[2]	10	10				
c	uptake	pupae[3]	3	0.75				
7	uptake	final instar	50	30	Algae	200	26,100	16
8	elimination	final instar	50	30	Algae	200	26,100	16
9	uptake	penultimate instar	40	37	Algae	200	26,100	16

1. All clearance phases of the elimination experiments were initiated after 10 d contaminated food exposures.
2. non-feeding pupae ("freely dissolved" uptake routes only)
3. dead pupae (cuticle adsorption)

1,2,3,4,6,7,8,9-octachloro[U-^{14}C]dibenzo-p-dioxin (OCDD) were obtained from Chemsyn Science Laboratories (Lenexa, Kansas, USA). Purification of TCDF and OCDD was carried out by reverse-phase HPLC (Nova Pak C_{18}, 15 cm x 3.9 mm id column, (Waters Associates, Milford, MA, USA) using an isocratic solvent system of methanol:water (9:1) at 1.5 mL min^{-1}. Recovery for OCDD exceeded ≥ 98.0% and ≥ 97.7% for TCDF. The specific activities of TCDF and OCDD were 124,524 dpm ng^{-1} (7.47 MBq ng^{-1}) and 569 dpm ng^{-1} (34,140 Bq ng^{-1}) respectively.

3.6 Food Sources: Nutra Fin and Algae

3.6.0 Nutra Fin.

Nutra Fin (Hagen Distributors, Taiwan) is a staple fish food with guaranteed 46% crude protein, 5% minimum crude fat, 2 % maximum crude fibre, and a maximum moisture content of 8%. Nutra Fin is formulated from fish meal, plankton, shrimp meal, soy flour, aquatic plants, kelp, oatflower, yeast, codfish meal, fish liver, and chlorophyll.

On June 27 1991, 1.009 g of Nutra Fin was homogenized into micrometer-sized particles with a Polytron (Kinematic, Littau-Luzern, Switzerland) in 10 mL of hexane in a Pyrex screw top culture tube. Thirty-six microliters of the stock solution ^{3}H-2,3,7,8-TCDF was introduced into the slurry with a syringe. The mixture was further mixed with the Polytron for 5 min. The excess hexane was evaporated with N_2 on a "N-EVAP" Evaporator (Organomation Association Inc., Shrewsbury, MA, USA) resulting in a fine powdery texture Nutra Fin.

For daily feeding, 10 mg of treated Nutra Fin or control food was weighed and homogenized in a 100 mL graduated cylinder with 100 mL of water with the polytron. The polytron was washed three times with acetone, and once with distilled water between runs.

A 3 mL subsample of the 100 mL food source was pipetted into scintillation vials.

AtomLight High Sample Capacity Scintillation Solution (12.0 mL) (Biotechnology Systems Research Products, Boston, MA, USA) was added prior to being counted on a LS 7500 Liquid Scintillation Counter (LSC)(Beckman Instruments Inc., Irving, CA, USA) with a quench curve set for 10 min counts. The remaining 97 mL food source was added to the test streams. Combustion of the food on a Packard 306 Oxidizer (Canberra-Packard Company, IL, USA) and counted by LSC indicated 60 ng of ^{3}H-TCDF g^{-1} of Nutra Fin (dry wt).

3.6.1 Algae. chlorella sp., obtained from L. Henzel, (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB, (June 16, 1991) was reared in bulk in a Coldstream coldroom. Large quantities of stock solution were prepared by combining the following nutrient solutions with Milli-Q Pore water (filtered 0.45 μm); 40 $\mu M \cdot L^{-1}$ $NaNO_3$, 1 $\mu M \cdot L^{-1}$ KH_2PO_4, 16 $\mu M \cdot L^{-1}$ KCL, 225 $\mu M \cdot L^{-1}$ $MgSO_4$ $7H_2O$, 500 $\mu M \cdot L^{-1}$ $CaCl_2$ $2H_2O$, 225 $\mu M \cdot L^{-1}$ $NaHCO_3$, 400 $\mu M \cdot L^{-1}$ Na_2SiO_3 $9H_2O$, 10 $\mu M \cdot L^{-1}$ Na_2SeO_3, and trace elements and vitamin mix and autoclaving before use (Hendzel 1991, pers. comm., Freshwater Institute, Winnipeg, MB).

For daily algal feeding, approximately 500 mL of algae-stock solution was placed in a 600 mL beaker. A magnetic stirring bar was added and the solution was stirred at moderate speeds with a PC-351 stirrer (Corning Glass Works, New York, USA). A measured volume of the solution was filtered on to a desiccated, preweighed GF/F Glass Microfibre filter (Whatman International Ltd., Maidstone, England) with a Pyrex Millipore Glassware apparatus. The wet algal paper was dried in a vacuum desiccator (≈ 4 h). To prevent algae growth during this time, the algae-stock solution was stored in the dark (by placing tin foil around the beaker) at 14 °C in the Exposure Room. One subsample was taken after 4 h to verify that no algae growth occurred.

The filter paper was reweighed when dried, and the weight of algae (dry wt) was calculated after correcting for weight changes in the paper due to filtration. The volume needed to introduce 10 mg algae (dry wt) $tank^{-1}$ was determined. This volume was placed into control and test treatment beakers. The solutions were stirred at moderate speeds (≈ 5 min) as described above and 0.25 mL of a TCDF and OCDD stock solution (described below) was slowly injected into the test treatment beakers.

Enough dual-labelled stock solution was prepared to add 0.25 mL subsamples daily to 10 mg of algae (dry wt) d^{-1} $tank^{-1}$ for the duration of the uptake and elimination Experiments # 7, 8, and 9. On April 28, 1992, 38.7 µL of ^{3}H-2,3,7,8-TCDF and 5.00 mL of ^{14}C-OCDD was transferred into a 25 mL volumetric flask coupled with a polyethylene stopper. Acetone was added to make up the solution to 25 mL. The mixing of 0.25 mL of the stock solution with the calculated volume of algae to produce 10 mg (dry wt) produced final dual-labelled contaminant concentrations of 200 ng of TCDF g^{-1} of algae (dry wt) and 26,100 ng of OCDD g^{-1} of algae (dry wt). These concentrations were determined by oxidizing dried treated algae.

On May 3, 1993, a second batch of stock solution was prepared to conduct Experiments # 5 and 6 by combining 241 µl of ^{3}H-2,3,7,8-TCDF and 1.27 mL of ^{14}C-OCDD as outlined above. Final concentrations of the dual-labelled compounds yielded 72 ng TCDF g^{-1} (algae dry wt) and 5,600 ng OCDD g^{-1} (algae dry wt). These concentrations were determined by counting 1.00 mL algae food subsamples with Atomlight fluor on the LSC.

3.6.2 Particle Sizes. The average size of the particles after homogenization was determined by measuring the widest cross-section of 600 particles using a Research Microscope (Reichert, Austria), with a method outlined by Hara et al. (1993). Microscope slide images were projected onto a Summagraphic Bit Pad Two Data Tablet® and distances

were measured with a "puck-like" cursor. Calibrations were made using the projected image of a micrometer slide (1.0 mm ruled to 0.01 mm). All measurements were relayed to a IBM computer and stored on SigmaScan software (Jandel Scientific, Corte Madera, CA, USA).

3.7 Sampling

3.7.0 Uptake Phase. Three hydropsychid larvae were collected from each experimental chamber at each sampling time. Individual hydropsychid larvae were placed in a clean scintillation vial with a thin film of dechlorinated tap water and placed in a freezer. Samples were collected on days 0, 1, 2, 5, 8, 14 or 16, 24, and 30 for the uptake phase of Experiment #s: 1, 2, 5, and 7.

3.7.1 Depuration Phase. H. bidens were fed contaminated food for 10 d as outlined above and then removed from exposure tanks with tweezers and placed into clean water. The water velocity was set at 5 cm s^{-1} for 3 h (to allow dispersal) prior to recalibrating to test velocities, either 16 or 24 cm s^{-1}. Daily, 10 mg of control (untreated) food was added to each system. Three organisms were sampled at days 0, 1, 2 or 3, 5, 10, and 18 for the depuration experiments (Experiment #s: 3, 4, 6, and 8).

3.7.2 Penultimate Instars. Two samples, were collected on days 0, 1, 2, 5, 10, 15, 32, and 37 for Experiment # 9. Each sample has a combined minimum wet wt of 10 mg.

3.7.3 Bioconcentration. A total of 15 quiescent pupae (non-feeding) were collected from the two experimental tanks (Experiment # 6b) on days 2, 5, 7, 8, and 10.

3.7.4 Cuticle Adsorption. The six dead final instar larvae (previously by freezing) were sampled from the two treated tanks in Experiment # 6 after an 18 h exposure.

3.7.5 Larvae Analysis. For analysis, thawed hydropsychids were blotted dry for 3 s on a paper towel prior to being weighed. Each larvae was oxidized using the oxidizer. Samples were assayed by using the LSC with 10 min counts.

3.8 Total Water Concentration of TCDF and OCDD

Whole water concentrations were determined by pipetting 5.00 mL of water daily from each tank prior to and 5 min after introducing the food. Each sample was placed into scintillation vials along with 12.0 mL of AtomLight. Radioactive counts (dpm) were calculated on the LSC and converted to mg L^{-1} concentrations by dividing total DPMs by specific activity (dpm ng^{-1}) to define the exposure milieu.

3.9 SPM, DOM, and "Freely Dissolved" (f_d) Concentrations

Concentrations of the TCDF and OCDD f_d and associated with POM or DOM using procedures outlined by Landrum et al. (1984) and Servos (1988). On day 30 of the experiment, 20.0 mL sample of water was pipetted from each of the contaminated systems into 25 mL Pyrex heavy duty round bottom centrifuge tubes with screw caps. To determine total water concentration a 4.00 mL subsample was set aside. The remaining 16 mL were centrifuged at 5000 g and 14 °C in a Superspeed RC2-B Automatic Refrigerated Centrifuge (Ivan Sorval Inc., Newtown, CT, USA) for 30 min to remove suspended solids. A 4.00 mL subsample was immediately taken after the 30 min centrifugation. A 4.00 mL sample of supernatant was passed through a reverse-phase C_{18} Sep-Pak Cartridge (Waters-Associates -

Millipore®, Milford, MA, USA) to obtain the TCDF or OCDD associated with DOM. Freely dissolved TCDF and OCDD were than eluted from the Sep-Pak into scintillation vials using 8.00 mL of methanol. The methanol was evaporated to dryness under a fume hood. Scintillation cocktail fluid was added to each sample vial prior to being assayed by the LSC.

3.10 Toluene Extractable Lipids; H. bidens, Nutra Fin, and algae

Freeze dried and pre-weighed H. bidens (n=32), Nutra Fin (n=4), and algae (n=8) samples were homogenized with the Polytron in 2.00 mL of toluene. Particles were allowed to settle for 12 h before pipetting a known quantity of the supernatant into a pre-weighed aluminum dish (Fisher Scientific, Winnipeg, MB). The dish was placed in a fume hood overnight and the excess solvent was evaporated. The lipid residues were weighed, and the percent of lipid was determined.

3.11 Organic Carbon Content (OC): Nutra Fin and Algae Particles

Samples were prepared for OC analysis by placing pre-weighed Nutra Fin and algae particles in 250 mL nalgene bottles. The OC of Nutra Fin and algae particles were determined in an Chemistry labatory (Freshwater Institute, Winnipeg, MB) using a standard technique (Stainton et al. 1977). The particulate matter is first captured on a pre-ignited glass fiber filter paper and combusted in oxygen helium atmosphere at 975 °C. The carbon is oxidized to CO_2 which is then quantified by an Elemental Analyzer (Leeman Labs Inc., Lowell, MA, USA).

3.12 Seine and Mesh Measurements

Nets, appearing like spider webs, were teased away from individual hydropyschids and mounted onto pre-cleaned microscope slides. Measurements of mesh dimensions (width x

length) and the thickness of individual strands were made using an microscope projection system developed by Hara et al. (1993). Black and white photographs were taken of the nets with a camera mounted on a microscope (Wild Leitz Canada Ltd., Ottawa, ON).

3.13 Data Analysis

3.13.0 Growth Rates

Correction for growth is essential in determining the equilibrium/steady state concentrations of contaminants in feeding experiments. If the body burden stayed the same and the growth doubled, this would show up as a "false" depuration if expressed as concentration (e.g., ng g^{-1} (wet wt)). Growth rates were determined using an exponential model (Muir et al. 1992) described by Equation (2).

$$\mathbf{\ln wt = \ln wt_o - g_c t} \qquad (2)$$

wt = final weight of an organism

wt_o = initial weight of an organism

g_c = growth rate constant (d^{-1})

t = time

3.13.1 Bioaccumulation Parameters

Bioaccumulation is the net transfer of a contaminant from both food and water sources into an organism. Biomagnification is the direct uptake of a chemical by an organism via food. Bioconcentration is the direct uptake via the respiration pathways (e.g.,

gills) from water into an organism (Geyer et al. 1993). The Biomagnification and Bioconcentration Factors are demonstrated in Equation 3 and 4 respectively. For river filter-feeders, bioavailability generally refers to the extent that contaminants bound to particulate matter are available for consumption by aquatic organisms.

$$\mathbf{BMF = C_B \div C_F} \qquad (3)$$

BMF = Biomagnification Factor

C_B = concentration in organism on a lipid normalized basis (ng g^{-1})

C_F = concentration in food on a lipid and OC normalized basis (ng g^{-1})

$$\mathbf{BCF = C_B \div C_w} \qquad (4)$$

BCF = Bioconcentration Factor

C_W = concentration in water (ng mL^{-1})

3.13.2 Pharmacokinetics

The first-order kinetics approach can utilize a single compartment (e.g., uptake from water) to quantify the contaminant accumulation in an organism (Figure 5), or it can utilize a two compartment model which incorporates both food and aqueous uptake routes (Figure 6) (Bruggeman et al. 1981, Connell 1990). First-order uptake and clearance are illustrated in Figure 7 (Connell 1990).

Equation 5 and 6 are used to describe uptake from both food and water uptake routes in a two compartment model (Bruggeman et al. 1981).

