TOXICITY OF PERFLUORINATED ORGANIC ACIDS TO SELECTED FRESHWATER ORGANISMS UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT

TOXICITY OF PERFLUORINATED ORGANIC ACIDS TO SELECTED FRESHWATER ORGANISMS UNDER LABORATORY AND FIELD CONDITIONS

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Very little toxicological data exists for perfluorinated organic acids (PFAs), even with the increased global detection of these compounds in air, water, and biota. PFAs are unique surfactants used in many applications. Single-species laboratory tests and multi-species microcosm studies were used to evaluate the toxicity of structurally similar PFAs to selected freshwater organisms. Eight PFAs of varying chain lengths were tested in laboratory studies on five organisms representing two trophic levels. Two PFAs were tested in separate outdoor microcosm studies on the zooplankton community and the floating macrophyte, *Lemna gibba*. Overall, evaluations showed that relative toxicities were influenced by carbon chain length. In general, PFAs > 7 carbons were relatively toxic to all organisms, however, perfluoropropionic acid, a three carbon PFA, was toxic only to plant species but not invertebrates. Under present environmental concentrations, there appears to be no immediate hazard to freshwater organisms, however, different sensitivities, determined here, requires further investigation of these extremely persistent compounds.

PREFACE

This thesis has been organized as a series of manuscripts either under review (Chapter 2 and 4) or to be submitted (Chapter 3) for publication in scientific journals. Some repetition of introductory and methodological material is unavoidable. The titles and authorship of these publications are listed below:

Chapter 2. Boudreau, T.M., Sibley, P.K., Mabury, S.A., Muir, D.C.G., and Solomon, K.R. 2002. Laboratory evaluation of the toxicity of perfluorooctane sulfonic acid (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna*, and *Daphnia pulicaria*. In press. *Archives of Environmental Contamination and Toxicology*

Chapter 3. Boudreau, T.M., Kobras, K., Janutka, R., Small, J., Sibley, P.K., Mabury, S.A., Muir, D.C.G., and Solomon, K.R. 2002. Toxicological investigation with perfluoroalkane carboxylic acids (PFCAs) in relation to carbon-chain length. To be submitted. *Environmental Toxicology and Chemistry*

Chapter 3 includes contributions from co-authors as described:
Ms. K. Kobras conducted *Daphnia* spp. and *Lemna gibba* laboratory tests with perfluorononanoic acid. Mr. R. Janutka conducted *Lemna gibba* range-finder and definitive tests with perfluorobutyric acid, perfluoropentanoic acid, perfluoroheptanoic acid, and perfluorooctanoic acid. Data were donated by Mr. J. Small who analysed perfluorooctanoic acid water residue samples with ion chromatography at ANALEST at the University of Toronto, Toronto, ON, Canada.

Chapter 4. Boudreau T.M., Wilson, C.J., Cheong, W.J., Sibley, P.K., Mabury, S.A., Muir, D.C.G., and Solomon, K.R. 2002. Response of the zooplankton community and environmental fate of perfluorooctane sulfonic acid (PFOS) in aquatic microcosms. Submitted. *Environmental Toxicology and Chemistry*

Chapter 4 includes major contributions from the co-authors as described:
Mr. C. Wilson helped identify and enumerate the zooplankton community field samples and helped with data inputs and CANOCO evaluation. Data were provided by Mr. W.J. Cheong who analysed PFOS water residue samples with ion chromatography at ANALEST at the University of Toronto, Toronto, ON, Canada. Also, Ms. Erin Smith who analysed the chlorophyll(a) samples using fluorospectrometry. This data was part of another evaluation and graciously provided for this manuscript.

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1 INTRODUCTION

1.1 THESIS OVERVIEW

Perfluorinated organic acids, or perfluoroalkanes, (PFAs) have been used for numerous applications since their initial synthesis in the 1920s. Recently, PFAs, especially perfluorooctane sulfonic acid (PFOS), have been detected in human serum and wildlife tissue samples from around the globe suggesting ubiquitous contamination. Current knowledge suggests that PFAs, particularly long-chained moieties, will favour partitioning into water, rather than air or soil. Therefore, aquatic environments could be a potential sink for PFA accumulation, and thus be subject to hazards from PFA contamination. The present state of knowledge of toxicity of PFAs to aquatic organisms is limited by the lack of published toxicity data.

This thesis investigates the potential toxicological impact of PFOS and several perfluoroalkane carboxylic acids (PFCAs) on aquatic organisms combining single-species laboratory studies and multi-species field microcosm studies. This thesis also addresses the relationship between carbon-chain length of PFCAs and toxicity in laboratory studies. The literature review will address PFA sources, chemistry, environmental persistence, and toxicity.

1.2 PERFLUORINATED ORGANIC ACID PRODUCTION AND USES

Short-chained PFAs were first produced in the 1920's and 1930's by either electrochemical fluorination or telomerisation processes [1-4]. The development of fluorocarbon chemistry led to the discovery of practical applications for PFAs, such as

lubricants, wetting agents, general surfactants, and fire fighting foams. Since then, the 3M Company has manufactured many long-chain perfluoroalkyl intermediates, such as perfluorooctanoic acid (PFOA) and PFOS (**Figure 1.1**), for use in several products. The 3M Co. has many production and manufacturing facilities in North America and Europe

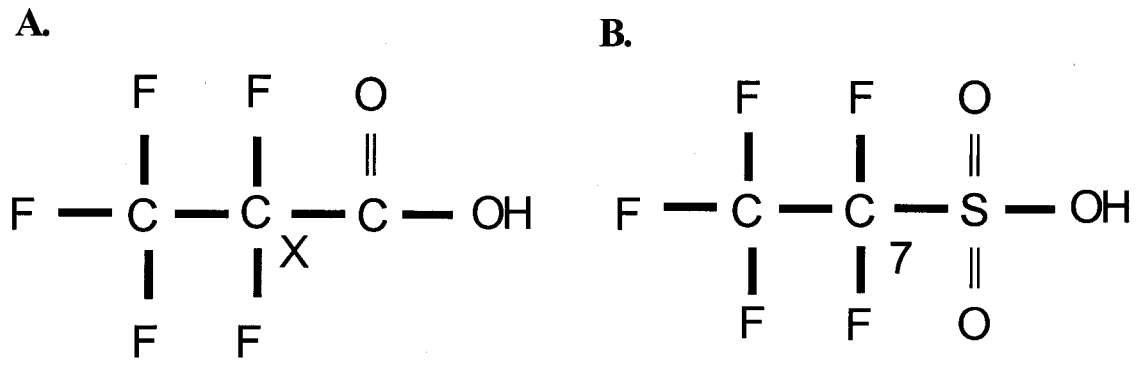

Figure 1.1 Basic structures of perfluorinated organic acids. (A) Generalized structure of perfluoroalkyl carboxylic acids; X can be 1 (PFPrA) to > 20. (B) Structure of PFOS.

and is still the largest producer of fluorochemicals, however, DuPont, Bayer, BASF, and Ciba-Geigy also produce PFAs for many applications throughout North America [4,5]. The basic building block for perfluoroalkane sulfonates (PFSAs), such as PFOS, is perfluorooctanesulfonyl fluoride (PFOSF) [6,7]. Under environmental conditions, PFOSF will readily hydrolyse to form PFOS [6]. Hydrocarbon acids or halogenated organic homologues comprise the subsequent backbone for fluorine substitution in PFCA production [2,3,8,9]. In 1979, total annual production of PFOS in USA was roughly 330,000 Kg [8] compared to ~ 5 million Kg worldwide production in 2000 [6]. 3M has committed to ending production of PFOA and has stopped production of PFOS as of December 2000 [10].

1.2.1 Industrial Applications

One of the main applications of fluorinated compounds are in formulations for aqueous fire-fighting foams [8,11,12]. This class of fluorinated compounds possesses excellent fire-extinguishing properties and widely used in fighting large-scale chemical fires with extreme temperatures [3,12]. Perfluorinated liquids offer unique properties ideally suited to reduce static environments and heat transfer, in such products as microprocessors, superconductors, and transformers [8]. They are also used as leveling or wetting agents in paints, polishes, pulp and paper, oil, gas and mining industries (see http://products.3m.com/us/electronics_mfg/products/). Some PFCAs are used as solvents for certain polymerization reactions as well as raw materials for general synthesis of other halogenated compounds [8,10,13]. They are also used in applications where corrosion and high-temperature protection is required, such as in electroplating, pipe coatings, and linings. Other applications include specialty gases and liquids for pharmaceutical and health care professions to dissolve and transport large quantities of drugs or gases (i.e. oxygen) to areas in the body [14,15,16]. PFOA is a processing agent used in the production of polytetrafluoroethylene, or Teflon®, DuPont's no-stick brandname, and is primarily used for anti-fouling coatings, insulation, anti-static, and superconducting properties in the wire and cable industry (see http://www.dupont.com/teflon/af/). Some formulations of fluorinated compounds are used in lubricants for automotive industries, and as sizing agents in paper corrugation. (see http://www.3M.com/market/industrial/fluids/).