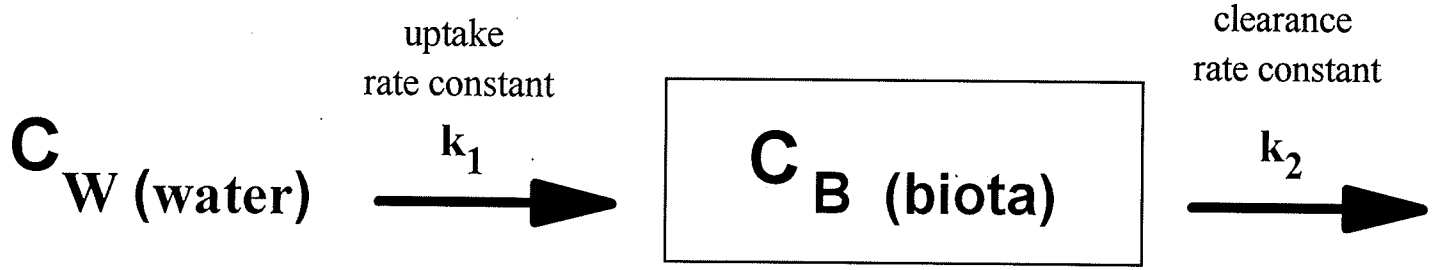


Figure 5. Single-compartment model for the uptake and clearance of a lipophilic chemical by an organism (Connell 1990)

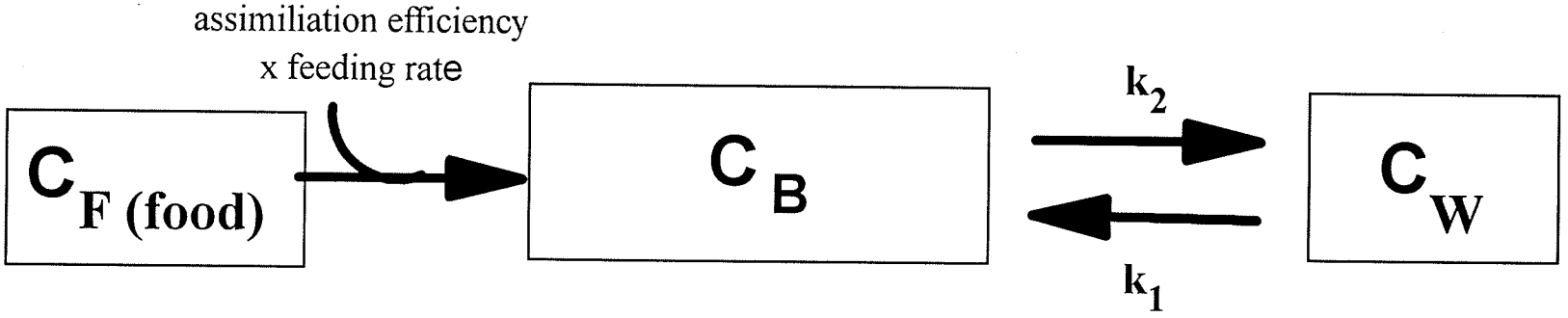


Figure 6. Two-compartment model for the uptake and clearance by an organism (Bruggeman et al. 1981)

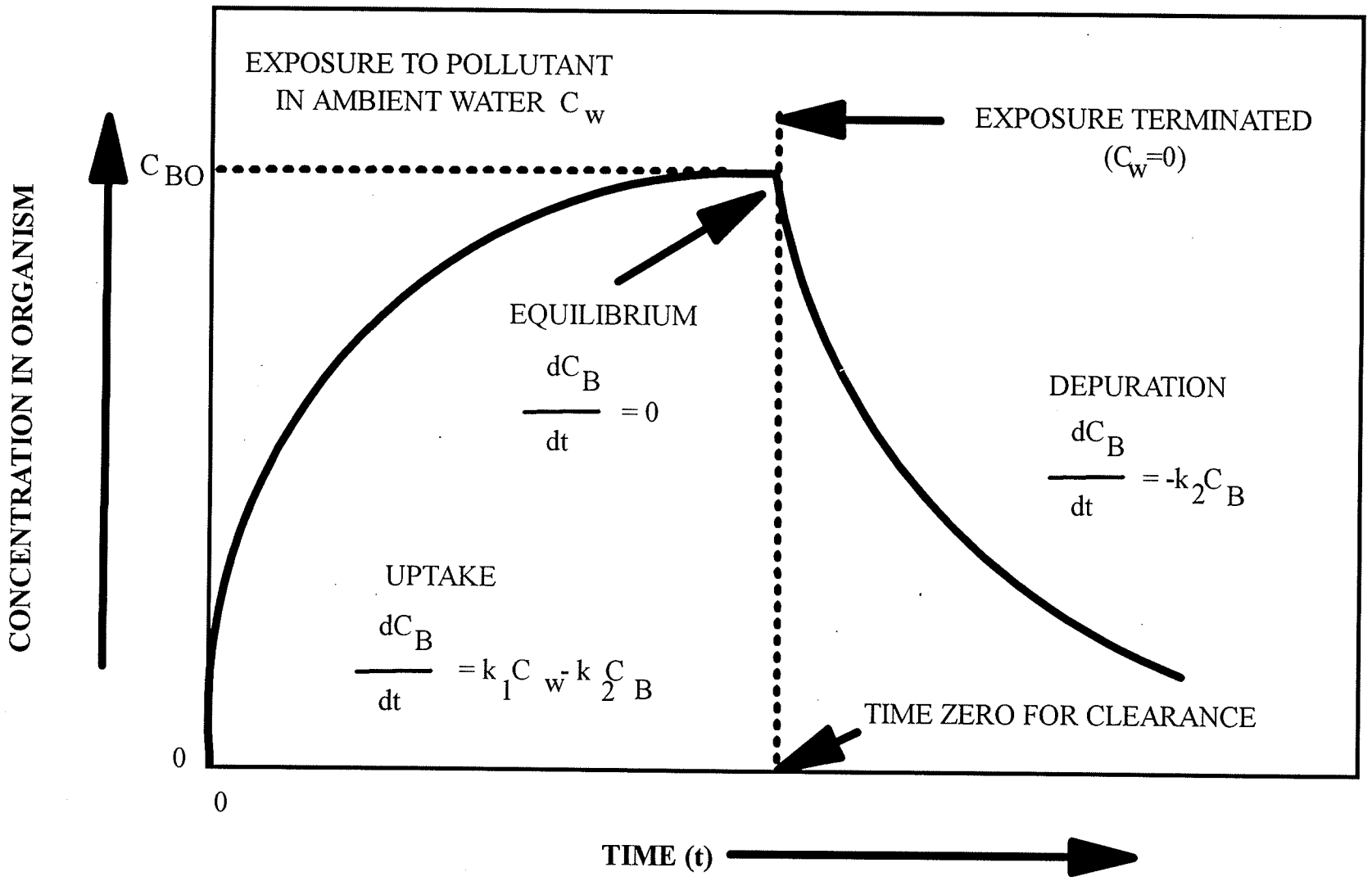


Figure 7. Uptake and clearance patterns of a lipophilic pollutant over time with an organism as represented by first-order kinetics (Connell 1990)

$$dC_B/dt = k_1C_W - k_2C_B \qquad (5)$$

C_W = concentration in water ($\mu g\ mL^{-1}$)

C_B = concentration in organism ($\mu g\ g^{-1}$)

t = time (h)

k_1 = uptake coefficient ($\mu g\ g^{-1}\ h^{-1}$)

k_2 = elimination rate constant (h^{-1})

$$dC_B/dt = \alpha\ FR\ C_F + k_1C_B - k_2C_W \qquad (6)$$

α = absorption efficiency for ingested chemical

FR = feeding rate (food wt x organism wt (g^{-1}) x time (h^{-1}))

During the elimination phase in clean water for the single compartment model, k_1C_w = 0 and therefore the concentration in the organism can be described as in Equation 7.

$$dC_B/dt = k_2C_B \qquad (7)$$

Integration of Equation 7 gives Equation 8.

$$\ln C_B = \ln C_o - k_2t \qquad (8)$$

C_O = concentration in organism at the start of the elimination

3.13.3 Half life (t½)

The biological t½ is represented by Equation 9.

$$\mathbf{t_{½} = (\ln 2)/k_2 \text{ or } 0.693/k_2} \qquad (9)$$

Where k_2 is equal to the slope of a plot of ln C_B vs t.

3.13.4 Assimilation Efficiencies (α)

Values of α were calculated from Equation 10 by using the NLIN Procedure (SAS Institute Inc., Cary, NC, USA). The NLIN Procedure fits nonlinear regression models by least squares. The regression expression was written by D.C.G. Muir (Freshwater Institute, Winnipeg, MB, pers. comm.). The variables necessary to carry out the non-linear regression for the dual-label uptake experiments were: FR, % lipid in the food and larvae, C_F, C_B and their respective sampling day, and k_2.

$$\mathbf{C_B = C_F\ \alpha\ FR\ /\ k_2\ *\ [1\text{-}\alpha(\text{-}k_2 t)]} \qquad (10)$$

Assimiliation efficiencies were also determined from Equation 11 by using the steady state concentrations obtained from dual-label experiments. For both equations the FR was assumed to be 0.089 g g^{-1} d^{-1} (dry wt basis).

$$\mathbf{\alpha = C_B\ k_2 / C_F\ FR} \qquad (11)$$

3.14. Statistical analysis

The results for TCDF and OCDD concentrations in the H. bidens larvae for the replicated treated tanks were analyzed for treatment effects using the ANOVA Procedure (SAS). Data were pooled for the replicated tanks (#1 and #2) if there were nonsignificant differences between uptake or depuration curves at the $p<0.05$ level. Data were expressed as means ± SE and not corrected for growth dilution. Student's t-tests ($p < 0.05$) were conducted on growth rates to determine if they were significantly different from zero. Student t-test ($p<0.05$) were conducted on mean individual BAFs at steady state (n=18) to examine the effects of water velocity, food quality, contaminant concentration, and different instars. Simple linear regression analysis (Lotus 123, Lotus Development Corporation, Cambridge, MA, USA) was conducted on the elimination data to determine the slopes and their respective k_2 values. Student t-tests were conducted on the slopes of the depuration curves, using the standard error of the X coefficients, and standard error of the Y estimate ($p<0.05$) (Steel and Torrie 1980) to determine if there was any differences in the rates of elimination for TCDF and OCDD.

4.0 RESULTS AND DISSCUSSION

4.1 Results: Biological Considerations in Experiments with H. bidens

Currently there have been no field or laboratory studies conducted on H. bidens (Ross). This section of the thesis was created to record species specific observations collected during this work.

4.1.0 Collection of H. Bidens

H. bidens (Ross) larvae were collected in the Assiniboine River in riffle velocities of 20.6 to 28.7 cm s^{-1}. Three distinct microhabitats were identified for H. bidens larvae:

1. Buried within an epilithic "Accumulations of Diatoms and Detritus (ADD)" coating rocks (Fuller 1980b).

2. Found in sand encapsulated homes along rock crevices. Fine sand granules are held together by the silk excreted by the silk gland of the larvae. The H. bidens pupae cases were also found associated with the hibernaculum of the larvae. The pupal cases were not much bigger than the organism itself and both the anterior and posterior ends had small holes for flow-through ventilation.

3. Lodged in some stationary water-logged sticks.

The openings of the H. bidens retreats were parallel and perpendicular to the current. When dislodged from their homes, H. bidens (Ross) drifted downstream with the current, slowly sinking and wiggling on their backs.

In the lab, H. bidens built silken retreats and uniform nets within the 3 d

acclimatization period indicating that 16 and 24 cm s^{-1} were within their velocity tolerance range. Within the test area, most of the hydropsychids built their hibernaculums on the screened diffuser. Hibernacula were less frequently built on the glass marble substrate and the glass sides of the aquarium.

One characteristic that aided in fast field collection of H. bidens was the distinct white "furry" frontoclypeal patches found on all larval instars. The V-shaped patch may be a result of a slight carapace depression "clothed in fine setae which either traps suspended particles or encourages fungal growth (P. Schefter, 1992, Royal Ontario Museum Entomology Curatorial Assistant, pers. comm.).

4.1.1 Feeding Behavior

Filter-feeding H. bidens larvae were observed to extend their thorax out of their retreats and sweep their nets for the lodged μm-sized particles. Glass et al. (1969) describe a similiar feeding behaviour of a Cheumatopsyche sp.

4.1.2 Body Attributes

The mean body length (9.3 ± 1.0 mm), head width (1.3 ± 0.23 mm), apotome length (1.08 ± 0.25), and wet wt (0.016 ± 0.0046 g) were determined for 63 final instar hydropsychids. Hydropsychid dry wt was determined to be 15.9 % ± 0.71 of its wet wt. No attempt was made to sex the larvae.

4.1.3 Initial Wet Weights

The mean wet weights for the hydropsychid placed initially into each tank ranged from 13.0 ± 2.6 to 27.3 ± 5.8 mg (Table 7). For each experiment the mean wet wt did not vary

Table 7. Mean weights (n=50) at the start of each experiment and % mortality.

Experiment #	Phase	Duration d	Velocity cm s^{-1}	Food	Tank	Wt (mg) ± SD	% Mortality
1	uptake	30	16	Nutra Fin	control	15.5 ± 4.6	40
					Rep #1	17.0 ± 4.9	44
					Rep #2	17.2 ± 4.6	46
2	uptake	30	24	Nutra Fin	control	17.9 ± 4.6	40
					Rep #1	17.3 ± 5.4	40
					Rep #2	16.3 ± 4.1	38
3	elimination	18	16	Nutra Fin	control	16.4 ± 3.0	48
					Rep #1	15.9 ± 3.4	54
					Rep #2	16.8 ± 3.3	48
4	elimination	18	24	Nutra Fin	control	13.0 ± 2.6	58
		18			Rep #1	14.4 ± 3.0	58
		52			Rep #2	14.3 ± 3.3	58
5	uptake	30	16	Algae	control	21.0 ± 4.5	36
					Rep #1	22.4 ± 6.2	32
					Rep #2	20.6 ± 4.7	46
6	elimination	18	16	Algae	control	23.7 ± 6.5	54
					Rep #1	27.3 ± 5.7	56
					Rep #2	26.5 ± 5.8	54
7	uptake	30	16	Algae	control	15.6 ± 3.1	42
					Rep #1	16.4 ± 3.5	42
					Rep #2	15.8 ± 4.4	38

more than one mg between the control and treatment tanks. The final instar hydropsychid larvae collected in 1993 (Experiment # 5 and 6) were larger than those in 1991 (Experiment # 1,2,3, and 4) and 1992 (Experiment # 7 and 8). Final instar was verified by comparing the physical attributes of the pupae (quiescent stage) to final instar larvae used in the experiment: body wt, size, apotome length and width.

4.1.4 Lipid Content

Toluene extractable lipids were 3.5 ± 1.6% in the final instar H. bidens (n=32) and 5.5 ± 1.6% (n=2) in penultimate instars.

4.1.5 Mesh Characterization

H. bidens (Ross), like many other filter feeding species, spin rectangular nets (Figure 8) of uniform size. The integrity of the H. bidens' nets do not appear to be influenced by the experimental conditions. The mean mesh sizes range from 53.9 x 121 μm at 16 cm s^{-1} to 64.3 x 123 μm at 24 cm s^{-1} (Table 8). The width to length ratio (W/L) is used to describe the mesh shape. The lower the W/L ratio, the greater the rectangular character, and as the W/L ratio approaches 1, the shape becomes more square. The W/L ratio ranges from 0.45 to 0.52 at the locations where the curent is slow and fast respectively. The total surface area for one net spun at 16 cm s^{-1} was determined to be 18.4 mm^2. The filtration rate (net area x current speed (Alstad 1982)) for this net was determined to be 29.4 cm^3 s^{-1}. The net surface area for sister species H. orris was 15 mm^2 (Wallace et al. 1977).

The nets of H. bidens can be described as having two structural components: a single exterior anchor strand (Figure 8A) and the finer strands that make up the lattice of the mesh (Figure 8B). The mean thickness of the fine mesh strands were similar at the two

Table 8. Final instar H. bidens (Ross) mesh attributes obtained during labatory experiments

	Mesh sizes μm ± SD	# Nets (n)	# Data points
16 cm s^{-1}			
Fine strand thickness	9.5 ± 2.4	13	206
Anchor strand thickness	32.8 ± 5.6	3	15
Mesh size	53.9 ± 20.2 x 121 ± 20.3	8	71
24 cm s^{-1}			
Fine strand thickness	9.4 ± 2.1	13	192
Anchor strand thicknes	25.7 ± 3.7	3	9
Mesh size	64.3 ± 15.8 x 123 ± 14.9	5	56

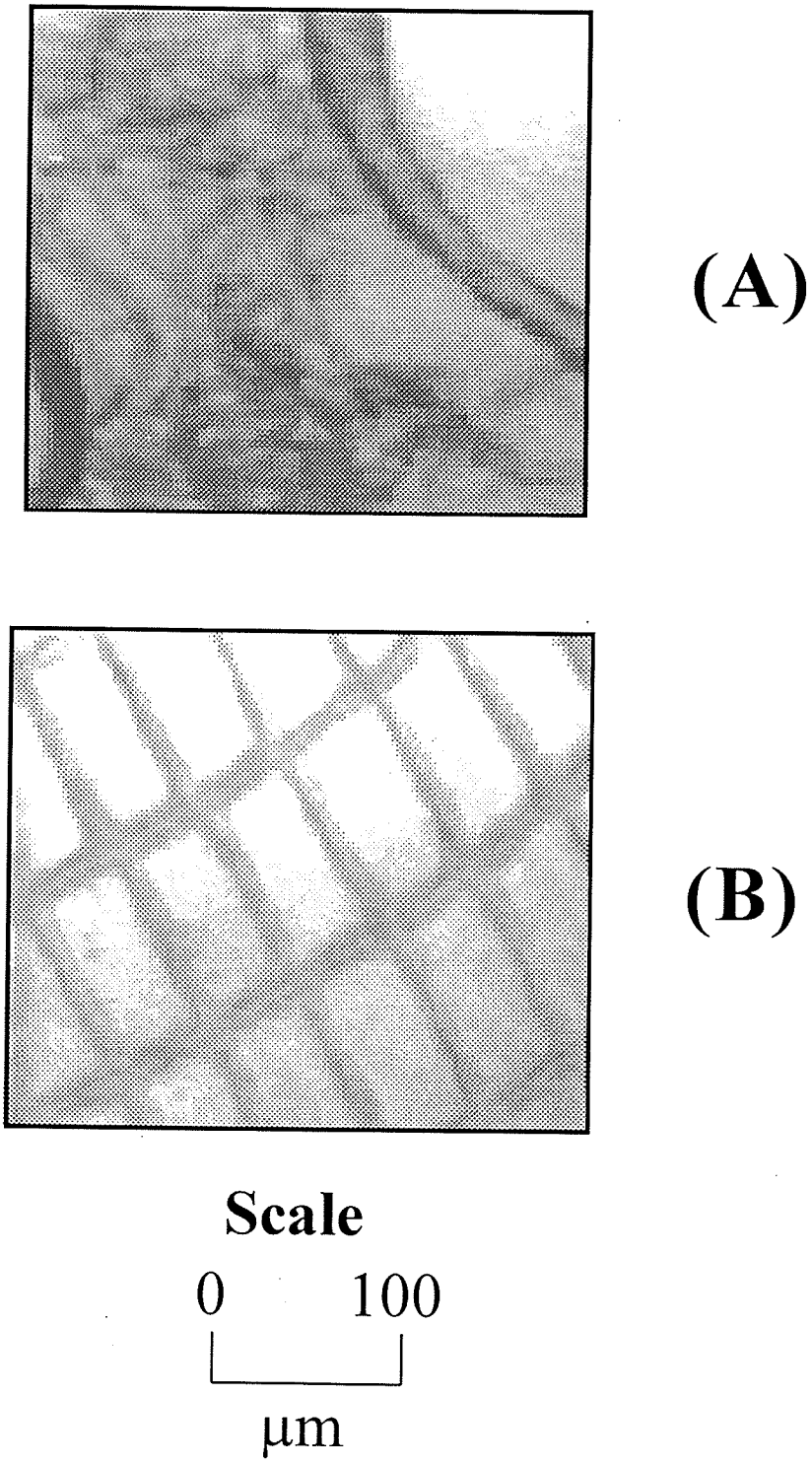


Figure 8. A scanned photographic image of H. bidens' net: (A) anchor strand, (B) finer mesh strands

experimental velocities (16 and 24 cm s^{-1}): 9.5 and 9.4 μm respectively (Table 8). The anchor strand however was approximately 20% thicker at 16 cm s^{-1} than at 24 cm s^{-1}.

4.2 Discussion

Adult H. bidens have been documented in Wisconsin (Longridge et al. 1973, Hilsenhoff 1975), Arkansas (Unzicker et al. 1970), and Minnesota (Denning 1943). In many respects H. bidens are indistinguishable from a southern sister species H. orris (Ross). H. orris is one of the most common Hydropsyche species in central and southeastern United States (Ross1944). Schuster (1978) identifies the difficulty differentiating between the two larvae.

Mackay (1978) indicated that multivoltine species "will be those having a maximum larvae head widths of 1.1 mm or less" in final instars. The mean head width for final instars of H. bidens was 1.3 mm, indicating possibly a bivoltine or multivoltine life strategy (Mackay 1979). Flannagan (1977), in contrast, identified univoltinism to be the main life strategy for the hydropsychid sp. assemblage on the Roseau River (MB): Hydropsyche bifada (Banks), H. bronta (Ross), H. slossonae (Banks), H. scalaris (Hagen), and H. walkeri (Betten & Mosley).

Like H. orris, H. bidens have thick net strands which are adapted for faster currents and have some of the smallest mesh sizes known for hydropsychids (Wallace 1977). The measured final instar mesh dimensions of 53 x 121 μm to 64 x 123 μm are comparable to H. orris' 63 x 137 μm. Field observations have shown that nets of H. orris' are effective at capturing animal remains (Wallace et al. 1977). Hydropsychid nets would have a greater efficiency for retaining larger particulate matter; however, smaller particles are generally not "under-represented" (Fuller et al. 1983, Wallace et al. 1980). Many studies have measured

particles which are smaller-sized than the minimum mesh size (see Table 9) and in the digestive tracts of caddisfly larvae (Wallace 1975, Wallace et al. 1977, Alstad 1978, Fuller et al. 1983).

The ability of aquatic filter-feeders to capture small particles is accomplished in many ways. Blackfly larvae utilize a mucosubstance (common to ocean filter-feeders) to line their filtering cephalic fans (Freeden 1964). Direct absorption to the mesh strands of the net has also been demonstrated for hydropsychids (Thorp et al. 1986) and this may be enhanced by the charge of the particle (LaBarbera 1978, Karickhoff et al. 1985). Lush and Hynes (1973) also emphasize the importance of capturing small particles as aggregates in nets.

There are certain evolutionary advantages to a rectangle mesh compared witha square mesh design with equal mesh widths (Wallace et al. 1976). First the narrow mesh widths for H. bidens (53.9 to 64.3 μm) allowing effective capture of small particles of at least 53.9 μm. Second, the longer lengths (121 to 123 μm) allow a reduction in the use of energetically costly protein strands (Fuller and Mackay 1980a), and in the time required to build the nets without compromising the lower limits of particle size retained in the nets. Another benefit is that the design allows less resistance to the current than a square design. It is known that nets do affect flow (Nowell et al. 1984).

Thicker anchoring strands were observed at 16 than 24 cm s^{-1}. One possible explanation is that thicker strands may increase the rigidity and architectural plasticity of the net at slower currents.

Gut contents of hydropsychids collected in the field appear to be variable, dependent on temporal and spatial variability of food (Georgian et al. 1981, Fuller et al. 1983, Alstad 1987b, Benke 1990). Hydropsychid growth rates and assimilation responses will be affected by the quality of detritus, and species of diatom or algae present (Fuller et al. 1988). Parker

Table 9. Mean mesh dimensions, and particle sizes captured in gut, of 5th instar Hydropsyche species

Mesh size (μm)	Species	Mean particle size captured (μm)[1]	Source
150 x 260	incomoda	125	Wallace 1975
63 x 137	orris	104	Wallace 1975
64 x 123	bidens	--	this study
145 x 250	macleodi	46.5	Wallace 1977
134 x 249	venularis	48.5	Wallace 1975
180 x 270	sparna	18.7	Wallace 1977

1. Determined as square root of mean particle size (μm^2) in their gut

(1983) established that even though the major component of gut contents was detritus, growth was attributed to biotic material. Fuller et al. (1991) showed a positive growth for hydropsychids that feed on diatoms and algae in the lab. In particular H. betteni grew well on a diet of algae compared to detritus at 14 °C. Another study by Fuller et al. (1988) showed that H. betteni feeding on a diet of Chlorella sp. had a negative growth weight change. The results of this latter study showed positive growth rates in 6 out of the 28 lab streams conducted with H. bidens larvae feeding on Chlorella (unidentified sp.).

The percentage of lipid in the test organism is important because lipophilic contaminants partition into lipid rich tissues. The lipid content of the penultimate instar on a dry wt basis (5.5 ± 1.6%) was similiar to those obtained for final instar (3.5 ± 1.6%). In general, however, the lipid content of the penultimate instars would be lower than final instars (Beenakkers et al. 1981). The final instar lipid reserves are utilized during metamorphosis from pupae to adult, and are essential for non-feeding activities, such as flight, diapause, and during famine (Chapman 1969, Beenakker et al. 1981, Downer 1981). Female hydropsychid larvae may also carry more lipids than males; lipids are needed for egg development (Beenakker et al. 1981). The large variability (± SD) measured in lipids may therefore be due to the sex of the caddisfly.

5. RESULTS AND DISCUSSION

5.1 Results: Uptake and Depuration Kinetics of TCDF and OCDD

5.1.0 Mortality

Mortality of H. bidens (Ross) in the uptake experiments ranged from 26 to 46% (Table 7) and was highest during the initial 3 d acclimatization period prior to adding contaminated food. Total deaths were similar between control and treatments for each experiment

A higher incidence of deaths (48 to 62%) occurred during the elimination experiments. Mortalities in controls and treatments were also similar.

5.1.1 Growth Rates

Weight changes for hydropsychids were significantly different from zero (student t-test, $p < 0.05$) for only 8 tanks out of 24 examined. Growth rates for only those experimental tanks (control and treated) that had significant changes in weights are listed in Table 10. For the remaining 16 experimental tanks, the data did not fit a positive or negative linear regression as indicated by insignificant r values.

All positive growth rate constants obtained were for experiments conducted at 16 cm s^{-1}. The only negative growth rate constants occurred during the elimination experiment at 24 cm s^{-1}. This data suggests that H. bidens' grows better at 16 cm s^{-1} than at 24 cm s^{-1} regardless of whether the diet consists of Nutra Fin or Chlorella particles.

5.1.2 Food Attributes

The two food types used in the bioaccumulation experiments fall into the smallest river

Table 10. Experimental tanks in which significant growth rates for H. bidens were obtained

Phase	Duration d	Food	Concentration TCDF ng g^{-1}	Concentration OCDD ng g^{-1}	Velocity cm s^{-1}	Tank #	Growth rate constant $d^{-1} \times 10^3 \pm$ SD	r
Uptake	30	Nutra Fin	60	---	16	Rep #1	5.63 ± 4.10	0.566
Uptake	30	Algae	72	5,600	16	Rep #1	11.2 ± 3.66	0.346
Uptake	30	Algae	200	26,100	16	Rep #2	10.0 ± 3.79	0.300
Elimination	18	Nutra Fin	60	---	24	Control	-20.0 ± 4.94	0.446
	18					Rep #1	-8.20 ± 1.96	0.444
	52					Rep #2	-7.80 ± 2.04	0.411
Elimination	18	Algae	72	5,600	16	Rep #1	16.8 ± 4.95	0.387
						Rep #2	19.0 ± 5.20	0.424

Rate constants ± standard error of the regression coefficient, for the model ln wt = a + b(time), where a = intercept and b = rate constant, based on entire data set.

particle class (<106 μm) (Table 11).

Algae contained 52.6% OC (dry wt) and 13.9 ± 7.3% toluene extractable lipids whereas Nutra Fin totalled 30.4% OC (dry wt) and 8.4 ± 1.1% toluene extractable lipids. The algae particles had approximately 40% greater OC and toluene extractable lipids than the Nutra Fin fish food source. Toluene solvent extracts lipophilic compounds, such as TCDF and OCDD, and the free lipids in which they are found (e.g., triacylglycerols or wax esters) and minimally structural lipids (e.g., phospholipids).

5.1.3 Water and Food Concentrations

The mean water (POM, DOM, and "f_d") and food concentration are summarized in Table 12. Two trends were evident for the aqueous contaminant exposure concentrations for the static streams. As the TCDF/OCDD concentration sorbed to the food particles increased, a corresponding elevation in the mean concentration was detected in the water. As a result, the uptake experiments (30 d) had higher final mean water concentrations than the elimination experiments (18 d).

5.1.4 Kinetic Data

For most experiments the data for the two replicated tanks could be pooled because treatment effects (time-concentration interactions) were not significant (ANOVA, $p < 0.05$). For the pooled data, each sampling day had 6 organisms (n=6), three from each of the replicated tanks. The steady state concentrations (mean ± SE) for the uptake experiments for both TCDF and OCDD were calculated from individual caddisflies levels (ng g^{-1}) collected on the last three sampling days (n=18).

Table 11. Nutra Fin (fish food) and Chlorella (algae) particle attributes: minimum, maximum, and mean particle sizes, % organic carbon (OC), and % of toluene extractable lipids

Food Type	Mean Diameter[1] ± SD (μm)	Particle Sizes[1] min (μm)	max (μm)	% OC	% Lipid ± SD
Nutra Fin	2.4 ± 2.3	0.39	23.3	30.4	8.4 ± 1.1
Algae	5.8 ± 1.1	0.82	7.6	52.6	13.9 ± 7.3

1. The mean diameter, and minimum and maximum particle sizes were determined from 600 Nutra Fin and 600 algae particles

Table 12. Mean concentration of TCDF and OCDD bound to daily food aliquots, and mean aqueous concentration of TCDF and OCDD in miniature lab streams before and after the daily food introduction for 7 out of 9 experiments conducted.

Exp. #	Tank #	Velocity cm s^{-1}	TCDF Concentration			OCDD Concentration		
			Aqueous(1) pg L^{-1}	Aqueous(2) pg L^{-1}	Food ng g^{-1}	Aqueous(1) ng L^{-1}	Aqueous(2) ng L^{-1}	Food ng g^{-1}
Uptake Experiments								
1	#1	16	118 ± 74.0	184 ± 78.0	61.8 ± 14.5	--	--	--
1	#2	16	76 ± 36.0	133 ± 74.0	64.8 ± 17.8	--	--	--
2	#1	24	49.8 ± 20.6	107 ± 23.2	58.4 ± 15.5	--	--	--
2	#2	24	65.4 ± 22.2	121 ± 36.1	67.2 ± 27.0	--	--	--
5	#1	16	156 ± 144	192 ± 91.5	72	29.5 ± 21.6	44.4 ± 28.9	5,600
5	#2	16	237 ± 180	342 ± 194	72	29.2 ± 24.0	47.4 ± 28.2	5,600
Elimination Experiments								
3	#1	16	64.0 ± 19.0	100 ± 35.0	50.0	--	--	--
3	#2	16	69 ± 9.1	108 ± 44.0	58.8	--	--	--
4	#1	24	49.0 ± 17	101 ± 76.0	38.3	--	--	--
4	#2	24	41.5 ± 13.0	86.0 ± 24.0	39.4	--	--	--
6	#1	16	76.7 ± 50.8	122 ± 43.7	72	11.7 ± 5.49	29.6 ± 16.8	5,600
6	#2	16	24.9 ± 12.4	138 ± 85.6	72	10.2 ± 5.41	25.2 ± 7.62	5,600
8	#1	16	87.6 ± 68.2	357 ± 147	200	19.8 ± 12.7	48.1 ± 14.3	26,100
8	#2	16	143 ± 73.5	357 ± 121	200	47.1 ± 8.56	65.4 ± 29.0	26,100

1. Total water concentration before daily food introduction
2. Total water concentration 5 min after food introduction

Note: Total water concentration measures TCDF or OCDD associated in all three phases: POM, DOM, and f_d

5.1.4.0 30 d Nutra Fin uptake experiments with TCDF (60 ng g^{-1}) at 16 and 24 cm s^{-1}

Although the 30 d uptake curves were similiar in shape for the two treated tanks at 16 cm s^{-1} (Figure 9A), significant differences in contaminant levels (over time) (ANOVA, $p < 0.05$) did not permit the data to be pooled (Figure 9A). Steady state concentrations of TCDF reached 424 ± 113 and 1790 ± 419 pg g^{-1} (wet wt) by 16 d.

At 24 cm s^{-1}, there were no significant (ANOVA, $p < 0.05$) treatment effects (time-concentration interactions between the two treated tanks for the 30 d experiments) permitting the data to be pooled. (Figure 9A). A steady state TCDF concentration of 671 ± 119 pg g^{-1} (wet wt) was reached for the pooled data within 16 d.

5.1.4.1 18 d Nutra Fin depuration experiment with TCDF (60 ng g^{-1}) at 16 and 24 cm s^{-1}

The data for both experiments (16 and 24 cm s^{-1}) were combined for the two replicated tanks because treatment effects were non-significant (Figure 9B). Elimination rates (k_d) following 10 d exposures, fitted a first order kinetics model with half-lives of 27.5 ± 6.9 d (k_d=0.025 d^{-1}) and 17.4 ± 2.8 d (k_d=0.040 d^{-1}) for the slow and fast currents, respectively (Figure 9B).

The exposure at 24 cm s^{-1} was extended to 52 d to examine long term trends in TCDF concentrations. A residual body burden, representing approximately 30% of initial concentrations remained in the hydropsychids from approximately day 30 to 52 (Figure 9B). Concurrently, a significant weight loss (g_c = -7.80 ± 2.04 d^{-1}) was detected during this experiment (see Table 10).