1.2.2 Commercial Applications

A number of commercial products are made from PFAs. These include fluorochemical additives for latex and alkyd paints and other coatings, as well as oil- and water-repellent treatments for stone and masonry applications [17]. Fluorochemicals are also used as a general soil and stain repellent applied to carpets and textiles, such as Scotchgard™ [6,17]. They are also used as aerosol propellants, lubricants, and release agents [18]. Food-wrapping containers such as microwave popcorn bags, utilize PFOS to prevent oil, grease, and water from ruining the walls of the container. The PFOS-derived Sulfuramide™ is used as an insecticide to control fire ants [18,19] and Sulfotine™ is used against wasps, hornets, and termites [20].

1.3 PHYSICAL CHEMICAL PROPERTIES OF PERFLUORINATED SURFACTANTS

1.3.1 General

PFAs are straight-chained polyfluorinated organic acids analogous to endogeneous fatty acids. These compounds are surface active agents and thus, are generally called surfactants. Surfactants can be ionic or nonionic. Anionic surfactants are the most important class of fluorinated surfactants. PFCAs and PFSAs are two main categories where, in aqueous medium, the acid terminal group dissociates to form an anion [17].

1.3.2 Fluorine-Carbon Bond

The wide utility of perfluorinated compounds is due to the unique properties of the fluorocarbon bond. Fluorine has a very low dissociation energy (37 kcal/mol) and is the most electronegative (4.0) element known [17,21]. Hence, fluorine is the most reactive element, combining energetically (covalently) with carbon to form an extremely strong bond [3,4,22]. Consequently, fluorinated compounds posses exceptional non-reactivity and generally inert to many different environments. For example, they are stable to hydrolysis, acids, alkali, oxidation, reduction [17], and biological breakdown [22-24]. Remediation of these recalcitrant compounds into less inert metabolites is very difficult. Most of these compounds, once deposited into the environment, will persist for an extremely long time. Also, the greater electronegativity of fluorine increases the acidity of PFCAs to a level equivalent to that of some mineral acids [3]. The acidity of perfluorodecanoic acid (carbon chain of 10) is equal to nitric acid [17].

1.3.3 Solubility

PFAs consist of a hydrophobic (nonpolar) fluorocarbon tail and hydrophilic (polar) head group (**Figure 1.1**). The hydrophilic group and small size of the fluorine atoms protecting the carbon chain allow the molecule to become soluble in aqueous medium [17]. PFCAs and PFSAs are two popular amphiphilic groups of PFAs where the subsequent carboxylic or sulfonic group functions as the soluble head group [8]. PFAs are more hydrophobic than their corresponding hydrocarbon cousins. Equivalent conductance measurements [2] have shown that PFCAs are completely dissociated in water. Studies with

PFCAs have shown that solubility tends to decrease with increased fluorination or chain length [16,25-27]. Solubility decreases because micelles (droplets) of PFA come out of solution once the critical micelle concentration (cmc) has been exceeded [27]. PFAs are insoluble in organic solvents. Consequently, PFAs have very low surface energies, referring to their ability to reduce the surface tension of a liquid at an interface. Homologous hydrocarbon surfactants have nearly 50% higher surface energies [22]. Furthermore, PFAs are also lipophobic in that the compound will not partition into lipids or fats.

1.3.4 Nomenclature and Structures

This thesis comprised research using several PFAs. **Table 1.1** describes the nomenclature for those PFAs mentioned in the thesis. The use of the prefix "perfluoro" means the replacement of all hydrogen atoms by fluorine on the carbon chain [3]. Acronyms used to name PFAs describe the chain length and the hydrophilic group. For example, PFPrA or perfluoropropionic acid (**Table 1.1**), is a perfluorinated (PF), propane or 3 carbon (Pr) carboxylic acid (A). Whereas, PFOS or perfluorooctane sulfonic acid stands for perfluorinated (PF), octane or 8 carbon (O), sulfonic acid (S).

Table 1.1 Compound name, acronym, structure, and anionic form of PFAs.

Compound Name	Acronym[a]	Structure	Anion
Perfluoroalkyl sulfonates	*PFSAs*	$CF_3(CF_2)_XSO_3H$	
perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_2OH$	$C_8F_{17}SO_3^-$
Perfluoroalkyl carboxylates	*PFCAs*	$CF_3(CF_2)_XCO_2H$	
perfluoropropionic acid	PFPrA	C_2F_5COOH	$C_2F_5COO^-$
perfluorobutyric acid	PFBA	C_3F_7COOH	$C_3F_7COO^-$
perfluoropentanoic acid	PFPeA	C_4F_9COOH	$C_4F_9COO^-$
perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	$C_6F_{13}COO^-$
perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	$C_7F_{15}COO^-$
perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	$C_8F_{17}COO^-$
perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	$C_9F_{19}COO^-$

[a] From [28]

1.4 ENVIRONMENTAL FATE

1.4.1

General

There are twenty-nine known [29] natural sources of fluorinated organics, and none with more than two fluorine atoms [18]. Production of natural organofluorines mainly occurs in few species of plants in the form of monofluoroacetate (MFA). Polyfluorinated compounds are only synthetic in origin.

PFAs have very long lifetimes because hydrolytic defluorination of compounds with more than one fluorine per carbon is too slow to be of environmental significance [6,18,30]. The only chemical interconversion of PFAs occurs at the carboxylic or sulfonic acid

functional groups, where the hydrogen is lost leaving the anionic form under environmental conditions. Most fully fluorinated organic acids are volatile. Long-range atmospheric transport of PFAs may likely be accomplished through these volatile precursors and allow for global distribution in many environmental compartments. Since, many PFAs would partition into aquatic environments, there is potential for risk to aquatic organisms.

1.4.2 Modes of Environmental Transport

Volatile intermediates, used in the manufacture of many long-chained PFAs, have been detected in air samples and may explain the existence of long-lived PFAs in remote regions. Martin *et al* [7] confirmed the presence of six fluorinated compounds from rural and urban locations, three of which are expected to degrade to PFOS. Furthermore, the thermolysis of fluoropolymers (through incineration) has been suggested as a potential route for more persistent PFCAs, such as trifluoroacetic acid (TFA), to enter the atmosphere [32]. Volatile intermediates can be emitted into the atmosphere at landfills containing fluorochemical waste, volatilization during production processes or degradation of transient compounds already present in the atmosphere [7,18].

Reported Henry's Law constants for PFAs [6] are very small, suggesting that recalcitrant PFAs will favour partitioning into aqueous environments, rather than air, soil or biota, and remain there due to the moderate solubility of the dissociated anion [33]. Environmental deposition may occur as a result of input from consumer products in municipal waste treatment facilities, wet or dry atmospheric deposition from transient volatile derivatives or direct wastewater inputs from fluorochemical production facilities.

1.4.3 PFA Detection and Analysis

Detection of PFAs in 1960's and 1970's consisted of nonspecific analytical methods of poor sensitivity [34-36] and was largely restricted to low-molecular weight PFAs [7]. Currently, there are many methods of detection consisting of liquid chromatography/mass-spectrometry [37], gas chromatography/chemical ionization mass-spectrometry [7,31], ^{19}F-nuclear magnetic resonance [38,39], and high performance liquid chromatography/tandem mass-spectrometry (electrospray HPLC MS/MS) [40] that refine compound selectivity, specificity, and sensitivity. These novel quantitative characterization methods allow detection of PFAs in several matrices such as water, fog, rain, soil, air, and biota.

1.4.4 Environmental Concentrations of PFAs

Known recorded environmental concentrations of PFAs consist primarily of measurements in aqueous media. However, air and soil samples have been monitored, but to a lesser degree [7,41]. PFAs have been detected in precipitation and surface waters [28,31,40]. Background concentrations have been detected up to 0.032 ± 0.010 μg/L for PFOS and as high as 0.017 ± 0.011 μg/L for PFOA [11,28,40]. Elevated concentrations of PFAs have been observed in close proximity to production and manufacturing facilities [40] or aqueous fire fighting activities [11,38]. The maximum concentrations of PFOS and PFOA in surface waters after a fire fighting foam spill near Toronto, Canada were 2210 μg/L and 11.3 μg/L, respectively [28]. PFOS and PFOA concentrations in the Tennessee River, USA downstream from a production facility reached a maximum of 0.14 μg/L and 0.6 μg/L, respectively [40]. Concentrations of PFCAs in groundwater near military fire fighting

facilities ranged from 3 to 7090 μg/L [11].