5.1.4.2 30 d algae uptake experiment with TCDF (200 ng g^{-1}) and OCDD (26,100 ng g^{-1})

Data were combined for the replicated tanks after treatment effects were shown to be

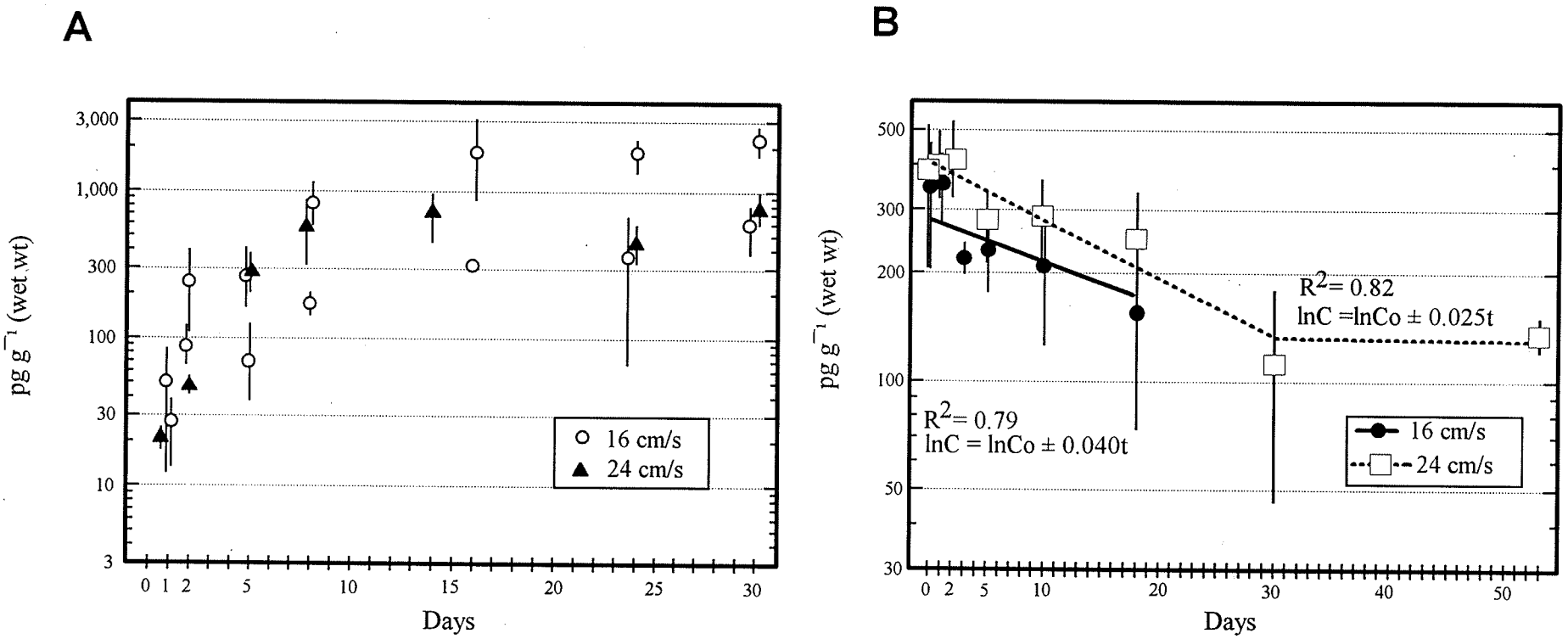


Figure 9. Uptake and elimination of TCDF (60 ng g^{-1}) by H . bidens feeding on Nutra Fin at 16 and 24 cm/s; bars indicate ± SE

non-significant (ANOVA $p < 0.05$). A steady state concentration of 1070 ± 152 pg g^{-1} (wet wt) was achieved for ^{3}H-2,3,7,8-TCDF (Figure 10A). Concurrently, H. bidens accumulated body burdens (wet wt) of 28,000 ± 2,680 pg g^{-1} of ^{14}C-OCDD at an exposure concentration of 26,100 ng g^{-1} (Figure 10B). Steady state concentrations were reached within 16 d for both TCDF and OCDD.

5.1.4.3 18 d algae depuration experiment with TCDF (200 ng g^{-1}) and OCDD (26,100 ng g^{-1})

Data for the replicated tanks were pooled because of non-significant treatment effects (ANOVA, $p < 0.05$). Elimination of TCDF followed a single first-order curve with a t½ of 10.0 ± 1.2 d (k_d=0.07) following the 10 d dual-labelled algae experiment (Figure 11A). In contrast the clearance curve for OCDD was biphasic with a fast initial phase with a t½ of 0.98 d (k_d=0.71) followed by a slow phase with a t½ of 24.2 ± 6.1 d (k_d=0.027) following a 10 d exposure at 26,100 ng g^{-1} (Figure 11B).

5.1.4.4 30 d algae uptake experiment with TCDF (72 ng g^{-1}) and OCDD (5,600 ng g^{-1})

Treatment effects (ANOVA, $p < 0.05$) were not significantly different for the replicated tanks. Data for the uptake experiment were therefore pooled. After 16 d, the steady state concentrations in the larvae (wet wt) were 617 ± 108 pg g^{-1} for TCDF and 9260 ± 1210 pg g^{-1} for OCDD (Figure 12A and 12B respectively).

5.1.4.5 Cuticle Adsorption and Bioconcentration Experiment with TCDF (72 ng g^{-1}) and OCDD (5,600 ng g^{-1})

The contribution due to two alternative uptake routes, cuticle adsorption and bioconcentration, was tested with non-feeding pupae and dead larvae respectively.

After a 10 d aqueous exposure, nine hydropsychid pupae reached steady state

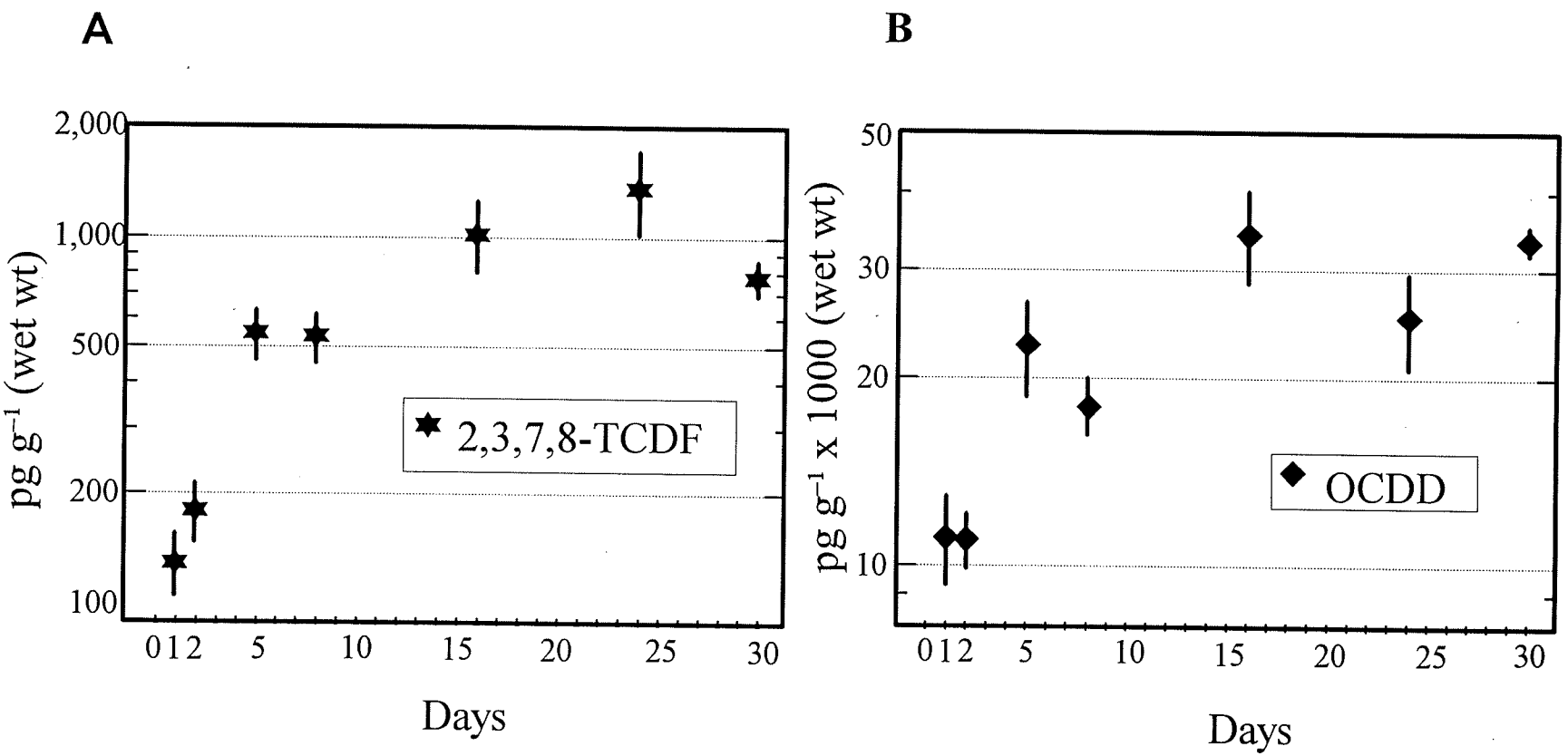


Figure 10. Uptake of algae-sorbed TCDF (200 ng g^{-1}) and OCDD (26100 ng g^{-1}); bars indicate ± SE

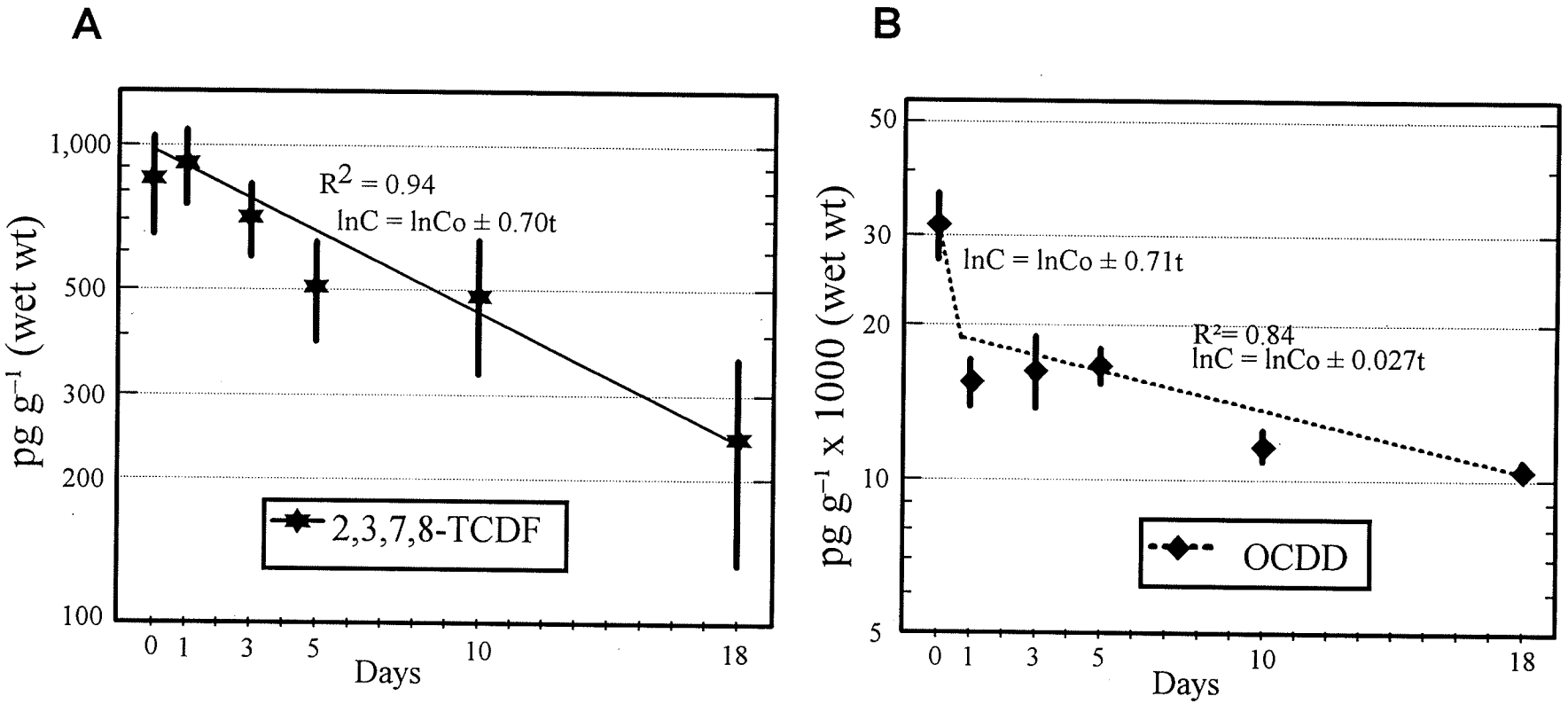


Figure 11. Elimination of algae-sorbed TCDF (200 ng g^{-1}) and OCDD (26100 ng g^{-1}); bars indicate ± SE

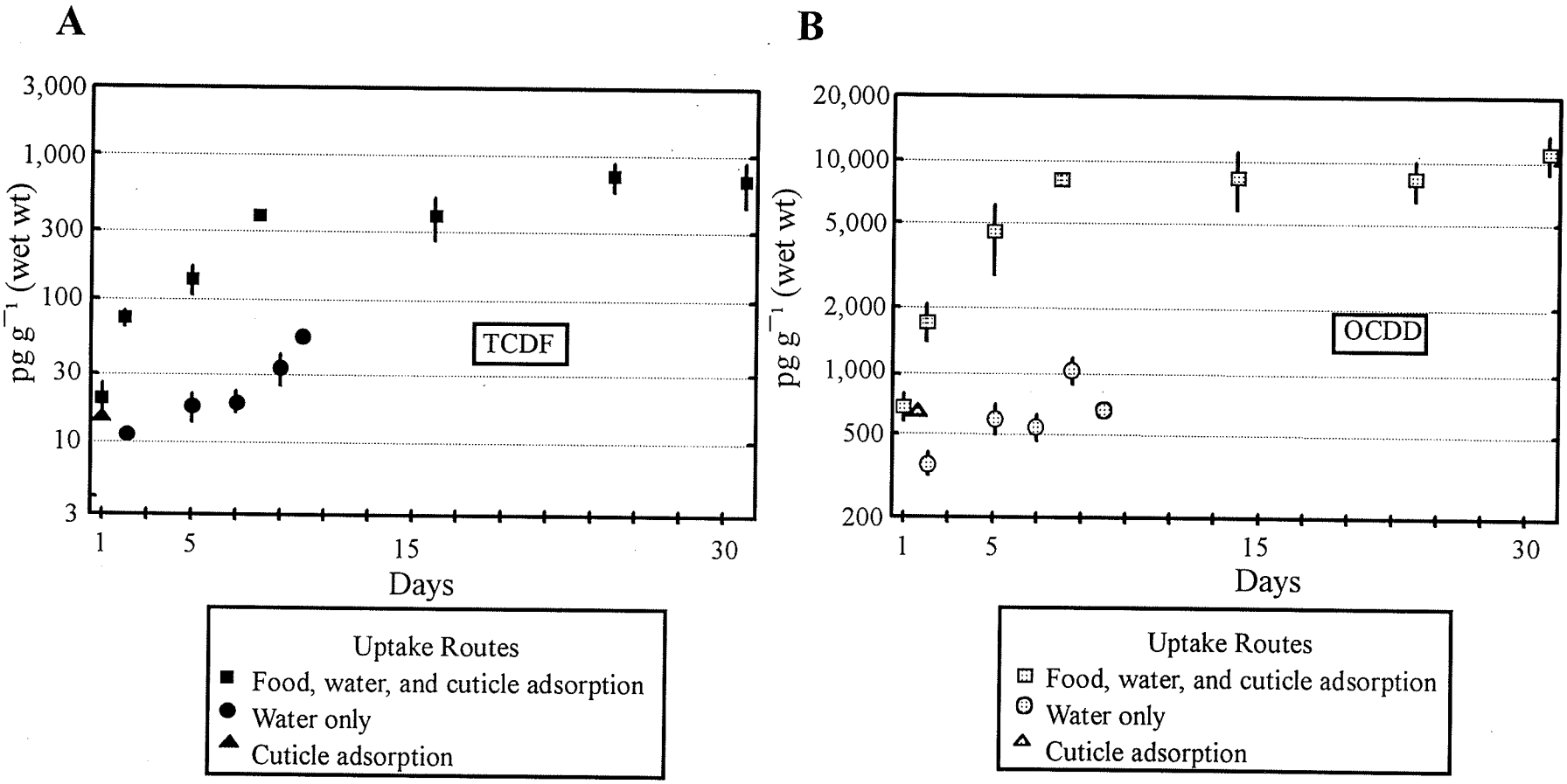


Figure 12. Bioaccumulation of algae-sorbed TCDF (72 ng g^{-1}) and OCDD (5600 ng g^{-1}) by larvae (food, water, and cuticle adsorption), pupae (water only), and dead pupae (cuticle adsorption); bars indicate ± SE

concentrations of 32.0 ± 5.09 pg g^{-1} for TCDF and non steady state body levels of 743 ± 100 pg g^{-1} for OCDD (Figure 12). Steady states levels for TCDF were reached within 5 d.