1.4.5 PFAs in Biological Matrices

1.4.5.1 Detection in humans

It had been known for some time that organic fluorine compounds were present in human blood and sera [34,36,42]. However, it wasn't until methods to identify the structure of PFAs in blood and other biological tissues that these substances were identified as longer-chained PFAs and that they were becoming more prevalent in human tissues [43-47]. Initially, PFAs were found in the blood of exposed 3M workers at concentrations roughly 100 times higher than the general public [37,41,48,49]. In 1995, male employees had serum concentrations ranging from non-detectable (ND) to 12.8 mg PFOS/L [37] and ND to 80.0 mg/L [49], respectively. No adverse health effects were determined for any employee over the duration of the study. Hansen *et al* [47] reported a compound-specific method to quantify PFAs in blood and livers and showed that PFAs are present in nonindustrially exposed humans. Additionally, blood bank samples were analysed from USA, China, Japan, Europe, and Canada and PFOS was found at concentrations between 10 and 100 μg/L [50]. Ubel *et al* [48] discovered a slow elimination process for PFOA in humans when excretion was monitored over 18 months. The elimination of PFAs is discussed in greater detail below.

1.4.5.2 Detection in wildlife

Global biological sampling recently detected certain PFAs, especially PFOS, in many wildlife tissues, such as marine mammals, birds, otters, mink, and fish from both urban and remote locations [51]. PFOS was detected in oysters from Chesapeake Bay, USA at concentrations ranging from < 0.042 to 1.23 μg/g, wet weight [52]. Almost every tissue sample from the continental United States, polar regions, and as far away as Midway Atoll in the middle of the Pacific Ocean, had measurable amounts of PFOS [53,54]. Although most samples contained measurable concentrations of PFOS, frequencies of detection and concentrations of PFOA and other PFAs were lower. PFOA and other PFAs varied in frequency and concentration among samples. Fish liver samples from a reference site in a Canadian creek detected PFHpA, PFOA, and PFDA at concentrations of 0.10, 0.09, and 0.19 μg/g, respectively [28]. PFOS concentrations were several-fold greater in bird and mink samples collected from urbanized and/or industrial areas [53,55]. Importantly, concentrations of PFOS were typically higher in predatory animals such as mink and fish-eating birds than concentrations in their diets [51]. These reports suggest a tendency for PFOS, and perhaps many related PFAs, to bioaccumulate to higher trophic levels.

1.4.6 Routes of Exposure

Routes of human exposure may arise from dermal absorption, ingestion, and inhalation through commercial and industrial products or occupational exposures. PFA-containing products such as microwave popcorn bags, fast-food wrappers, stain-repellants on carpets and textiles, car polishes, and household lubricants may act as a common source

of PFAs for humans. However, the general public is not exposed to PFAs to the same degree as they are to hydrocarbon surfactants [17].

The primary routes of exposure for wildlife occur through the diet [51,56] and, in the case of aquatic organisms, from their environment [28,57]. Authors have shown that concentrations of PFOS are greater in higher trophic levels, such as birds, mink, dolphins, and polar bears than their primary food source, fish [51,53,54].

1.4.7 Retention and Metabolism of PFAs

The primary target organs of PFAs are the liver and blood plasma. PFOS [54], perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) [58,59] have been stated to bind to specific proteins in liver and blood then undergo enterohepatic re-circulation. This property allows PFNA, PFOS, and PFDA to remain in biological systems for very long periods. In contrast, PFAs < 9 chain length do not experience enterohepatic re-circulation, but under go a slow renal elimination. In general, the elimination rate for most PFAs is slow and they remain as the parent molecule. Known half-life ($t_{1/2}$) values for PFOS in humans has been estimated at approximately four years [6,60], likewise, a review by the United States Environmental Protection Agency (USEPA) indicated PFOA has a mean serum $t_{1/2}$ in humans of 4.4 years [5]. The major route of elimination for most PFAs in humans is through urine and feces [46].

Routes of elimination for PFAs in wildlife are similar to humans, and rates are smaller. No metabolism of PFA has been observed in laboratory mammals and they are excreted as the parent molecule [58,61]. Reported $t_{1/2}$ for PFOS in biota have been estimated

to be 180 d for monkeys [62], and 89 d for male rats [62,63]. PFDA has been detected in liver of male rats for more than 30 days after a single dose of 50 mg/kg [64]. PFDA has been shown to have a long $t_{1/2}$ in rat tissues [58]. PFCAs < PFNA have been shown to have faster urinary elimination rates in rodents and rhesus monkeys [65]. PFOA was detected in male rats up to 26 days [59,66-68].

1.5 TOXICITY OF PERFLUORINATED ORGANIC ACIDS

1.5.1 General

The current state of knowledge of PFA toxicity is primarily laboratory terrestrial mammal data. Very little published toxicity data exists for aquatic organisms. The most commonly utilised PFA moieties are ≥7 carbons in length, which include PFHpA, PFOA, PFOS, PFNA, and PFDA. A list of some known toxicity data with laboratory organisms is presented in **Table 1.2**.

1.5.2 Perfluorinated Carboxylates

The shortest fluoroorganic acid, trifluoroacetic acid (TFA) has been extensively investigated by others [30,69-71]. TFA has been shown to exhibit low to moderate phytotoxicity to macrophytes, like *Lemna gibba*, under laboratory and field conditions [69,70]. No toxicity is apparent for microbial communities [71], invertebrates, and freshwater algae [30]. Longer-chained PFCAs such as PFOA and PFDA have been shown to be potent peroxisome proliferators [72-76] and PFDA is the most potent

Table 1.2 Summary of laboratory toxicity data for three commonly tested PFAs.

Compound	Test Organism	Effect Endpoint[a] (95% C.I. if available)	Reference
PFOA	rat (Charles River)	LD50 = 540 mg/Kg (389, 749)	[5]
	rat (Fisher 344)	LD50 = 189 mg/Kg	[93]
Ammonium	*Pimephales promelas*	LC50 = 301 mg/L	[5]
salt of PFOA	*D. magna*	EC50 = 221 mg/L (immobility)	[5]
	S. capricornutum	EC50 = 310 mg/L (growth)	[5]
PFOS	rat (NA)	LD50 = 251 mg/Kg	[62]
	Rhesus monkey	LD50 = 4.5 mg/Kg/d	[62]
	P. promelas	LC50 = 9.5 mg/L	[94]
	D. magna	LC50 = 61 mg/L	[94]
	S. capricornutum	EC50growth = 71 mg/L	[94]
PFDA	mouse	LD50 = 103 mg/Kg (66.9, 134)	[90]
	hamster	LD50 = 100 mg/Kg	[90]

[a] Endpoints denoted by LD50 refers to lethal dose (either intraperitoneal or gavage), LC50 refers to lethal concentration, EC50 refers to effect concentration.

peroxisome proliferator described [77]. In contrast, TFA has not been shown to induce proliferation of peroxisomes in rats [78]. PFCAs with a chain length > 7 were shown to disrupt peroxisomal enzymatic activities in the β-oxidation fatty acid metabolic pathway in rodent livers [63,78-82]. The disruption of the peroxisomal fatty acid β-oxidation increases production of free radicals, such as hydrogen peroxide and superoxide, to a greater degree than the enzymes which metabolize them [78,82,83]. Increased amounts of free radicals has been linked to carcinogenesis observed in PFOA-exposed rodents [83,84] and human cells [85]. In addition, increased free radicals can attack and cause oxidative damage to

biomolecules, particularly polyunsaturated fatty acyl chains of phospholipids in biological membranes [86] leading to loss in membrane fluidity and function. Furthermore, the observed down-regulation of gap junction intercellular communication (GJIC) in rats [87] and human kidney cells [18] by PFCAs between 7 and 10 carbons in length has also been hypothesized to initiate hepatocarcinogenesis. Upham *et al* [87] have also shown that non-fluorinated fatty acids with analogous chain lengths (C7 to C10) do not inhibit GJIC. PFCAs interfered dramatically with hepatic lipid (and cholesterol) metabolism [88,89] and regulation of fatty acid binding proteins [73,85]. In particular, PFOA and PFDA were shown to induce cytochrome P450 4A in a dose-dependant manner [81] and PFOA is one of the most powerful inducers of total microsomal cytochrome P450 in mice [78]. Exposure to either PFOA or PFDA has been shown to induce hepatomegaly and decreased food-intake leading to wasting syndrome [25,77,80,90]. Lethality in 50% of the test population was observed in male rats at 189 mg/Kg and 41 mg/Kg of PFOA and PFDA, respectively [77].

Recently, Sanderson *et al* [91] showed that PFOA significantly reduced representative species of a zooplankton community at concentrations between 10 and 70 mg/L. At these concentrations, community diversity was significantly reduced and was dominated by rotifer species. A review by the USEPA reported fathead minnow 96 h LC50 values between 70 and 843 mg/L and *Daphnia magna* 48 h EC50 values ranged from 39 to 1000 mg/L [5]. The report indicated that *Selenastrum capricornutum*, the most sensitive organism, had 96 h EC50 values ranging between 1.2 and 670 mg/L [5].

There is no known mechanism of action for PFAs. The toxicity of MFA, not a perfluorinated compound, is explained by the synthesis of fluoroacetate to fluorocitrate,

which blocks the Krebs tricarboxylic acid cycle at the stage of citric acid [92], and can lead to paralysis of muscles in humans [3]. Researchers using TFA and PFCAs have not been able to mimic this mechanism in biota.