5.1.4.5a Cuticle adsorption

After an 18 hr exposure the total TCDF sorbed to the cuticle of dead H. bidens equalled 17.8 ± 8.04 pg g^{-1}. The total OCDD sorbed to the cuticle was 682 ± 224 pg g-1 (Table 13).

5.1.4.5b Bioconcentration

The total uptake of TCDF and OCDD attributed to bioconcentration was calculated from Equation 12. This equation assumes that uptake by non-feeding pupae was due only to water exposure (bioconcentration).

$$\mathbf{C_{BFC} = C_P - C_C} \qquad (12)$$

C_{BFC} = Concentration attributed to bioconcentration

C_P = Concentration in pupae

C_C = Concentration due to cuticle adsorption

The accumulation attributed to bioconcentration was determined to be 62.2 pg g^{-1} for TCDF and 625 pg g^{-1} for OCDD (Table 13).

5.1.4.5c Biomagnification

The concentrations of TCDF and OCDD in hydropsychids attributable to dietary

Table 13. The percentage of TCDF and OCDD accumulated in H. bidens attributed to food and water pathways, and cuticle adsorption

Uptake Routes	TCDF pg g^{-1} ± SE	TCDF %	OCDD pg g^{-1} ± SE	OCDD %
Food	537	87.0	7,950	85.8
Water[1]	62.2	10.1	625	6.75
Cuticle Adsorption	17.8 ± 8.04	2.88	682 ± 224	7.35
Bioaccumulation [2]	617 ± 108	100	9,260 ± 1,210	100

1. Body burdens (pg g^{-1} wet wt) after a 10 d exposure (n=1)
2. Bioaccumulation = total uptake from all routes (food and water pathways, and cuticle adsorption)

sources was calculated by subtracting concentrations in larvae exposed to food from concentrations taken up from water or by adsorption:

$$C_M = C_{BAF} - (C_P + C_C) \qquad (13)$$

C_M = Concentration in organism attained from food only

C_{BAF} = Concentration in organism from all uptake routes

The total attributed to dietary uptake is therefore 87.0% and 85.8% of the observed bioaccumulation steady states for TCDF and OCDD, respectively (Table 13).

5.1.4.6 18 d algae elimination experiment with TCDF (72 ng g^{-1}) and OCDD (5,600 ng g^{-1})

The results from only one experimental tank were used. One tank was lost to apparent ^{3}H-TCDF and ^{14}C-OCDD contamination during the clearance phase of the experiment based on measuring increases in TCDF and OCDD levels in H. bidens after they have been placed in clean water. These results cannot be explained by a loss in body weight during the experiment because significant growth rates were detected for both replicated tanks in Experiment 8 (see Table 10).

Elimination curves for H. bidens for both TCDF and OCDD appeared to be biphasic (Figure 13A+B). Both curves were characterized by a fast initial phase of clearance followed by a slower final one. Half-lives for the fast initial phase were 4.6 ± 0.21 d (k_d=0.15) for TCDF and 2.9 ± 1.5 d (k_d=0.24) for OCDD. The final slower phase of elimination for TCDF had a t½ of 63 ± 16 d (k_d=0.011). During the final elimination phase for OCDD, a constant body burden of approximately 7 ng g^{-1} was maintained for the last 15 d of the experiment.

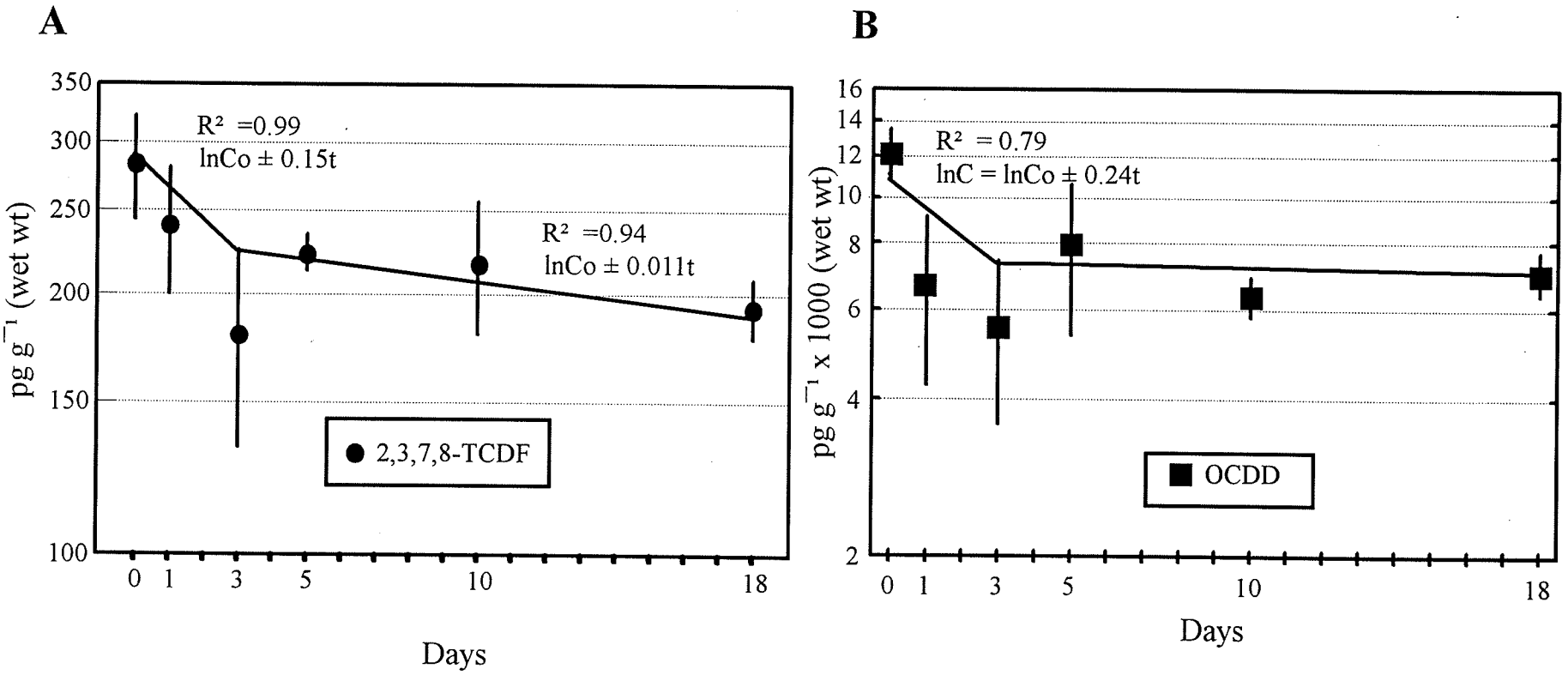


Figure 13. Elimination of algae-sorbed TCDF (72 ng g^{-1}) and OCDD (5600 ng g^{-1}); bars indicate ± SE

5.1.4.7 37 d uptake experiment with penultimate instars

This experiment consisted of only one treated tank operating at 16 cm s^{-1}. Hydropsychids were fed algae at contaminant concentrations of 200 pg g^{-1} (TCDF) and 26,100 pg g^{-1} (OCDD). Steady state concentrations of TCDF and OCDD for seven hydropsychid pupae were 694 ± 121 pg g^{-1} (wet wt) and 23,600 ± 2,070 pg g^{-1} (wet wt), respectively (Figure 14A+B). These values were lower than those obtained for final instars (1,070 pg g^{-1} and 28,000 pg g^{-1} for TCDF and OCDD respectively) feeding on the same food and contaminant concentrations.

5.1.5 Half-lives (t½)

Half-lives of TCDF and OCDD for TCDF and OCDD are listed in Table 14. Although the t½ for TCDF in H. bidens feeding on Nutra Fin (60 ng g^{-1}) appears to be faster at 24 cm s^{-1} (t½ = 17.4 ± 2.8 d) than at 16 cm s^{-1} (t½ = 27.5 ± 6.9 d), elimination rates were not significantly different (student t-test, $p<0.05$). The depuration curve for TCDF for H. bidens exposed to a 10 d dietary algae exposure of 200 ng g^{-1} fits a first-order kinetic model with a t½ of 10.0 ± 1.2 d. Conversely, exposing H. bidens to algae with TCDF concentrations of 72 ng g^{-1} generated an elimination curve with biphasic characteristics: an initial fast phase (t½ = 4.6 ± 0.21 d), followed by a slower phase of elimination (t½ = 63 ± 16 d). Elimination curves for H. bidens feeding on algae at both OCDD concentrations, 5,600 and 26,100 ng g^{-1}, were biphasic. The initial fast elimination rates are similar: t½ of 2.9 and 0.98 d respectively. The final phase of elimination for OCDD at 26,100 ng g^{-1} was slower (t½ = 24.2 ± 6.1 d). At OCDD concentrations of 5,600 ng g^{-1} there was no decrease in body burden (wet wt) for H. bidens for the last 15 d of the elimination experiment.

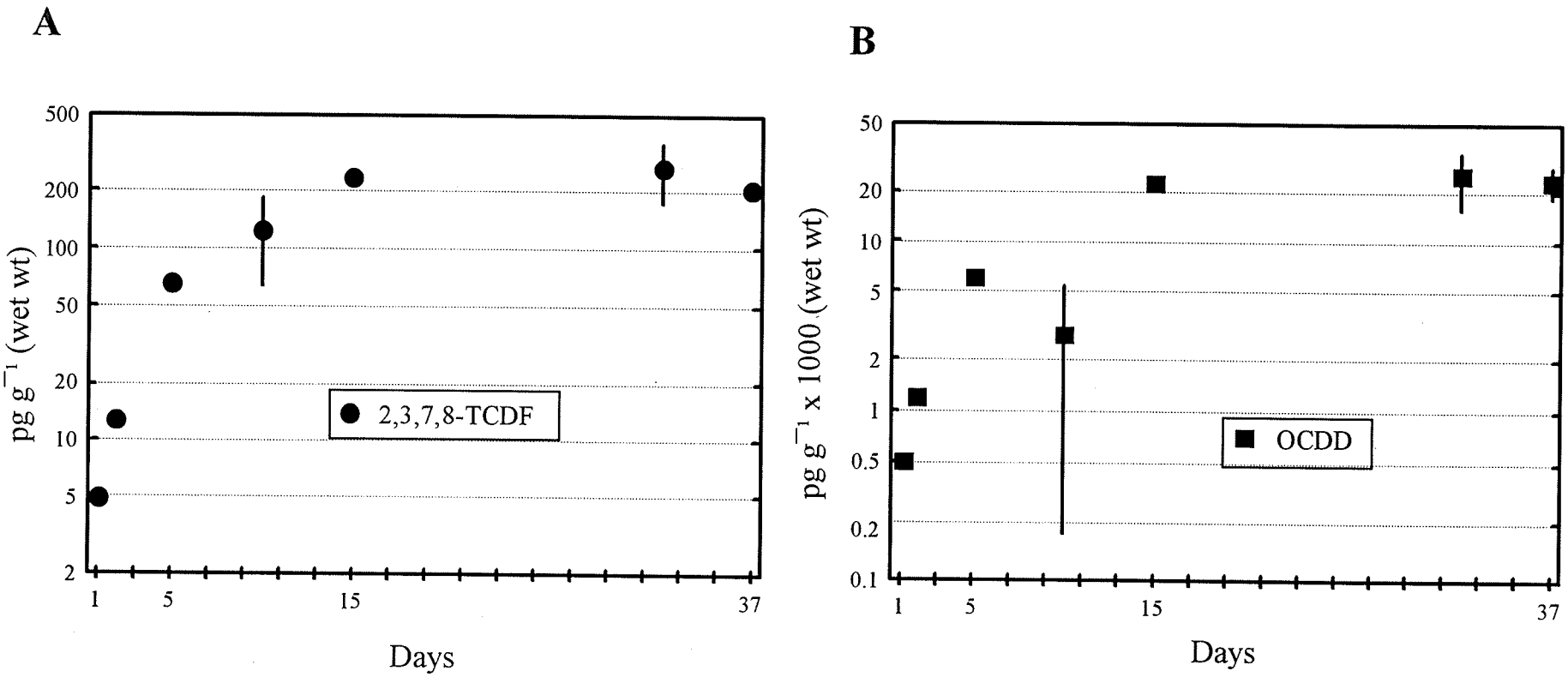


Figure 14. Uptake of algae-sorbed TCDF (200 ng g^{-1}) and OCDD (26100 ng g^{-1}) by penultimate instar; bars indicate ± SE

Table 14. Elimination half-lives ($t_{½}$) and bioaccumulation factors (BAFs) for final and penultimate H. bidens instars

Instar	Food	Treatment	Concentration ng g^{-1}	Velocity cm s^{-1}	Half-life d ± SE	BAF[1] ± SD (n=18)
Final	Nutra Fin	TCDF	60	16	27.5 ± 6.9	345 ± 161 1850 ± 999
Final	Nutra Fin	TCDF	60	24	17.4 ± 2.8	640 ± 442
Final	Algae	TCDF	72	16	4.6 ± 0.21[2] 63 ± 16[3]	859 ± 581
Final	Algae	TCDF	200	16	10.0 ± 1.2	531 ± 293
2nd Last	Algae	TCDF	200	16	---	219 ±102
Final	Algae	OCDD	5,600	16	2.9 ± 1.5[2] ∞[4]	164 ± 83.0
Final	Algae	OCDD	26,100	16	0.98[2] 24.2 ± 6.1[3]	117 ± 39.8
2nd Last	Algae	OCDD	26,100	16	---	57.3 ± 13.1

1. BAF = concentration in organism on a lipid (dry wt) basis/food OC (dry wt)
2. initial fast phase
3. slower final phase
4. no significant decrease in final phase

5.1.6 BAFs

The mean BAFs for TCDF and OCDD for each experiment were calculated from individual BAFs obtained from the last three sampling periods for each experiment (Table 14).

Four-fold lower BAFs were calculated for OCDD in comparison to TCDF for any one experiment. Significantly lower BAFs (Student t-test, $p < 0.05$) were found at higher TCDF and OCDD concentration (Table 14). The penultimate instar also had significantly lower BAFs than final instar larvae feeding on the same contaminated food. Note that in this calculation the toluene extractable lipids were higher for early instars (5.5%) than final instars (3.5%).

In the Nutra Fin experiment with 60 ng TCDF g^{-1} at 16 cm s^{-1}, the replicated tanks had significantly different BAFs: 345 and 1850. The BAFs obtained for both the Nutra Fin experiment conducted with TCDF at 24 cm s^{-1} (633) and for the dual-labelled algae experiment with 72 ng TCDF g^{-1} (967) fell within the mid-range of the values obtained for 16 cm s^{-1}.

5.1.7 Percentage of TCDF and OCDD Associated with POM, DOM, and "Freely Dissolved" (f_d)

The percent of chemical in each phase (POM, DOM, and f_d) is difficult to determine owing to current limitations in analytical techniques and therefore could only be estimated by a reverse-phase Sep Pak procedure. The percent of TCDF and OCDD found associated with the three phases from water samples taken from day 30 of the dual-label uptake Experiment #7 are summarized in Table 15. Most of 2,3,7,8-TCDF and OCDD was found associated with POM (> 90%), followed by DOM (> 2%). The fraction dissolved was < 5%.

5.1.8 BCFs

BCFs for TCDF and OCDD were determined for H. bidens pupae. Because of the

Table 15. Percent of TCDF and OCDD found associated with three phases: POM, DOM, and f_d

	Percent in Each Phase[1]	
Phases	TCDF ± SD	OCDD ± SD
Particulate Organic Matter	93.4 ± 4.3	90.3 ± 4.7
Dissolved Organic Matter	2.1 ± 2.6	4.8 ± 3.0
Freely Dissolved	4.4 ± 2.2	4.9 ± 2.1

1. At the end of the dual-labelled uptake experiment # 7.

difficulty in obtaining accurate f_d values for chlorinated compounds, f_d concentrations were assumed to be 4.4% of the mean experimental water concentration for experiment # 6 (see Table 12) for TCDF (0.0904 ng L^{-1}) and 1 % for OCDD (19.2 ng L^{-1}) based on Muir et al. (1992a), and Servos (1988), and present work. TCDF reached a concentration of 17.8 ± 4.64 pg g^{-1} wet wt following a 10 d exposure period. After a 10 d exposure, OCDD reached a non-steady state body burden of 118 pg g^{-1} wet wt. The BCF_{ss} for TCDF was calculated to be 1.02 x 10^5 and non-steady state BCF for OCDD was 9.32 x 10^3 (Table 16).