1.5.3 Perfluorooctane Sulfonate

PFOS has gained considerable attention since 3M's decision to phase-out production of PFSAs [6,50,95] on the basis of extreme persistence. Physiological effects due to exposure of PFOS include inhibition of gap junction intercellular communication in rat [87] and dolphin liver epithelial cells [96], uncoupling of oxidative phosphorylation in rabbit cells [18,97], increased liver enzymes, wasting syndrome, hepatocellular hypertrophy, and death in rats dosed at ≥2 mg/kg/day over 19 days [6,90]. Studies in Rhesus monkeys showed lethality at 7 weeks for a dose of 4.5 mg/kg/day [6].

Fish 96 h lethality testing by The Environmental Agency [94] reported LC50 values of 9.5, 11, and 68 mg/L for fathead minnow, rainbow trout, and bluegill sunfish, respectively (**Table 1.2**). Fish leukocytes lost membrane integrity in a concentration dependent fashion in studies with PFOS by Hu *et al* [98]. It has been proposed that acute toxicity to fish species was due to complications related to surface tension [17]. The 48 h acute toxicity test using PFOS with *Daphnia magna* was 61 mg/L [94]. In contrast, the lithium salt of PFOS showed a 48 h lethality to *D. magna* of 210 mg/L [94]. The 96 h EC50 with freshwater algae (*Selenastrum capricornutum*) was 71 mg/L [94].

1.5.4 Governmental Regulatory Status

Recently, regulatory efforts through the USEPA has focused on PFAs, especially PFCAs and PFSAs. The 3M Co., the major manufacturer of PFOS, decided to discontinue production and use of PFOS, especially in their popular Scotchgard™ line in March 2000 [6,41,50]. The USEPA published the Toxic Substances Control Act (TSCA) Priority Testing List (Section 4e) in May 2000, which included 50 perfluorinated chemicals. The listing was based on potential for long-range atmospheric transport, persistence, bioaccumulation, and bioconcentration as well as the lack of toxicological data [99]. In March 2002, the final rule dealing with any new manufacture or import for any use of PFSAs after December 31, 2002 as a significant new use was first published in the Federal Register No. 67 FR 11008 [6]. The rule prohibits any new use or importation of PFSA-containing products unless otherwise confirmed or stated [100]. The UK has also participated in toxicological assessment of perfluorooctanyl-based chemicals, like PFOS [94].

1.6 RESEARCH OVERVIEW AND HYPOTHESES

1.6.1 Laboratory Studies

1.6.1.1 Acute toxicity testing

Evaluation of carbon-chain length and relative toxicity to common test organisms was examined. Standard acute test protocols were used to evaluate toxicity toward two green algae, one floating macrophyte, and two daphnid species. Chain lengths of PFCAs tested are shown in **Table 1.1**. Endpoints were used to compare the relative sensitivity of each test organism to each PFCA, and to determine if any relationship existed with respect to the

length of the carbon chain. The toxicity of a perfluorinated sulfonate, PFOS (see Chapter 2) was compared with the structurally similar PFCAs, PFNA and PFOA (see Chapter 3), on the organisms mentioned above.

1.6.1.2 Chronic toxicity testing

To evaluate toxicity over larger exposure periods, effects of PFOS and PFNA, on the survival and reproduction of *D. magna* and *D. pulicaria* over 21 d were conducted. This evaluation was performed to compare the sensitivities of homologous compounds (chain length = C8) to overall survival and fecundity during the daphnid life-cycles.

1.6.2 Microcosm Studies

The toxicity of PFOA under natural conditions was evaluated over a 35-day exposure period in the summer of 2000. Assessment included biological sampling of the zooplankton community and *L. gibba* for changes in community structure as well as dynamics and growth, respectively. A second 35-d microcosm study to evaluate the toxic effects of PFOS was also conducted during the summer of 2000. Assessment included the same criteria as the PFOA study. Identification and enumeration of field zooplankton samples took place in the laboratory. Water samples were taken for fate analysis and chlorophyll(a) concentration. Chlorophyll(a) measurements from both microcosm studies were part of separate evaluations, but data were used as a surrogate for phytoplankton in both microcosm studies to improve the overall zooplankton evaluation.

1.6.3 Hypotheses

a) Ho: Relative toxicity of PFAs does not vary as a function of chain length.
Ha: Relative toxicity will vary with increasing chain length.

b) Ho: There is no difference in relative sensitivities between representative secondary and primary trophic levels towards PFAs.
Ha: There is a difference in relative sensitivities between primary and secondary trophic levels.

c) Ho: PFOS will not be the most toxic PFA to freshwater organisms.
Ha: PFOS will be the most toxic to freshwater organisms.

1.7 REFERENCES

1 Ameduri B, Boutevin B. 1997. Telomerisation reactions of fluorinated alkenes. In: Chambers RD, ed. *Organofluorine chemistry: fluorinated alkanes and reactive intermediates*, Springer-Verlag Berlin, Germany. p 165-223.

2 Kauck EA, Diesslin AR. 1951. Some properties of perfluorocarboxylic acids. *Ind Engine Chem* 43:2332-2339.

3 Hudlicky M. 1971. *Organic fluorine chemistry.* Plenum Press, New York, NY, USA

4 Banks RE. 1979. *Organofluorine chemicals and their industrial applications.* Banks RE, ed, Ellis Harwood Ltd., West Sussex, UK 255 p.

5 USEPA. 2002. Hazard assessment of perfluorooctanoic acid and its salts. Report No.: Office of Pollution Prevention and Toxics; U.S. Environmental Protection Agency. Washington, D.C.

6 USEPA. 2000. Perfluorooctyl sulfonates; proposed significant new use rule. Report No. No. 40 CFR 721; OPPTS-50639: USEPA. Washington DC, USA.

7 Martin JW, Muir DCG, Moody CA, Ellis DA, Kwan WC, Solomon KR, Mabury SA. 2002. Collection of airborne fluorinated organics and analysis by gas chromatography/ chemical ionization mass spectrometry. *Anal Chem* 74:584-590.

8 Fielding HC. 1979. Organofluorine surfactants and textile chemicals. In: Banks RE, ed. *Organofluorine chemicals and their industrial applications*, Ellis Horwood Ltd. West Sussex, UK. p 255.

9 Saunders BC. 1972. Chemical characteristics of the carbon-fluorine bond. In: foundation C, ed. *Carbon-fluorine compounds: chemistry, biochemistry, and biological activities*, Associated Scientific Publishers (Elsevier) Amsterdam. p 413.

10 3M News. 2000. 3M phasing out some of it's specialty materials. Report No.: USEPA OPPT. St. Paul, MN, USA.

11 Moody CA, Field JA. 1999. Determination of perfluorocarboxylates in groundwater impacted by fire-fighting activity. *Environ Sci Technol* 33:2800-2806.

12 Moody CA, Field JA. 2000. Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams. *Environ Sci Technol* 34:3864-3870.

13 Karsa DR, ed. 1989. *Industrial applications of surfactants II.* Whitstable Litho Printers Ltd., Whitstable, Kent, UK, 401 p.

14 Filler R. 1979. Fluorine-containing drugs. In: Banks RE, ed. *Organofluorine chemicals and their industrial applications*, Ellis Horwood Ltd. West Sussex, UK. p 255.

15 Alliance Pharmaceutical Corp. 2001. Core Technologies; perfluorochemicals Alliance Core Technologies August 16, 2001.

16 Chittofrati DL, Sanguineti A, Visca M, Gambi C, Senatra D, Zhen Z. 1989. Perfluorinated surfactants at the perfluoropolyether-water interface. *Colloids and Surfaces* 41:45-59.

17 Kissa E. 2001. *Fluorinated surfactants and repellents.* Hubbard AE, ed, Marcel Dekker, Inc., New York, NY, USA 615 p.

18 Key BD, Howell RD, Criddle CS. 1997. Fluorinated organics in the biosphere. *Environ Sci Technol* 31:2445-2454.

19 Manning RO, Bruckner JV, Mispagel ME, Bowen JM. 1991. Metabolism and disposition of sulfluramid, a unique polyfluorinated insecticide, in the rat. *Am Soc Pharm Exp Therap* 19:205-211.

20 USEPA. 1999. EPA new pesticide fact sheet; lithium perfluorooctane sulfonate (LPOS). Report No. EPA-730-F-99-009: United States Environmental Protection

Agency. Washington D.C., USA.

21 Sharpe AG. 1971. The physical properties of the carbon-fluorine bond. In: Ciba foundation, ed. *Carbon-fluorine compounds: chemistry, biochemistry and biological activities*, Associated Scientific Publishers London, UK. p 33-54.

22 Bryce HG. 1964. General Chemistry. In: Simons JH, ed. *Fluorine Chemistry*, Academic Press Inc. New York, NY, USA. p 506.

23 Key BD, Howell RD, Criddle CS. 1998. Defluorination of organofluorine sulfur compounds by *Pseudomonas* sp. strain D2. *Environ Sci Technol* 32:2283-2287.

24 Remde A, Debus R. 1996. Biodegradability of fluorinated surfactants under aerobic and anaerobic conditions. *Chemosphere* 32:1563-1574.

25 Goecke-Flora CM, Wyman JF, Jarnot BM, Reol NV. 1995. Effect of the peroxisome proliferator perfluoro-*n*-decanoic acid on glucose transport in the isolated perfused rat liver. *Chem Res Toxicol* 8:77-81.

26 Sharma R. 1995. Surfactant adsorption and surface solubilization: an overview. In: Sharma R, ed. *Surfactant adsorption and surface solubilization*. 615 ed, American Chemistry Society, by Maple Press, York, PA Washington, DC, USA. p 400.

27 Sugihara G, Nagadome S, Yamashita T, Kawachi N, Takagi H, Moroi Y. 1991. Cationic surfactants with perfluorocarboxylates as counterion: solubility and micelle formation. *Colloids and Surfaces* 61:111-121.

28 Moody CA, Martin JW, Kwan WC, Muir DCG, Mabury SA. 2002. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. *Environ Sci Technol* 36:545-551.

29 Gribble GW, Worman JJ. 2000. Into the 21st Century: natural sources of organohalogen compounds. Book of abstracts for the SETAC 21st annual meeting, Nashville, TN, USA. SETAC. p 334.

30 Boutonnet JC, Bingham P, Calamari D, de Rooij C, Franklin J, Kawano T, Libre JM, McCulloch A, Malinverno G, Odom JM, Rusch GM, Smythe K, Sobolev I, Thompson RS, Tiedje JM. 1999. Environmental risk assessment of trifluoroacetic acid. *Hum Ecol Risk Assess* 5:59-124.

31 Scott BF, Moody CA, Spencer C, Martin JW, Sargeant DB, Muir DCG, Mabury SA. 2002. Analysis for perfluoroalkanoic acids/ anions in surface waters and precipitation using GC/MS and LC/MS/MS. *To be submitted.*

32 Ellis DA, Mabury SA, Martin JW, Muir DCG. 2001. Thermolysis of fluoropolymers as a potential source of halogenated organic acids in the environment. *Nature* 412:321-324.

33 Wujcik C, Cahill TM, Seiber JN. 1998. Extraction and analysis of trifluoroacetic acid in environmental waters. *Anal Chem* 70:4074-4080.

34 Taves DR. 1968. Evidence that there are two forms of fluoride in human serum. *Nature* 217:1050-1053.

35 Sharma R, Pyter R, Mukerjee P. 1989. Spectrophotometric determination of perfluoro carboxylic acids (heptanoic and decanoic) and sodium perfluorooctanoate and decyl sulfate in mixtures by dye-extraction. *Analyt Lett* 22:999-1007.

36 Guy WS. 1976. Inorganic and organic fluorine in human blood; 1976; Washington, DC. American Chemistry Society. p 117.

37 Olsen GW, Burris JM, Mandel JH, Zobel LR. 1999. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. *J Occup Environ Med* 41:799-806.

38 Moody CA, Kwan WC, Martin JW, Muir DCG, Mabury SA. 2001. Determination of perfluorinated surfactants in surface water samples by two independent analytical techniques: liquid chromatography/ tandem mass spectrometry and 19F NMR. *Anal Chem* 73:2200-2206.

39 Ellis DA, Martin JW, Muir DCG, Mabury SA. 2000. The development of an 19F NMR method for the analysis of fluorinated acids in the environment. *Anal Chem* 72:726-731.

40 Hansen KJ, Johnson HO, Eldridge JS, Butenhoff JL, Dick LA. 2002. Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. *Environ Sci Technol* 36:1681-1685.

41 Weber J. 2000 June 5, 2000. 3M' s big cleanup. Business Week:73.

42 Guy WS, Taves DR, Brey WS. 1976. Organic fluorocompounds in human plasma: prevalence and characterization. In: Filler R, ed. *Biochemistry Involving Carbon-Fluorine Bonds; ACS Symposium Series No 28*, American Chemistry Society Chicago, IL. p 117-134.

43 Belisle J, Hagen DF. 1980. A method for the determination of perfluorooctanoic acid in blood and other biological samples. *Anal Biochem* 101:369-376.

44 Venkateswarlu P. 1982. Sodium biphenyl method for determination of covalently bound fluorine in organic compounds and biological materials. *Anal Chem* 54:1132-1137.

45 Greene RJ, Brashear WT, Auten KL, Mahle DA. 1992. Confirmation of a carboxylic acid metabolite of perchlorotrifluoroethylene and a method for its GC-ECD analysis in biological matrices. *J Anal Toxicol* 16:28-32.

46 Kissa E. 1994. Fluorinated surfactants in blood. *J Fluorine Chem* 66:5-6.

47 Hansen KJ, Clemen LA, Ellefson ME, Johnson HO. 2001. Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ Sci Technol* 35:766-770.

48 Ubel FA, Sorenson SD, Roach DE. 1980. Health status of plant workers exposed to fluorochemicals - a preliminary report. *Am Ind Hyg Assoc J* 41:584-590.

49 Olsen GW, Gilliland FD, Burluv MM, Burris JM, Mandel JS. 1998. An epidemiologic investigation of reproductive hormones in men with occupational exposure to perfluorooctanoic acid. *J Exper Med* 40:614-622.

50 Brown D, Mayer CE. 2000 May 17, 2000. 3M to pare Scotchgard products. The Washington Post.

51 Giesy JP, Kannan K. 2001. Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* 35:1339-1342.

52 Kannan K, Hansen KJ, Wade TL, Geisy JP. 2002. Perfluorooctane sulfonate in Oysters, *Crassostrea virginica*, from the Gulf of Mexico and the Chesapeake Bay, USA. *Arch Environ Contam Toxicol* 42:313-318.

53 Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP. 2001. Perfluorooctane sulfonate in fish-eating birds including bald eagles and albatrosses. *Environ Sci Technol* 35:3065-3070.

54 Kannan K, Corsolini S, Falandysz J, Oehme G, Focardi S, Giesy JP. 2002. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from the coasts of the Baltic and the Mediterranean Seas. *Environ Sci Technol* 36:3210-3216.

55 Kannan K, Newsted J, Halbrook RS, Giesy JP. 2002. Perfluorooctane sulfonate and related fluorinated hydrocarbons in mink and river otters from the United States. *Environ Sci Technol* 36:2566-2571.

56 Martin JW, Mabury SA, Solomon KR, Muir DCG. 2003. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 22:189-195.

57 Martin JW, Mabury SA, Solomon KR, Muir DCG. 2003. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout. *Environ Toxicol Chem* 22:196-204.

58 Vanden Heuvel JP, Kuslikis BI, Van Rafelghem MJ, Peterson RE. 1991. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J Biochem Toxicol* 6:83-92.

59 Goecke-Flora CM, Reo NV. 1996. Influence of carbon chain length on the hepatic effects of perfluorinated fatty acids. A ^{19}F- and ^{31}P-NMR investigation. *Chem Res Toxicol* 9:689-695.

60 3M. 2000. 3M fluorochemical EPA submissions. Report No. 1: 3M Center Ltd. St. Paul, MN, USA.

61 Goecke CM, Jarnot BM, Reo NV. 1992. A comparitive toxicological investigation of perfluorocarboxylic acids in rats by fluorine-19 NMR spectroscopy. *Chem Res Toxicol* 5:512-519.

62 3M. 2000. Sulfonated perfluorochemicals in the environment: sources, dispersion, fate, and effects. U.S. Environmental Protection Agency public docket No. AR226-0620: 3M Co. St. Paul, MN.

63 Vanden Heuvel JP, Kuslikis BI, Shrago E, Peterson E. 1991. Inhibition of long-chain acyl-CoA synthetase by the peroxisome proliferator perfluorodecanoic acid in rat hepatocytes. *Biochem Pharm* 42:295-302.

64 Van Rafelghem MJ, Van Heuvel JP, Menahan LA, Peterson RE. 1988. Perfluorodecanoic acid and lipid metabolism in the rat. *lipids* 23:671-678.

65 Griffith FD, Long JE. 1980. Animal toxicity studies with ammonium perfluorooctanoate. *Am Ind Hyg Assoc J* 41:576-583.

66 Ophaug RH, Singer L. 1980. Metabolic handling of perfluorooctanoic acid in rats. *Proc Soc Exp Biol Med* 163:19-23.

67 Hanhijarvi H, Ophaug RH, Singer L. 1982. The sex-related difference in perfluorooctanoate excretion in the rat. *Proc Soc Exp Biol Med* 171:50-55.

68 Hanhijarvi H, Ylinen M, Kojo A, Kosma V. 1987. Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the wistar rat. *Pharm Toxicol* 61:66-68.