5.1.9 Assimilation Efficiencies (α) and Feeding Rates (FR)

Because the experimental feeding rate of H. bidens was unknown, a rate of 0.089 g g^{-1} d^{-1} (dry wt basis) was assumed (McCullough et al. 1979) in order to calculate α. Assimilation values for TCDF and OCDD for the dual-labelled algae experiments are summarized in Table 17.

Assimiliation values were estimated to be < 10% for both TCDF and OCDD from both Equations 11 and 12. An increase in the concentration of TCDF in food from 72 to 200 ng g^{-1} resulted in a three-fold reduction in α values from 9.2 to 3.4%. A reverse trend holds true for rOCDD where the value of α at a 5,600 ng g^{-1} exposure was 2.8% compared to 4.8% at the higher exposure concentration of 26,100 ng g^{-1}.

Table 16. BCFs (10d non steady states) for H. bidens pupae exposed to algae-sorbed TCDF (72 ng g^{-1}) and OCDD (5600 ng g^{-1}).

Compound	C_O ng Kg^{-1} (lipid dry wt)	freely dissolved concentration (ng L^{-1})	BCF[1]
TCDF	406	0.00398	1.02×10^5
OCDD	1770	0.19	9.32×10^3
OCDD	1770	0.074	2.39×10^4 [2]

1. BCF = C_O (ng Kg^{-1})/C_W (ng L^{-1})
2. Assuming OCDDs f_d concentration can not exceed its C_s of 0.074 ng L^{-1}

Table 17. Estimated assimiliation efficiencies (α) assuming a FR of 0.089 g g^{-1} d^{-1} (dry wt basis)

Compound	Food Concentration ng g^{-1} (dry wt)	Steady State Concentration ng g^{-1} (dry wt)	k_2	α % [1]	α % ± SD [2]
TCDF	72	0.617	0.15[3]	5.7	9.2 ± 1.3
TCDF	200	1.07	0.07	1.7	3.4 ± 0.34
OCDD	5600	9.26	0.011[3]	0.08	2.8 ± 0.17
OCDD	26,100	28.0	0.71[3]	3.4	4.9 ± 0.33

1. Calculated from Equation 12 ($\alpha = C_O\ k_2/C_F$ FR).
2. Calculated from Equation 11 ($C_O = C_F\ \alpha$ FR / k_2 * [1-α(-k_2t)]).
3. The initial fast k_2 value was used for biphasic eliminations.

5.2 DISCUSSION

The results of this study support the hypothesis that food chain accumulation is the most important pathway of uptake for both TCDF and OCDD in river ecosystems for hydropsychid larvae. This is the first study to successfully derive pharmacokinetic parameters for particle-bound TCDF and OCDD for an aquatic filter-feeder in miniature laboratory streams.

Hydropsychids are holometabolous apneustic (obtaining oxygen from the water) and have special respiratory organs permeable to oxygen. These thin walled projections are called laminar and papillae gills and are believe to be potential routes for entrance of "freely dissolved "chlorinated hydrocarbons (Connel 1990). Accumulation of "freely dissolved" TCDF and OCDD by H. bidens pupae was relatively low when compared to the amount uptaken by dietary sources by filter-feeding larvae.

H. bidens larvae are different in appearance to their pupae (known as holometabolous). Metamorphosis from larvae to pupae encompasses extensive reconstruction of tissues, including the development of wing pads (Chapman 1969). No current information is known that compares the respiration rates between caddisfly larvae and pupae. The lack of differences in body undulations for Hydropsyche pupae and larvae (Dr. R. Mackay, 1994, University of Toronto, pers. comm.) suggests similar respiration rates. Both pupae and larvae lead relatively sedentary life styles with limited ability for body movements (Dr. R. Mackay, 1994, University of Toronto, pers. comm.) In addition they probably have similar total diffusible surface area and gill membrane design. It can therefore be hypothesized that the accumulation of TCDF and OCDD by bioconcentration pathways would be comparable for the pupae and larvae life stages.

Adsorption of TCDF and OCDD to the cuticle of dead H. bidens was also shown to be

a relatively minor pathway of uptake. Fry et al. (1990) showed for non-feeding Chironomus riparius that uptake by cuticle adsorption and bioconcentration routes was relatively low for two hydrophobic compounds, 5,5',6-trichlorobiphenyl-PCB (log K_{ow} = 6.72) and p,p'-DDE (log K_{ow} = 5.69). Sebastien and Lockhart (1981) showed the filter-feeding blackfly larve accumulated 68 times more methoxychlor when introduced in particulate than emulsion form.

Although algae and Nutra Fin particles are of similar sizes, their nature, behavior, and chemical partitioning characteristics may be different. Nutra Fin (i.e., dried fish food) was made-up from both biotic and abiotic components. Some biotic particles (i.e., algae used in this experiment) are known as "truly" suspended and have a greater resistance to settling. Floating particles are known to have higher transportability which can ultimately affect their bioavailability and rate of ingestion by organisms (Lopez 1980).

Hydrophobic compounds readily adsorb to the suspended particles of diameter < 64 μm in size (Eadie et al. 1982a, 1982b) and this pattern is also seen for decreasing sediment particle sizes (Förstner 1987). Smaller particles contain higher concentrations of organic carbon than larger sized particles and have larger surface areas. For instance in a study by Naiman (1983), organic matter content ranged from 32 to 82% for very fine particulate matter (0.5 to 53 μm), 21 to 35% for fine particulate matter, and 42 to 64% for coarse particulate matter (< 1 mm). Suspended biotic particles (e.g., phytoplankton) have been reported to contain 40% OC dry wt, abiotic particles to contain 0.1 to 10% carbon dry wt, and sediment and soil particles to contain between 0.1 to 5% OC dry wt (Authenreith et al. 1991, Suter 1993). The biotic particles (Chlorella sp.) in this experiment contained slightly higher OC content (52.6%). The experimental abiotic particles, Nutra Fin (30.4% OC dry wt), had conservatively three-fold higher OC content than abiotic particles found naturally in river ecosystems.

It is known that a fast net exchange occurs for contaminants bound to low OC particles (e.g., detritus) compared to high OC materials (e.g., biotic particles). For example, Authenreith et al. (1991) showed that between 54 to 96% of suspended sediment-bound 2,4,5,2',4',5'-hexachorobiphenyl (HCBP) was found associated with the algae after 24 h, and Power et al. (1987) showed a 72% transfer of Aroclor 1254 from abiotic particles to laboratory reared diatoms within 4 h. These studies support the hypothesis that high organic content microseston (e.g., algae) would be important transport vectors for chlorinated hydrocarbons in river ecosystems.

It appears that the ability of an organism to capture and consume either biotic and detrital microseston may influence its potential for contaminant transfer in river ecosystems. If contaminants are bound mainly to biotic particles, those organisms that selectivley capture and feed on these particles, such as hydropsychid larvae, may have higher contaminant loads. In this study it has been demonstrated that H. bidens can readily bioaccumulate algae-bound TCDF and OCDD which could be the important link to explain the levels of PCDD/Fs in insectivorous fish downstream from pulp and paper mills. In 1991, 3,708 adult Trichoptera m^{-2} emerged $season^{-1}$ from the riffle areas located near Beaudry Park. Of these, 512 were H. bidens, contributing to a significant portion of the total Trichoptera biomass.

Feeding rates and assimilation efficiencies are key uptake parameters for predicting bioaccumulation of TCDF and OCDD by the hydropsychid larvae. Unfortunately, both dietary parameters are difficult to measure for small filter-feeders and are usually estimated by modellers. Thomann (1981) estimates feeding rates of 1.7% (body wt d^{-1}) for small fish (0.05 - 50 g) and 0.9% for large fish (5 - 5000 g). Similar feeding rates were found for some aquatic macroinvertebrates (Table 18); 1.9% body wt for Mysis relicta feeding on Daphnia

Table 18. Feeding rates reported for aquatic invertebrates.

Organism	Food	Feeding Rate $g\ g^{-1}\ d^{-1}$ (dry wt)	Source
Mysis relicta (migrating omnivore)	Daphinia pulex (zooplankton)	0.019	Landrum et al. (1992)
	Tubellaria flocculosa (algae)	0.046	
Chironomid		0.1	Davies et al.
Hexagenia (deposit-feeder)		0.3	(1975)
Chironomid (deposit-feeder)	Lake bottom sediments	0.62 (5° C) 4.8 (22° C)	Johannson 1980
Lepidostoma (shredder)	Alder leaves	0.6	Grafius et al. (1979)
Hydropsychidae (filter-feeders)	Natural river seston	0.209 (1)	Georgian et al. (1981)
Simulidae	Diatoms	0.0326	McCullough et al.
Hydropsychidae	Diatoms	0.033 - 1.3	(1979)
(filter-feeders)	Diatoms and algae	0.089	

1. Calculated - assuming hydropsychid mean dry weight = 3.20 mg (this study), and seston capture rate = 134 mg d^{-1}, and ingestion rate = 200 x capture rate.

pulex (Landrum et al. 1992), and between 8.9% to 130% for hydropsychid larvae consuming a diet of algae and diatoms and algae respectively (McCullough et al. 1979). For the late instar caddisfly in the Tallulah River, it was established that they would capture approximately 200 times more seston than would be required for ingestion. Assuming that hydropsychid larvae have a mean dry wt of 3.20 mg, and that their seston capture rate is 134 mg d^{-1}, the feeding rate for insitu river caddifly would equal 0.209 g g^{-1} d^{-1} (dry wt basis). Other studies have measured much higher ingestion rates for deposit-feeders, as seen for chironomids ranging from 10% to 480% body wt (Davies et al. 1975, Johannson 1980).

Water-column feeders such as the migratory omnivore Mysis relicta and the filter-feeding hydropsychid larvae have feeding rates between 0.046 to 0.089 g g^{-1} d^{-1} (dry wt basis), approximately an order of magnitude lower than detritivores when consuming a diet of predominately algae. Freshwater deposit feeders can consume in excess of their total body wt d^{-1} (Hargrave 1972, Johannsson 1980). Hargrave (1972) compiled the feeding rates for 17 deposit feeders, and found that they ranged from 0.11 to 26 g g^{-1} d^{-1} (dry wt).

Feeding rates can be controlled by food preferences, bioavailability of food at the individual level, particle sizes and concentration, transportability, and gut passage time which affects pinocytosis. Pinocytosis, the process of exchange from tissue membranes to insect haemolymph, may be enhanced in higher lipid foods such as algae relative to detritus (Opperhuizen et al. 1986, Chapman 1992). Such factors as gut passage time can be influenced by temperature, species of aquatic organism and size, meal size, meal frequency, food particle size and surface, digestibility, and fat content (Windell 1978). For example, in a study conducted by Fisher et al. (1990), finely ground food had reduced gut passage time which in turn led to lower α.

In the literature, α for TCDF by aquatic organisms (Table 19) ranged from 3.6% for

Table 19. Literature t½ and α for TCDF and OCDD for aquatic organisms.

Organism	Compd	Food ng g^{-1}	t½	α %	Source
Invertebrates	TCDD	--	--	50	Kleeman et al. (1986)
Rainbow trout	OCDD	94	13 ±12	18 ± 17	Muir et al. (1992c)
	TCDF	0.36	73 ± 5	59 ± 3	
	TCDF	7.2	70 ± 3	55 ± 3	
Rainbow trout fry	OCDD	721	5 to 7	--	Muir et al. (1986)
Minnows	OCDD	721	13	--	Muir et al. (1986)
Rainbow trout	OCDD	0.05	--	8	Brugeman et al. (1981)
Chironomid	TCDF	--	35	15	Muir et al. (1992)
Guppies	TCDF	17	--	3.6	Loonen et al. (1991)
	TCDF	753	--	3.6	

guppies (Loonen et al. 1991), 15% for chironomids, hexagenia, and other emerging insects (Muir et al. 1992a), and up to 59 ± 3% for rainbow trout (Muir et al. 1988). Kleeman et al. (1986) reported α values as high as 50% for TCDD in aquatic organisms. Landrum et al. (1992) calculated very high assimilation efficiencies for xenobiotics (> 100%) when determining α as the ratio of uptake clearance (k_d) to that of the feeding rate for an aquatic macroinvertebrate, Mysis relicta.

The current study estimated that the α for H. bidens consuming a diet of algae ranged from 1.7 to 4.5% for TCDF at food concentrations of 200 and 72 ng g^{-1} respectively. For OCDD, α was lower, ranging from 1.4 to 2.4% for OCDD at food concentrations of 5,600 and 26,100 ng g^{-1} respectively. Both these values are lower than the above literature values. The variation in α values for both TCDF and OCDD maybe influenced by the high levels of TCDF and OCDD used in this experiment. A study by Muir et al. (1992) however, showed that the α for TCDF in rainbow trout was independent of levels in the food over a 100 fold-range of concentrations from 0.36 to 42.8 ng g^{-1}.

The results of this study indicate that > 90% of the TCDF and OCDD was associated with POM. The fractions associated with DOM or freely dissolved concentrations were low (≈ 4%). The "freely dissolved" values for TCDF and OCDD may be underestimated due to a net loss to the glassware from the dissolved phase. Both the studies of Servos (1988) and Muir et al. (1992) obtained similar values for 2,3,7,8-TCDF and 1,3,6,8-TCDD (≈ 4%), but lower values for OCDD (< 1%) using the same Sep Pak method for extraction. Some limitations for the Sep Pak extraction method have been identified. Trapped microparticles or colloids may occur in the reverse phase cartridge, potentially over-estimating "freely dissolved" concentration; e.g., up to 10% has been measured for T_4CDD (Servos et al. 1989a).

The experimental design made it difficult to determine if particle concentrations

affected uptake rates. The particle concentrations were similar for both the algae and Nutra Fin exposures because 10 mg of food was added to each system daily and particle sizes were similar. In each experiment, 50 organisms initially fed daily on 10 mg of food introduced into each tank. By the end of the experiment, with fewer organisms due to sampling and mortality, the final organisms were exposed to higher particle concentrations. It has been shown that reduced particle concentrations resulted in lower survival and growth rates for blackfly larvae Simulium vittatum (Fuller et al. 1988). In one experiment conducted on hydropsychids, reduced growth rates were positively correlated with lower diatom concentrations (Fuller et al. 1988).

The current experiments were inconclusive as to whether there was a net positive or negative growth effect due to food type or contaminant concentration. However, H. bidens grew better at 16 cm s^{-1} than at 24 cm s^{-1} regardless of whether the diet consisted of Nutra Fin or Chlorella particles.

Mortality in hydropsychids due to intra-species aggression has been well documented (Ross 1944, Fuller et al. 1981). The cause of mortality in this study appeared to be mainly aggressive and carnivorous behaviour while H. bidens larvae sought suitable substrate for attachment and net spinning activities especially during the acclimatization period. Fewer deaths occurred for the remaining bioaccumulation and clearance experiments. Overall, higher mortalities were detected in the clearance experiments. The greater loss of larvae is probably due to the additional antagonistic behavior caused by placing the larvae into the clean tanks for the depuration phase of the experiment.

High individual variability of TCDF and OCDD concentrations in H. bidens resulted in high variance of uptake and elimination parameters (high standard errors) for all toxicokinetic experiments conducted. Large variability in the accumulation of TCDF and OCDD may be

attributed to individual differences in ability to capture, process, and assimilate TCDF/OCDD food (biomagnification routes) and/or patchy distributions in the cages. Lower variability would be expected if uptake processes were controlled by physical/chemical parameters such as bioconcentration and cuticle adsorption (Larsson 1984). Variability can also be accounted for by the high individual differences in lipids usually detected in final instar larvae as seen in this experiment (Mackay 1984, Bush et al. 1985).