69 Berends AG, Boutonnet JC, Rooij CG, Thompson RS. 1999. Toxicity of trifluoroacetate to aquatic organisms. *Environ Toxicol Chem* 18:1053-1059.

70 Hanson ML, Sibley PK, Mabury SA, Solomon KR, Muir DCG. 2002. Trichloroacetic acid (TCA) and trifluoroacetic acid (TFA) mixture toxicity to the macrophytes *Myriophyllum spicatum* and *Myriophyllum sibiricum* in aquatic microcosms. *Sci Total Environ* 285:247-259.

71 Benesch JA, Gustin MS, Cramer GR, Cahill TM. 2002. Investigation of effects of trifluoroacetate on vernal pool ecosystems. *Environ Toxicol Chem* 21:640-647.

72 Intrasuksri U, R F. 1991. Comparison of the effects of selected monocarboxylic, dicarboxylic and perfluorinated fatty acids on peroxisome proliferation in primary cultured rat hepatocytes. *Biochem Pharm* 42:184-188.

73 Issemann I, Prince R, Tugwood J, Green S. 1992. A role for fatty acids and liver fatty acid binding protein in peroxisome proliferation. *Biochem Soc Trans* 20:824-827.

74 Vanden Heuvel JP. 1996. Perfluorodecanoic acid as a useful pharmacologic tool for the study of peroxisome proliferation. *Gen Pharm* 27:1123-1129.

75 Reo NV, Narayanan L, Kling KB, Adinehzadeh M. 1996. Perfluorodecanoic acid, a peroxisome proliferator, activates phospholipase C, inhibits CTP: phophocholine cytidylytransferase, and elevates diacylglycerol in rat liver. *Toxicol Lett* 86:1-11.

76 Intrasuksri U, Rangwala SM, O'Brien M, Noonan DJ, Feller DR. 1998. Mechanisms of peroxisome proliferation by perfluorooctanoic acid and endogenous fatty acids. *Gen Pharm* 31:187-197.

77 Harrison EH, Lane JS, Luking S, Van Rafelghem MJ, Andersen ME. 1988. Perfluor-*n*-decanoic acid: induction of peroxisomal oxidation by a fatty acid with dioxin-like toxicity. *lipids* 23:115-119.

78 Permadi H, Lundgren B, Andersson K, DePierre JW. 1992. Effects of perfluoro fatty acids on xenobiotic-metabolizing enzymes which detoxify reactive forms of oxygen and lipid peroxidation in mouse liver. *Biochem Pharm* 44:1183-1191.

79 Borges T, Gauert HP, Chen L, Robertson LW. 1990. Effect of the peroxisome proliferator perfluorodecanoic acid on growth and lipid metabolism in Sprague

Dawley rats fed three dietary levels of Selenium. *Arch Toxicol* 64:26-30.

80 Borges, Robertson LW, Peterson RE, Glauert HP. 1992. Dose-related effects of perfluorodecanoic acid on growth, feed intake and hepatic peroxisomal B-oxidation. *Arch Toxicol* 66:18-22.

81 Diaz MJ, Chinje E, Kentish P, Jarnot B, George M, Gibson G. 1994. Induction of cytochrome P4504A by the peroxisome proliferator perfluoro-n-octanoic acid. *Toxicol* 86:109-122.

82 Kawashima Y, Suzuki S, Kozuka H, Sato M, Suzuki Y. 1994. Effects of prolonged administration of perfluorooctanoic acid on hepatic activities of enzymes which detoxify peroxide and xenobiotic in the rat. *Toxicol* 93:85-97.

83 Glauert HP, Srinvasan S, Tatum VL, Chen L, Saxon DM, Lay LT, Borges T, Baker M, Chen LH, Robertson LW, Chow CK. 1992. Effects of the peroxisome proliferators ciprofibrate and perfluorodecanoic acid on hepatic cellular antioxidants and lipid peroxidation in rats. *Biochem Pharm* 43:1353-1359.

84 Abdellatif AG, Preat V, Taper HS, Roberfroid M. 1991. The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. *Toxicol Appl Pharm* 111:530-537.

85 Maloney EK, Waxman DJ. 1999. trans-Activation of PPAR and PPAR by structurally diverse environmental chemicals. *Toxicol Appl Pharm* 161:209-218.

86 Josephy PD, Mannervik B, Oritz de Montellano P. 1997. *Molecular toxicology.* Oxford University Press, New York, Oxford 368 p.

87 Upham BL, Deocampo ND, Wurl B, Trosko JE. 1998. Inhibition of gap junction intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. *Int J Cancer* 78:491-495.

88 Davis JW, Vanden Heuvel JP, Peterson RE. 1991. Effects of perfluorodecanoic acid on *de novo* fatty acid and cholesterol synthesis in the rat. *Lipids* 26:857-859.

89 Reo NV, Goecke CM, Narayanan L, Jarnot BM. 1994. Effects of perfluoro-n-octanoic acid, perlfuoro-n-decanoic acid, and clofibrate on hepatic phosphorus metabolism in rats and guinea pigs *in vivo*. *Toxicol Appl Pharm* 124:165-173.

90 Van Rafelghem MJ, Mattie DR, Bruner RH, Andersen ME. 1987. Pathological and hepatic ultrastructure effects of a single dose of perfluoro-n-decanoic acid in the rat, hamster, mouse and guinea pig. *Fundam Appl Toxicol* 9:522-540.

91 Sanderson H, Boudreau TM, Mabury SA, Solomon KR. 2002. Impact of perfluorooctanoic acid on the structure of the zooplankton community in indoor microcosms. *Aquat Toxicol* Accepted August 1.

92 Peters SR. 1972. Some metabolic aspects of fluoroacetate especially related to fluorocitrate. In: Ciba foundation, ed. *Carbon-fluorine compounds; chemistry, biochemistry, and biological activities*, Associated Scientific Publishers Amsterdam, The Netherlands. p 417.

93 Olson CT, Andersen ME. 1983. The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. *Toxicol Appl Pharm* 70:362-372.

94 The Environmental Agency (UK). 2000. Review of environmental effects data of perfluorooctanylsulfonate (PFOS)-based substances. Report No.: National Centre for Ecotoxicology and Hazardous Substances. Wallingford, Oxfordshire, UK.

95 Weber J. 2000 June 5, 2000. The path to a painful choice. Businessweek.

96 Hu W, Jones PD, Upham BL, Trosko JE, Lau C, Giesy JP. 2002. Inhibition of gap junction intracellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines *in vitro* and Sprague-Dawley rats *in vivo*. *Toxicol Sci* 68:429-436.

97 Schellmann RG. 1990. The cellular effects of a unique pesticide sulfluramid (N-ethylperfluorooctane sulphonamide) on rabbit renal proximal tubules. *Toxicol In Vitro* 4:71-74.

98 Hu W, King LE, Jones PD, Giesy JP. 2000. Measurements of effects of perfluorinated compounds on cell membrane fluidity by flow cytometry. Book of abstracts for the SETAC 21st Annual Meeting in Nashville, TN, USA. 314 p.

99 USEPA, Interagency Testing Committee. 2000. Forty-sixth report of the TSCA interagency testing committee to the administrator, US environmental protection agency. Report No. 46: USEPA. Washington, DC.

100 USEPA. 2002. Perfluorooctyl sulfonates; significant new use rule; final rule and supplemental proposed rule. Report No. Federal Register: 40 CFR Part 721; OPPTS-50639; FRL-6745-5: USEPA. Washington, DC.

2 LABORATORY EVALUATION OF THE TOXICITY OF PERFLUOROOCTANE SULFONATE (PFOS) ON *SELENASTRUM CAPRICORNUTUM, CHLORELLA VULGARIS, LEMNA GIBBA, DAPHNIA MAGNA*, AND *DAPHNIA PULICARIA*

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2.1 ABSTRACT

Perfluorooctane sulfonate (PFOS) is an anthropogenic compound found in trace amounts in many environmental compartments far from areas of production. This, along with the highly persistent nature of PFOS, presents a concern for possible effects in aquatic ecosystems. The objective of this study was to determine the toxicity of PFOS in representative freshwater organisms. Toxicity testing using standard laboratory protocols was performed on the green algae *Selenastrum capricornutum* and *Chlorella vulgaris*, the floating macrophyte *Lemna gibba*, and the invertebrates *Daphnia magna* and *Daphnia pulicaria*. The no observable effect concentration (NOEC) values were generated from the most sensitive endpoints for all organisms. Autotroph inhibition of growth NOEC values

were 5.3, 8.2, and 6.6 mg/L for *S. capricornutum*, *C. vulgaris*, and *L. gibba*, respectively. The 21-day immobility NOEC values for *D. magna* and *D. pulicaria* were 0.8 and 13.6 mg/L, respectively. In comparison to immobility, the 21-day lethality NOEC for *D. magna* was 5.3 mg/L. Based on effect (immobility) values, the most sensitive of all test organisms was *D. magna*. The most sensitive organism based on 50% inhibition of growth (IC50) was *L. gibba*, with an IC50 value of 31.1 mg/L determined from wet weight. This is 4.3 times less than the LC50 for *D. pulicaria*, which was 134 mg/L. Significant adverse effects ($p \leq 0.05$) were observed for all organisms in concentrations > 134 mg/L. The results indicate that, under laboratory conditions, PFOS is acutely toxic to freshwater organisms at concentrations at or near 100 mg/L. Based on known environmental concentrations of PFOS, which occur in the low ng/L to low µg/L range, there is no apparent risk to freshwater systems. However, further work is required to investigate long-term effects in these and other freshwater organisms.