Maximum concentrations of one ng g^{-1} (wet wt) for TCDF were reached within 16 d for final instar H. bidens independent of food concentrations ranging from 60 to 250 ng g^{-1}. OCDD steady state concentration was 9.26 ng g^{-1} (wet wt) at a food concentration of 5,600 ng g^{-1} and 2.80 ng g^{-1} (wet wt) at food concentration of 26,100 ng g^{-1}. The steady state body burdens obtained in this experiment are approximately 5 times greater than the levels found in benthic invertebrates (unspecified sp.) sampled immediately downstream from kraft pulp and paper mills (140 pg g^{-1}) at Hinton and Grand Prairie and three mills located in the Kootenay River Basin (Dwernychuk 1991, Noton 1991). The TCDF levels found in invertebrates sampled in channels receiving direct seepage from mill settling ponds (Dwernychuk 1991) were the same as those obtained in this experiment: 1,000 pg g^{-1} (TCDF) and 17 pg g^{-1} (TCDD). The TCDF levels obtained in this experiment are higher than those detected in fish species (< 4.4 pg g^{-1}) sampled downstream from the Grand Prairie paper mills including kokanee, dolly varden, cutthroat and rainbow trout, large scale and longnose suckers, and mountain whitefish. Whittle et al (1990) measured levels of 400 pg g^{-1} in bottom feeding fish sampled downstream from pulp and paper mills.

Sublethal effects for TCDD in some fish species are detected at concentrations as low as one ng g^{-1}. The body burdens accumulated in this study of 1,000 pg g^{-1} for TCDF (TCDD TEQs = 0.1) are not likely to be toxic (e.g., hazardous effect to reproduction or growth) to H.

bidens because the literature evidence suggests aquatic macroinvertebrates are less sensitive than fish (USEPA 1993).

Elimination of OCDD by H. bidens was best fitted by use of a biphasic first-order pharmacokinetic model. The elimination of TCDF by H. bidens generally fitted a single phase first-order kinetics model as seen for rainbow trout (Muir et al. 1992d). However, elimination of TCDF in the dual label Experiment (#6) appears to have biphasic characteristics. Larger deviation from single first-order curves was seen in the experiments at lower TCDF food concentrations; 60 to 72 ng g^{-1}. The TCDF levels in these experiments approached the detection limits of TCDF which might explain the departure from linearity during the clearance phase of the experiment. Similarly, excretion of TCDD appears to be primarily first-order for most aquatic species (World Health Organization 1989, Kleeman et al. 1986): however, one study conducted on rats also resulted in a biphasic rate of elimination (Olson and Bittner 1983).

In the literature an initial rapid loss of contaminant has been explained by a loss from the gut lining or from the elimination of the peritrophic membrane surrounding the faecal matter (Abedi et al. 1961). The quantities of OCDD cleared in the fast initial phases of elimination could also be explained by the elimination of the peritrophic membrane and gut contents, assuming the Hydropsyche larvae is able to eat 8.9% daily of its body weight d^{-1} in the miniature lab stream.

It was not possible to determine from the results of this study whether a one third increase in velocity (from 16 to 24 cm s^{-1}) or differences in food quality (e.g., algae or nutra fin particles) affected BAFs or the half-lives for either TCDF or OCDD. Other studies have shown that the accumulation of xenobiotics was affected by the quality of ingested food (Landrum et al. 1992).

Rapid rates of elimination for both TCDF and OCDD (≈ 30 d) help to explain the fast steady states and lower BCFs and BAFs attained in this experiment. Half-lives appear to be species specific, dependent on total lipid content, life stage of the organism, and other attributes such as size (Keuhl et al. 1987, Cook et al. 1990, USEPA 1993). Half-lives of TCDD in adult carp with 16% lipid was 320 d (k_d=0.0022) and small carp with 9% lipid was 63 d (k_d=0.011). A smaller fish, the medaka, with 8% lipid had a t½ of 175 d (k_d=0.0067) (Schmieder et al. 1992). Elimination half-lives for TCDF are approximately the same as those obtained in a natural lake mesocosm study for organisms of the same size; chironomids and emerging insects (35 d) and Hexagenia (25 d) (Muir et al. 1992b).

No studies on the biodegradation and biotransformation capabilities of TCDF and OCDD have been conducted with aquatic invertebrates. It was once believed that metabolism of hydrophobic organic compounds was restricted to higher organisms, i.e., fish, birds, and mammals most likely due to method problems when trying to measure enzymatic and synthetic enzymes of the smaller-sized lower invertebrates (Dick et al. 1989). The role of the invertebrate fat body (collective lipid cells) is analogous to that of the vertebrate liver, the major center for intermediary metabolism. Studies on Daphnia magna have measured Cytochrome P450 at 0.03 nmoles/mg microsomal liver protein (Ade et al. 1983). Cytochrome P450 is a group of enzymes capable of catalytic reactions, initiating oxidation and detoxification of certain xenobiotic and biogenic compounds.

For this experiment the best estimate of the "freely dissolved" concentration was taken to be 4.4% of the mean total water concentration for TCDF and 1% for OCDD; 0.0904 ng L^{-1} and 19.0 ng L^{-1}, respectively. The f_d concentration was calculated to be 0.00398 ng L^{-1} for OCDD and 0.19 ng L^{-1} for OCDD. The actual C_s of TCDF is 419 ng L^{-1} and for OCDD is 0.074 ng L^{-1} (see Table 8). The freely dissolved concentration is 2.5 times higher than OCDD

actual C_s, therefore underestimating its true BCF. Lipid normalized BCFs (10 d nonsteady states) were 1.02 x 10^5 for TCDF and 9.32 x 10^3 for OCDD. These results indicate that "freely dissoved" TCDF is more bioavailable for bioconcentration than OCDD.

Literature BCFs for aquatic organisms are generally calculated as the ratio of tissue concentration (wet wt)/water concentration (freely dissolved). BCFs for TCDD range from 380 to 2075 for algae (Isensee 1978) to 4.5 x 10^4 in fresh water organisms: snails, water fleas, and mosquito fish (Adams 1986). Yockim (1978) reported BCFs for water fleas to be between 1.7 to 7.1 x 10^3 for TCDD. Steady state BCFs for TCDD in fish species ranged from 9.27 x 10^4 (ratio of uptake to depuration rate constant) for small rainbow trout (Branson et al. 1985) to 4.30 x 10^6 for medaka (Schmieder et al. 1992). Mehrle et al. (1988) obtained an average BCF value of 26,700 (organism wet wt) for rainbow trout exposed to aqueous TCDD concentrations of 0.038 to 0.382 ng L^{-1}. This same study obtained a higher BCF for rainbow trout (wet wt) exposed for 28 d to TCDD concentrations of 0.382 ng L^{-1} (2.87 x 10^4) than to a 28 d exposure of 0.41 ng L^{-1} (6.05 x 10^3).

Lipid-normalized BCF values for TCDD for the fathead minnow ranged from 5.1 x 10^5 to 8.37 x 10^5. These BCFs are five to eight times higher than the 10 d non steady state lipid-normalized BCF value obtained for TCDF (1.02 x 10^5) for the hydropsychids in this experiment.

Geyer (1992) estimated a BCF (fish wet wt) for OCDD of 4.3 x 10^6. Measured BCFs (wet wts) were much lower than Geyers' estimate, ranging from 705 to 1.5 x 10^3 for small rainbow trout (Servos et al. 1989), to 8.5 x 10^3 for rainbow trout fry (Muir et al. 1985), and 2.2 x 10^4 for fathead minnows (Muir et al. 1985). Non steady state lipid normalized BCFs for the hydropsychid pupae (9.32 x 10^3) would therefore be lower than for fish.

Direct cuticular adherence of TCDF and OCDD to the cuticle of the organism accounts

for a small amount of the total accumulated in this experiment. For OCDD, cuticle adsorption appears to be a more important uptake route than for TCDF. It is known for algae, for example, that chemicals bind to the cell wall because of its higher lipid content (Authenreith et al. 1991). The lipid constituents of the outer integument may be important binding sites. Physical diffusion through the cuticle was expected to be relatively small for both TCDF and OCDD.

The lower BAFs attained for OCDD (117 to 164) relative to TCDF (531 to 1850) could be explained by the hypothesis that OCDD has difficulty in entering membrane pores due to its molecular size and shape (Opperhuizen et al 1985).

The concentration of TCDF or OCDD sorbed to the food particles significantly influenced the BAFs obtained for H. bidens. Higher BAFs were measured at lower contaminant concentrations. This may be a result of either greater bioavailability of TCDF and OCDD at lower concentrations, or a physiological impairment (i.e., respiration, metabolism, or feeding) at higher concentrations (Muir et al. 1986, 1992).

Significant differences in BAFs also occurred for different hydropsychid instars (final and penultimate) and levels of contaminant in the food. Although early instar have smaller mesh sizes and are more efficient at capturing smaller particles, they have lower BAF values than final instars. Willis and Hendricks (1992) measured two distinct growth phases for H. slossonae: from eggs to 4^{th} instar (0.007 mg d^{-1}), and fifth instar (0.148 mg d^{-1}). For the increase in growth in the final instar there would have to be a concurrent increase in feeding rate. A greater consumption of 2,3,7,8-TCDF and OCDD-bound-algae particles would explain the higher BAFs (Table 17) seen in the later instars. Many aquatic insects have faster growth rates at the end of their larval development (Beenakker et al. 1981, Willis et al. 1992) and these could be important life-stage phases for accumulation of contaminants.

Muirhead-Thomson (1987) has noted that the successful maintenance or culture of different stream biota under laboratory conditions is a subject which has for too long been neglected. Nowel et al. (1984) found that all lab streams had limitations in their designs in terms of uniform and steady currents. The suspended load in the size region occupied by the suspension feeder is not well characterized and larvae are known to select for specific flow environments on a rock (Nowell et al. 1984). Even within systems with calibrated flows of 16 and 24 cm s^{-1}, spatial variation will occur in the test areas and each organism may be exposed to slightly different current regimes. This will affect the rate food in which food is trapped and therefore help to explain high variablilities in individual body burdens for TCDF and OCDD.

The exposure environment for recirculating lab streams is difficult to characterize because of daily and temporal fluctuations in TCDF and OCDD concentrations. The lab-streams were "static systems" (recirculating the same water), which potentially allowed for the build-up of biological wastes and TCDF and OCDD concentrations. TCDF and OCDD water concentrations were lowest at the beginning of the experiment and highest at the end of the experiment. At the beginning of each day contaminated food was added to each system. At the end of the day, the total water concentration declined due to feeding organisms, loss to adsorption to the tank, and settling. By the end of the experiment, the total number of feeding organisms was reduced due to sampling and mortality. Hydropsychid feeding studies conducted by Fuller et al. (1981) showed that it was only necessary to change the water in low volume (15 cm diameter) circular Plexiglas labstreams when the food source added was animal matter, not algae, diatoms, or detritus.

CONCLUSION

As far as the author is aware, this bioaccumulation study is the first of its kind to integrate aquatic filter-feeders (single-species), miniature laboratory streams, and microseston-bound TCDF and OCDD. The experimental design is recommended for future toxicity studies requiring constant flow conditions as the pharmacokinetic results were reproducible and the miniature lab streams were economical to set up and operate. In addition, H. bidens (Ross) has proven to be an excellent test organism, especially the non-feeding pupae life stage (≤ 21d) which permits long term bioconcentration experiments to be conducted. Accurate knowledge of respiration rates and membrane permeability is needed for both caddisfly larvae and pupae in order to assess contaminant uptake by bioconcentration pathways.

This results of this study showed filter-feeding H. bidens (Ross) larvae were able to bioaccumulate micro-seston bound TCDF and OCDD under flowing conditions. The portion of the total body burden attributed to bioconcentration or cuticle adsorption pathways was determined to be low for non-feeding H. bidens pupae and dead larvae respectively lending further support for food chain transfer as the primary uptake rout for filter-feeders. The bioaccumulation of contaminants appears to be a function of both life history and feeding habits of aquatic organisms; for example, compounds that behave like TCDF and OCDD will not be bioavailable to aquatic organisms except by direct contact with contaminant-bound suspended particulate matter.

Feeding rates and assimilation efficiencies appear to be key dietary parameters for contaminant uptake. This study showed lower steady state body burdens for both TCDF and OCDD for penultimate hydropsychid instars with lower feeding rates than final instar larvae. It is apparent that to accurately model food chain transfer of chlorinated hydrocarbons in

riverine ecosystems, future research is needed to determine feeding rates and assimilation efficiencies for small-sized aquatic filter-feeders. It is also necessary to identify the specific Cytochrome P450 enzyme detected in some lower invertebrates, as only certain ones, such as P4501A1 have the capabilities to degrade planar aromatics.

From the results of this study it is also possible to conclude:

1. Filter-feeding organisms, such as caddisfly larvae, are sensitive biomarkers for lotic ecosystems.

2. The higher levels of PCDD/F congeners detected in fish that consume insects rather than detritus downstream from pulp and paper mills discharging chlorinated byproducts can be explained by a biomagnification pathway from contaminants bound to suspended particulate matter to filter-feeders to insectivorous fish.

3. As hydropsychids dominate the macroinvertebrate assemblage in river ecosystems, emergence can be an important means of transport of PCDD/Fs from aquatic into terrestrial ecosystems.

4. Higher chlorinated PCDD/F congeners are less likely to magnify.

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APPENDIX 1

A Raw data for the 30 d Nutra Fin uptake experiment with TCDF (60 ng/g) at 16 cm/s: A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g (wet wt)
1	A	1	0.0141	0.01
2	A	1	0.0224	0.01
3	A	1	0.0244	0.01
4	B	1	0.0110	4.02
5	B	1	0.0191	18.38
6	B	1	0.0200	12.13
7	C	1	0.0130	20.95
8	C	1	0.0184	121.27
9	C	1	0.0178	5.01
10	A	2	0.0222	0.01
11	A	2	0.0153	0.05
12	A	2	0.0120	5.76
13	B	2	0.0117	85.95
14	B	2	0.0106	143.14
15	B	2	0.0123	50.22
16	C	2	0.0218	502.60
17	C	2	0.0181	6.43
18	C	2	0.0132	260.50
19	A	5	0.0121	0.10
20	A	5	0.0140	1.72
21	A	5	0.0141	0.01
22	B	5	0.0196	49.09
23	B	5	0.0258	19.96
24	B	5	0.0120	166.53
25	C	5	0.0230	55.70
26	C	5	0.0228	333.02
27	C	5	0.0204	470.75
28	A	8	0.0177	0.01
29	A	8	0.0177	0.01
30	A	8	0.0144	0.01
31	B	8	0.0120	208.63
32	B	8	0.0093	114.67
33	B	8	0.0105	196.60
34	C	8	0.0246	343.91
35	C	8	0.0163	1225.76
36	C	8	0.0259	1086.26
37	A	16	0.0174	0.01
38	A	16	0.0194	0.01
39	A	16	0.0142	0.01
40	B	16	0.0127	314.90
41	B	16	0.0221	365.95
42	B	16	0.0132	274.43
43	C	16	0.0157	4061.34
44	C	16	0.0173	1411.57
45	C	16	0.0096	440.34

APPENDIX 1A (CONTINUED): Raw data for the 30 d Nutra Fin uptake experiment with TCDF (60 ng/g) at 24 cm/s

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g
46	A	24	0.0089	27.53
47	A	24	0.0170	0.01
48	A	24	0.0131	0.06
49	B	24	0.0135	968.56
50	B	24	0.0187	106.87
51	B	24	0.0083	29.42
52	C	24	0.0168	1534.18
53	C	24	0.0170	2660.38
54	C	24	0.0188	1232.80
55	A	30	0.0110	0.01
56	A	30	0.0107	0.01
57	A	30	0.0124	0.01
58	B	30	0.0177	947.79
59	B	30	0.0140	235.05
60	B	30	0.0137	581.48
61	C	30	0.0215	2235.66
62	C	30	0.0192	3128.26
63	C	30	0.0102	1437.67