2.2 INTRODUCTION

Perfluorooctane sulfonic acid (PFOS) is a unique substance that reduces surface tension and is thus generally known as a surfactant. PFOS has extreme thermal, biological, and chemical stability as well as hydrophobic and lipophobic characteristics. These properties make it superior to 'conventional' hydrocarbon surfactants (Banks 1979) in that it is more resistant to breakdown processes and requires less product to perform the same task. It is widely used in commercial and industrial applications such as insecticides, food wrapper coatings, stain-repellents, corrosion inhibitors, and especially, aqueous fire fighting

foams (Karsa 1989; Moody *et al.* 2002). Fluorinated surfactant properties are influenced by organofluorine chemistry. The carbon-fluorine bond is the strongest halogen-carbon bond. Hydrolytic defluorination of the fluorocarbon chain has not been observed (Key *et al.* 1997) so fluorinated surfactants are judged to have extremely long life-times. The only publicly available data on PFOS persistence in biota is half-life ($t_{1/2}$) values reported to be approximately 1428 d (USEPA 2000a), 180 d, and 89 d (USEPA 2000b) for humans, monkeys, and male rats, respectively. In the environment, PFOS is metabolically and chemically inert, resisting both biotic (Remede and Debus 1996; Key *et al.* 1998) and abiotic degradation (Sharpe 1971; Boudreau *et al.* 2002). These properties have resulted in growing public and scientific concern regarding potential hazards to aquatic environments.

PFOS has recently been detected in wildlife, including fish, birds, and mammals, from urban and remote areas around the world (Giesy and Kannan 2001; Kannan *et al.* 2001). Researchers also reported that PFOS was detected in human blood samples from the United States, Japan, China, and Europe (Olsen *et al.* 1999; Brown and Mayer 2000; Weber 2000) at levels between 10 and 100 parts per billion. Short-chain fluorinated compounds like trifluoroacetic acid (Wujcik *et al.* 1998; Boutonnet *et al.* 1999; Ellis *et al.* 2001) and chlorodifluoroacetic acid (Martin *et al.* 2001) have also been detected in precipitation and environmental waters. Moreover, Martin *et al.* (2002a) have detected high molecular-weight fluoroorganics in urban and rural air samples that are possible precursors to PFOS. In 2000, the US production volume of PFOS chemicals used in consumer applications totaled 5.6 million pounds (USEPA 2000a). After finding detectable amounts in blood of exposed workers (Ubel *et al.* 1980; Olsen *et al.* 1999), combined with the recalcitrant nature of PFOS,

the major manufacturer, 3M (St. Paul, MN), announced that it would phase out production of this compound (Brown and Mayer 2000; Renner 2001).

PFOS was reported to have a very low Henry's Law Constant indicating that volatilization from water to air is highly improbable (USEPA 2000a; USEPA 2000c). Therefore, aquatic environments could be a significant potential sink for PFOS. Moreover, bioaccumulation studies on PFOS in rainbow trout by Martin *et al.* (2002b; 2002c) have reported a steady state bioconcentration factor and biomagnification factor for whole carcass, based on wet weight, of 1100 ± 150 L/kg and 0.32 ± 0.05, respectively. This indicates a potential for accumulation in fish. Evidence to support this is reported by Giesy and Kannan (2001) where fishes collected from Michigan waters contained between 17 and 170 ng/g PFOS in their livers (primary target organ). Furthermore, PFOS production plants, product treatment plants, and fire fighting training facilities are often located in close proximity to both freshwater and groundwater systems (Moody and Field 1999). Hansen *et al.* (2002) detected measurable levels (mean was 32 ± 11 ng/L) of PFOS upstream from a fluorochemical manufacturing facility in Decatur, AL, USA. Large historical production volumes and extreme persistence of PFOS put some aquatic environments at potential risk of PFOS contamination. Moody *et al.* (2002) detected PFOS in the range of 2.0 - 72.9 μg/g in fish liver samples following an accidental release of fire-fighting foam into a creek (Etobicoke, Canada).

Very little is known about the toxicity of PFOS in aquatic ecosystems. To date, PFOS toxicity has been studied mainly on rodents and monkeys with little emphasis on aquatic organisms. Laboratory testing of PFOS, identified as a priority chemical, is required

before the United States Environmental Protection Agency can make regulatory decisions under the mandate of the Toxic Substances Control Act (USEPA 2000c; Sanderson *et al.* 2002). The focus of this research, therefore, was to investigate and compare the relative toxicity of PFOS on freshwater laboratory bioassay organisms.

2.3 MATERIALS AND METHODS

2.3.1 Test Compound

The potassium salt of perfluorooctane sulfonate ($(CF_3)(C_7F_{14}SO_3\text{-}K)$) was used for all toxicity tests and was supplied by 3M Co. (3M Center, St. Paul, MN). PFOS-K purity was 95% dry mass. All treatment concentrations were based on the PFOS anion (without K+). Due to the hydrophobic nature of PFOS, aggregation into micelles occurs in water beyond the critical micelle concentration (CMC) and could confound exposures in aqueous medium. PFOS has a CMC of 370 mg/L for freshwater and 570 mg/L for pure water (USEPA 2000a). In our tests, laboratory-grade distilled water was used for all solutions with maximum concentrations derived from stock solutions no greater than 450 mg/L. Formation of micelles was not evident in any of the test concentrations.

2.3.2 Organisms

Selenastrum capricornutum, recently renamed *Pseudokirchneriella subcapitatum* (UTCC 37) and *Chlorella vulgaris* (UTCC 266) were obtained as slants from the University of Toronto Culture Collection (UTCC; Toronto, ON, Canada). Axenic stocks of algae were cultured according to American Society for Testing and Materials (ASTM) guidelines

(Designation E 1218-97a; ASTM 1999a) and a standard operating procedure (SOP) developed by the Centre for Toxicology. The Centre for Toxicology had an existing axenic laboratory culture of *Lemna gibba* G3, maintained according to Marwood *et al.* (2001) and originally acquired from the University of Waterloo (Waterloo, ON, Canada). *Daphnia magna* were obtained from a brood stock (Dm99-23) at ESG International (Guelph, ON, Canada). *Daphnia pulicaria* were acquired from a brood stock maintained in the Department of Zoology at the University of Guelph (Guelph, ON, Canada). *Daphnia* spp. were cultured for more than three months prior to testing in the laboratory according to a combination of ASTM guidelines (Designation E 1193-97; ASTM 1999c) and a SOP developed by the Centre for Toxicology.

2.3.3 Lab Toxicity Testing

All toxicity testing involved four replicates per treatment plus controls. Toxicity testing consisted of initial range-finder tests followed by at least two definitive tests. Polypropylene or polyethylene containers were used for all stock solutions and test vessels due to our preliminary observations that PFOS has potential to adsorb to glass and Teflon® surfaces. Experiments were conducted in environmental chambers (Balley Refrigeration of Canada Ltd., Brockville, ON, Canada). The pH and temperature of all test solutions were measured at the beginning and termination of all tests.

2.3.4 Green Algae Toxicity Tests

Acute algal growth inhibition tests followed protocols found in ASTM (Designation E 1218-97a; ASTM 1999a) and Geis *et al.* (2000). Test solution volumes for all algae studies were 20 mL in 60 x 15 mm polyethylene disposable petri dishes (Fisher Scientific, Nepean, ON, Canada). Dilution medium for both algal species consisted of Bristol's algal growing media. Nominal concentrations for final testing with *S. capricornutum* and *C. vulgaris* are shown in **Table 2.1**. Endpoints for effects on algae were derived from inhibition of growth, namely cell density and chlorophyll(a) content. Both endpoints were determined using absorbance readings with a Baush & Loumb Spectronic 20 colorimeter/ spectrophotometer (Rochester, NY, USA) at the termination of all tests. Cell density for *S. capricornutum* was calculated using equation [1]:

$$[1] \qquad X = \frac{(Y + a)}{b} \ (\text{x } 10^7 \text{ cells/mL})$$

where Y is the absorbance reading at 460 λ, a is the Y-intercept for *S. capricornutum* standard curve, b is the slope of the standard curve, and X is the concentration of algae in solution. Cell density for *C. vulgaris* was calculated using equation [2]:

$$[2] \qquad X = \frac{(Y - a)}{b} \ (\text{x } 10^7 \text{ cells/mL})$$

Initial inoculation of algal tests was done such that cell density equaled 1.5 x 10^4 cells/mL. Equation [3], below, was used to determine initial test inoculation (Clesceri *et al.* 1989):

$$[3] \qquad A = \frac{(1.5 \text{ x } 10^4 \text{ cells/mL})(V_t)}{C_a}$$

where V_t is the volume of one test vessel, C_a is the original concentration of culture algae,

Table 2.1 Bioassay methods and nominal concentrations used in PFOS laboratory tests.