APPENDIX 2

A Raw data for the 30 Nutra Fin uptake experiment with TCDF (60 ng/g) at 24 cm/s: A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g
1	A	0	0.0159	0.76
2	A	0	0.0191	0.00
3	A	0	0.0183	4.87
4	B	0	0.0159	0.00
5	B	0	0.0127	0.00
6	B	0	0.0216	0.00
7	C	0	0.0169	0.00
8	C	0	0.0133	0.00
9	C	0	0.0168	0.00
10	A	1	0.0199	1.37
11	A	1	0.0162	0.10
12	A	1	0.0180	2.45
13	B	1	0.0212	21.94
14	B	1	0.0158	12.66
15	B	1	0.0092	31.78
16	C	1	0.0138	29.68
17	C	1	0.0166	19.64
18	C	1	0.0161	11.13
19	A	2	0.0164	3.67
20	A	2	0.0205	0.00
21	A	2	0.0147	2.70
22	B	2	0.0105	44.42
23	B	2	0.0170	53.53
24	B	2	0.0142	35.30
25	C	2	0.0188	62.68
26	C	2	0.0173	58.87
27	C	2	0.0165	31.25
28	A	5	0.0171	10.19
29	A	5	0.0151	8.56
30	A	5	0.0127	8.54
31	B	5	0.0197	518.75
32	B	5	0.0160	277.71
33	B	5	0.0178	551.24
34	C	5	0.0198	60.97
35	C	5	0.0151	139.21
36	C	5	0.0154	187.76
37	A	8	0.0195	16.48
38	A	8	0.0089	24.55
39	A	8	0.0294	15.79
40	B	8	0.0154	1652.79
41	B	8	0.0145	1089.21
42	B	8	0.0241	90.29
43	C	8	0.0164	547.75
44	C	8	0.0143	99.42
45	C	8	0.0210	45.29

APPENDIX 2A (CONTINUED): Raw data for the 30 d Nutra Fin uptake experiment with TCDF (60 ng/g) at 24 cm/s

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g
46	A	14	0.0224	11.22
47	A	14	0.0190	13.11
48	A	14	0.0185	32.82
49	B	14	0.0144	216.64
50	B	14	0.0145	1130.65
51	B	14	0.0127	656.23
52	C	14	0.0192	1941.01

APPENDIX 3

A Raw data for 18 d Nutra Fin elimination experiment with TCDF (60 ng/g) at 16 cm/s: A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g
1	A	0	0.0124	2.5
2	A	0	0.0190	3.3
3	A	0	0.0276	1.5
4	B	0	0.0228	917.0
5	B	0	0.0169	147.8
6	B	0	0.0122	159.4
7	C	0	0.0189	427.4
8	C	0	0.0214	120.5
9	C	0	0.0149	219.1
10	A	1	0.0202	8.5
11	A	1	0.0167	7.4
12	A	1	0.0231	5.2
13	B	1	0.0156	302.0
14	B	1	0.0160	630.8
15	B	1	0.0086	531.7
16	C	1	0.0112	190.9
17	C	1	0.0087	218.8
18	C	1	0.0089	248.0
19	A	3	0.0167	0.0
20	A	3	0.0141	3.9
21	A	3	0.0154	0.0
22	B	3	0.0144	221.7
23	B	3	0.0212	190.7
24	B	3	0.0124	167.8
25	C	3	0.0145	176.2
26	C	3	0.0158	278.2
27	C	3	0.0097	285.0
28	A	5	0.0080	61.9
29	A	5	0.0186	2.0
30	A	5	0.0113	2.7
31	B	5	0.0088	31.7
32	B	5	0.0081	275.9
33	B	5	0.0134	1790.2
34	C	5	0.0105	324.5
35	C	5	0.0137	292.3
36	C	5	0.0147	222.1
37	A	10	0.0225	5.4
38	A	10	0.0144	2.1
39	A	10	0.0147	2.4
40	B	10	0.0145	152.0
41	B	10	0.0176	186.6
42	B	10	0.0063	97.9
44	C	10	0.0202	648.3
45	C	10	0.0202	91.0

APPENDIX 3A (CONTINUED): Raw data for 18 d elimination experiment with TCDF (60 ng/g) at 16 cm/s

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g
46	B	18	0.0150	13.3
47	B	18	0.0246	378.7
48	C	18	0.0121	183.8
49	A	18	0.0187	0.0
50	C	18	0.0153	50.1
51	A	18	0.0215	55.0
52	A	18	0.0162	0.0
53	A	18	0.0157	0.0

APPENDIX 4

A Raw data for 18 d elimination experiment with TCDF (60 ng/g) at 24 cm/s: A (control), B (treated tank #1), C (treated tank #2)

OBS	STREAM	DAY	Weight g (wet)	Concentration of Furan pg/g
1	A	0	0.0151	8.5
2	A	0	0.0163	13.8
3	A	0	0.0183	7.1
4	B	0	0.0100	174.4
5	B	0	0.0180	123.3
6	B	0	0.0092	187.9
7	C	0	0.0101	1094.2
8	C	0	0.0086	414.8
9	C	0	0.0130	161.8
10	A	1	0.0179	14.6
11	A	1	0.0225	11.8
12	A	1	0.0139	33.8
13	B	1	0.0169	613.8
14	B	1	0.0170	338.5
15	B	1	0.0222	138.6
16	C	1	0.0106	391.3
17	C	1	0.0201	273.0
18	C	1	0.0175	688.6
19	A	2	0.0096	27.9
20	A	2	0.0226	11.9
21	A	2	0.0181	0.0
22	B	2	0.0154	370.2
23	B	2	0.0203	544.4
24	B	2	0.0127	273.2
25	C	2	0.0177	349.5
26	C	2	0.0164	850.9
27	C	2	0.0196	157.9
28	A	5	0.0207	10.9
29	A	5	0.0157	14.9
30	A	5	0.0104	15.6
31	B	5	0.0173	111.8
32	B	5	0.0163	130.7
33	B	5	0.0166	416.7
34	C	5	0.0174	468.6
35	C	5	0.0212	227.6
36	C	5	0.0143	291.0
37	A	10	0.0182	8.4
38	A	10	0.0225	17.1
39	A	10	0.0163	12.0
40	B	10	0.0130	204.3
41	B	10	0.0200	118.0
42	B	10	0.0183	118.0
43	C	10	0.0155	573.5
44	C	10	0.0133	436.9
45	C	10	0.0104	263.0

APPENDIX 4A (CONTINUED): Raw data for 18 d elimination experiment with TCDF (60 ng/g) at 24 cm/s

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g
46	A	18	0.0166	0.0
47	A	18	0.0180	7.9
48	A	18	0.0175	2.2
49	B	18	0.0196	135.5
50	B	18	0.0199	184.5
51	B	18	0.0147	241.0
52	C	18	0.0161	164.6
53	C	18	0.0192	127.4
54	C	18	0.0155	658.0
55	B	30	0.0243	433.8
56	B	30	0.0122	22.2
57	B	30	0.0173	109.1
58	C	30	0.0180	58.9
59	C	30	0.0164	43.4
60	C	30	0.0136	6.8
61	B	53	0.0202	14.5
62	B	53	0.0225	120.6
63	B	53	0.0243	145.9
64	C	53	0.0187	81.9
65	C	53	0.0257	146.8
66	C	53	0.0256	219.1

APPENDIX 5

A Raw data for 30 d uptake experiment with TCDF (200 ng/g) and OCDD (26,100 ng/g): A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	C	1	0.0099	98.6	10222.2
2	C	1	0.0124	88.4	8376.3
3	C	1	0.0197	55.2	4995.9
4	B	1	0.0182	147.1	10535.1
5	B	1	0.0101	216.9	16809.1
6	B	1	0.0140	178.2	15453.2
7	B	2	0.0183	233.1	10458.4
8	B	2	0.0155	208.1	12404.3
9	B	2	0.0153	282.2	13221.2
10	C	2	0.0178	114.8	9360.0
11	C	2	0.0142	60.9	6745.2
12	C	2	0.0177	186.4	13632.8
13	B	5	0.0150	750.6	31786.8
14	B	5	0.0158	408.9	10144.4
15	B	5	0.0139	780.5	17296.5
16	C	5	0.0133	658.0	34022.6
17	C	5	0.0149	284.2	16147.5
18	C	5	0.0109	396.6	26281.4
19	B	8	0.0175	903.0	18488.6
20	B	8	0.0104	536.0	24874.9
21	B	8	0.0133	428.5	15381.2
22	C	8	0.0156	572.3	20616.4
23	C	8	0.0142	362.3	17525.2
24	C	8	0.0218	400.1	11568.7
25	B	16	0.0151	1490.2	53503.9
26	B	16	0.0115	536.8	33009.9
27	B	16	0.0169	1596.7	48481.2
28	C	16	0.0183	1480.3	18602.3
29	C	16	0.0096	547.2	31469.7
30	C	16	0.0110	504.2	20754.1
31	B	24	0.0199	2517.7	32703.1
32	B	24	0.0144	785.4	32964.8
33	B	24	0.0077	1390.9	3954.5
34	C	24	0.0072	455.7	25605.3
35	C	24	0.0126	2267.9	29627.0
36	C	24	0.0126	771.7	25636.7
37	B	30	0.0145	830.9	37428.0
38	B	30	0.0144	1053.4	29047.1
39	B	30	0.0145	668.0	31137.5
40	C	30	0.0125	787.8	32674.9
41	C	30	0.0117	556.7	37207.3

APPENDIX 6

A Raw data for 18 d elimination experiment with TCDF (200 ng/g) and OCDD (26,100 ng/g): A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	g (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	B	0	0.0152	1549.5	33334.1
2	B	0	0.0104	494.6	21596.6
3	B	0	0.0155	776.5	27949.4
4	C	0	0.0107	495.3	19594.9
5	C	0	0.0109	447.6	50321.7
6	C	0	0.0161	1318.7	36347.8
7	B	1	0.0175	1111.1	19312.1
8	B	1	0.0191	1537.9	17952.0
9	B	1	0.0188	775.7	10292.4
10	C	1	0.0174	1001.0	12696.2
11	C	1	0.0121	641.4	19491.9
12	C	1	0.0132	416.2	13447.3
13	B	3	0.0146	454.9	15082.9
14	B	3	0.0136	922.7	24119.8
15	B	3	0.0145	922.7	24119.8
16	C	3	0.0177	777.7	11537.7
17	C	3	0.0129	234.4	9073.4
18	C	3	0.0157	936.7	14194.1
19	B	5	0.0104	264.1	19720.8
20	B	5	0.0108	204.4	17477.1
21	B	5	0.0138	361.2	18173.3
22	C	5	0.0131	680.9	19291.9
23	C	5	0.0138	976.9	13830.5
24	C	5	0.0144	560.8	11777.5
25	B	10	0.0158	430.1	12480.3
26	B	10	0.0165	294.0	8957.8
27	C	10	0.0186	467.8	10062.9
28	C	10	0.0150	1187.2	13427.1
29	C	10	0.0091	117.2	11568.4
30	C	10	0.0135	397.5	13682.2
31	B	18	0.0132	253.0	9293.3
32	B	18	0.0108	108.2	11260.8
33	B	18	0.0161	148.7	12204.0
34	C	18	0.0136	125.1	10002.1
35	C	18	0.0132	47.2	9998.9
36	C	18	0.0135	787.3	10128.2

APPENDIX 7

A Raw data for 30 d uptake experiment with TCDF (72 ng/g) and OCDD (5600 ng/g): A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	B	1	0.0189	8.82	669.51
2	B	1	0.0185	13.64	436.99
3	B	1	0.0113	10.25	575.45
4	C	1	0.0152	11.54	1109.98
5	C	1	0.0284	33.01	507.44
6	C	1	0.0235	39.75	732.90
7	B	2	0.0240	70.10	1991.80
8	B	2	0.0152	88.85	3017.76
9	B	2	0.0176	31.47	808.84
10	C	2	0.0221	67.05	1829.04
11	C	2	0.0245	83.09	1829.20
12	C	2	0.0185	90.98	797.99
13	B	5	0.0162	94.78	3102.69
14	B	5	0.0171	47.45	637.21
15	B	5	0.0217	70.40	1708.88
16	C	5	0.0213	178.89	5354.92
17	C	5	0.0188	233.57	11479.64
18	C	5	0.0196	190.49	4877.87
19	B	8	0.0191	457.22	8575.71
20	B	8	0.0198	422.08	9346.54
21	B	8	0.0152	405.54	7064.56
22	C	8	0.0195	332.99	6984.81
23	C	8	0.0199	354.87	8027.84
24	C	8	0.0218	307.49	8190.77
25	B	16	0.0161	603.71	17585.61
26	B	16	0.0243	127.76	2936.35
27	C	16	0.0196	745.68	8939.78
28	C	16	0.0136	110.10	4031.84
29	C	16	0.0168	341.30	8745.50
30	B	24	0.0236	693.55	7409.67
31	B	24	0.0130	138.91	6475.60
32	B	24	0.0144	1405.16	16793.60
33	C	24	0.0189	819.80	6192.99
34	C	24	0.0203	754.79	5765.88
35	C	24	0.0233	633.65	6863.94
36	B	30	0.0209	1364.58	21056.00
37	B	30	0.0096	152.02	9007.03
38	B	30	0.0146	215.30	12494.88
39	C	30	0.0195	1013.38	8372.76
40	C	30	0.0197	1217.52	9509.96
41	C	30	0.0135	149.38	5584.85

APPENDIX 8

A Raw data for 18 d elimination experiment with TCDF (72 ng/g) and OCDD (5600 ng/g): A (control), B (treated tank #1), C (treated tank #2)

OBS	Stream	Day	Weight g (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	B	0	0.0196	159.58	3590
2	B	0	0.0243	163.46	4170
3	B	0	0.0222	625.89	6110
4	C	0	0.0231	251.66	13600
5	C	0	0.0239	243.60	9460
6	C	0	0.0271	357.86	13120
7	B	1	0.0259	395.73	4340
8	B	1	0.0211	328.67	3960
9	B	1	0.0251	509.55	5520
10	C	1	0.0295	182.36	3450
11	C	1	0.0176	218.96	5240
12	C	1	0.0173	317.22	11370
13	B	3	0.0193	222.46	3240
14	B	3	0.0233	519.84	6350
15	B	3	0.0190	383.05	4280
16	C	3	0.0223	88.03	1670
17	C	3	0.0257	211.57	7230
18	C	3	0.0173	236.84	7680
19	C	5	0.0170	204.78	13010
20	C	5	0.0205	241.85	6170
21	C	5	0.0181	224.80	4610
22	B	5	0.0193	309.94	4030
23	B	5	0.0164	248.05	2980
24	B	5	0.0147	599.13	15040
25	B	10	0.0245	351.97	4730
26	B	10	0.0172	561.13	7320
27	B	10	0.0215	333.56	3950
28	C	10	0.0188	290.47	7450
29	C	10	0.0186	162.17	6040
30	C	10	0.0155	202.69	5610
31	C	18	0.0145	173.64	7850
32	C	18	0.0223	223.53	5730
33	C	18	0.0251	181.62	7670
34	B	18	0.0211	561.96	6860
35	B	18	0.0215	649.12	9760
36	B	18	0.0239	325.09	4460

APPENDIX 9

A Raw data for 10 d "freely dissolved'' uptake experiment with TCDF (72 ng/g) and OCDD (5600 ng/g)

OBS	Day	g (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	2	0.0245	11.11	437.57
2	2	0.0313	11.57	342.51
3	5	0.0371	14.42	544.77
4	5	0.0349	16.54	417.97
5	5	0.0271	21.27	544.75
6	5	0.0203	36.48	865.75
7	7	0.0332	19.57	645.82
8	7	0.0336	27.23	486.44
9	8	0.0269	55.50	1541.87
10	8	0.0226	44.81	738.76
11	8	0.0126	22.08	1018.22
12	10	0.2640	62.22	625.77

APPENDIX 10

A Raw data for 18 h uptake experiment with frozen caddisfly larvae at TCDF (72 ng/g) and OCDD (5600 ng/g)

OBS	Weight w (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	0.0230	14.13	550.16
2	0.0206	12.16	554.54
3	0.0241	26.96	940.72

APPENDIX 11

A Raw data for 37 d uptake experiment for penultimate instar with TCDF (200 ng/g) and OCDD (26100 ng/g)

OBS	Day	g (wet)	Concentration of Furan pg/g	Dioxin pg/g
1	1	0.0094	4.9	521.0
2	1	0.0103	4.7	500.0
3	2	0.0102	11.4	1110.0
4	2	0.0107	13.4	1250.0
5	5	0.0114	70.0	6150.0
6	5	0.0100	62.2	6221.4
7	10	0.0105	184.9	176.0
8	15	0.0103	222.0	21500.0
9	15	0.0104	254.0	24500.0
10	32	0.0111	176.0	15900.0
11	32	0.0106	356.0	33600.0
12	37	0.0073	203.0	27800.0
13	37	0.0110	203.0	18500.0