Species	Classification	Test Method	Test Period	Nominal Concentration Range (mg/L)
S. capricornutum	green algae	Geis *et al.* 2000	96 h	0, 28, 56, 113, 225, and 450
		ASTM E 1218-97a	96 h	0, 12.5, 25, 50, 100, 200, and 400
C. vulgaris	green algae	Geis *et al.* 2000	96 h	0, 28, 56, 113, 225, and 450
		ASTM E 1218-97a	96 h	0, 12.5, 25, 50, 100, 200, and 400
L. gibba	duckweed	ASTM E 1415-91	7 d	0, 10, 20, 40, 80, and 160
D. magna	water flea	ASTM E 729-96	48 h	0, 31, 63, 125, 250, and 450
		ASTM E 1193 -97	21 d	0, 6, 13, 25, 50, and 100
D. pulicaria	water flea	ASTM E 729-96	48 h	0, 31, 63, 125, 250, and 450

and A is the volume of original culture algae needed to inoculate each test vessel.

Chlorophyll(a) was measured according to Clesceri *et al.* (1989). Test temperature was maintained at 23° ± 1° C and continuously illuminated with cool-white fluorescent light between 3800 and 4200 lx measured at start and termination of each test with a Sper Scientific LUX light meter (Fisher Scientific, Nepean, ON, Canada). Suspension of algae cells was accomplished by careful manual shaking of vessels twice a day during testing.

2.3.5 Lemna gibba 7-day Toxicity Test

Acute testing on *Lemna gibba* followed the method developed by Greenburg *et al.* (1992) and Marwood *et al.* (2001). Test solution volumes were 10 mL and the bioassays were conducted in 60 X 15 mm polyethylene disposable petri dishes. Dilution water for all test solutions consisted of Hunter's growing media. Temperature during the test was 25° ± 1° C. Illumination was provided with continuous cool-white fluorescent lighting in the range

of 5800 - 6200 lux. Nominal concentrations of final test solutions are shown in **Table 2.1**. Endpoints used to determine inhibition of growth were mean frond number and biomass, measured as wet weight. The qualitative physical appearance (chlorosis, necrosis, and relative root length) of all treatment plants was recorded.

2.3.6 Daphnia Acute Toxicity Tests

Daphnia acute testing was derived from ASTM guidelines (Designation: E 729-96; ASTM 1999b). Acute testing with *Daphnia* consisted of 48 h exposure periods with neonates < 24 h old. Endpoints were lethality and immobility. Immobility was measured by evidence of appendage movement after gentle probing (ASTM 1999b). If this did not illicit a response, visual inspection by microscope for evidence of a beating heart within a two minute period (ASTM 1999c) was conducted. Test vessels consisted of 225 mL polypropylene disposable containers (Fisher Scientific, Nepean, ON, Canada). Test volume was set at 150 mL for 10 neonates per replicate such that loading density did not exceed 15 mL medium per neonate. Temperature was held constant at 21° ± 1° C with a 16:8 h light:dark photo period under cool-white fluorescent light between 380 and 480 lx. Organisms were not fed during acute tests. Dilution water was clean well water obtained from ESG International. Hardness was softened by addition of distilled deionized water to achieve a range of 200 to 225 mg/L of $CaCO_3$. Nominal concentrations of final test solutions are provided in **Table 2.1**.

2.3.7 Daphnia magna Life-cycle Test

The chronic test with *D. magna* was conducted using ASTM guidelines (Designation: E 1193-97; ASTM 1999c). The procedure consisted of a 21 d reproduction test beginning with < 24 h old neonates. Endpoints included adult survival, number of days to produce first

brood, mean number of young per adult, and mean number of young produced at each brood. Three neonates were exposed in 120 mL solution per replicate such that loading density did not exceed 40 mL per neonate. Test conditions, dilution water, and test vessels were the same as acute testing. The test medium was replaced weekly. Organisms were fed a contaminant-free mixture of *S. capricornutum* and *C. vulgaris* combined with a yeast-trout chow-cerophyll supplement three times per week. Nominal concentrations of final test solutions are shown in **Table 2.1**.

2.3.8 Statistical Analysis

All data were characterized for normality and equal variance by a Shapiro-Wilk's test in ANOVA. If the test failed, data was square root or natural log transformed to meet assumptions. If significance was found ($p \leq 0.05$) using ANOVA, treatments were compared to controls using Dunnett's test in Sigmastat 2.0 (Jandel 1995; San Rafel, CA, USA). Inhibition of growth (IC50) was determined using the linear interpolation method (ICp version 2.0) developed by EPAstats (Norberg-King 1993; Duluth, MN, USA). A surrogate value for the NOEC, was used by calculating an IC10 value using the ICp program. This surrogate has been recommended as an accurate estimation of the no observed effect concentration in general toxicity testing when inverse regression techniques cannot be used (Van Der Hoeven 1997). Trimmed Spearman-Karber 1.5 or Probit 1.5 (EPAstats; Duluth, MN, USA) were used to evaluate invertebrate lethality values. LC50 and LC10 (again used for NOEC), as well as EC50s for immobility, were calculated in this manner.

2.4 RESULTS

All results reported were based on nominal concentrations because actual test solutions have not been analysed to date. Only significant results are reported.

2.4.1 Algal Toxicity Tests

PFOS was found to significantly ($p \leq 0.05$) inhibit growth in both *S. capricornutum* and *C. vulgaris* in 4-day growth inhibition tests at concentrations ≥ 50 mg/L. *Selenastrum capricornutum* showed a cell density IC50 of 48.2 mg/L and chlorophyll(a) concentration IC50 of 59.2 mg/L (**Table 2.2**). *Chlorella vulgaris* was slightly less sensitive than *S. capricornutum*, with IC50s of 81.6 and 88.1 mg/L for cell density and chlorophyll(a), respectively. *Selenastrum capricornutum* had a NOEC of 5.3 mg/L, derived from cell density. A NOEC of 8.2 mg/L for *C. vulgaris* was also derived from cell density. *Selenastrum capricornutum* was slightly more sensitive with a NOEC value 35% lower than that of *C. vulgaris*. Chlorophyll(a) content was the least sensitive endpoint for both *S. capricornutum* and *C. vulgaris*. Cell growth in both algal species was not evident in test concentrations ≥ 200 mg/L.

Table 2.2 Endpoint effect values (mg/L) for PFOS laboratory tests.

Species name	Endpoint[a]	IC50, LC50, EC50 in mg/L (Confidence Interval)	NOEC value[b] in mg/L (Confidence Interval)
S. capricornutum	cell density	48.2 (45.2, 51.1)	5.3 (4.6, 6.8)
	chlorophyll(a)	59.2 (50.9, 67.4)	16.6 (8.5, 28.1)
C. vulgaris	cell density	81.6 (69.6, 98.6)	8.2 (6.4, 13.0)
	chlorophyll(a)	88.1 (71.2, 104)	9.6 (7.6, 16.5)
L. gibba	frond number	59.1 (51.5, 60.3)	29.2 (27.5, 32.1)
	wet weight	31.1 (22.2, 36.1)	6.6 (4.5, 13.6)
D. magna	48 h survival	130 (112, 136)	33.1 (32.8, 34.1)
	48 h immobility	67.2 (31.3, 88.5)	0.8 (0.6, 1.3)
	21 d adult survival	42.9 (31.7, 56.4)	5.3 (2.5, 9.2)
D. pulicaria	48 h survival	169 (136, 213)	46.9 (33.1, 65.3)
	48 h immobility	134 (103, 175)	13.6 (2.2, 33.1)

[a] The two most sensitive results for all test organisms, calculated from EPAstats, are reported here.

[b] NOEC, based on the most sensitive endpoint, is calculated with ICp value of 10.

2.4.2 Lemna gibba Toxicity Tests

Acute testing with *L. gibba* resulted in IC50 estimates of 59.1 mg/L for frond number and 31.1 mg/L for wet weight (**Table 2.2**). Significant ($p \leq 0.05$) results for frond number were seen at concentrations ≥ 40 mg/L, and ≥ 20 mg/L for wet weight. The NOEC for *L. gibba*, derived from wet weight, was 6.6 mg/L. Visual evidence of an adverse effect was observed after day 4 in concentrations ≥80 mg/L. Plants in the highest concentration (160 mg/L) exhibited a high percentage of chlorosis (≥57% measured by percent of total fronds) as well as necrosis (dead white fronds, not measured). At termination, all plants in the 160 mg/L concentration were dead and plants in the 80 mg/L concentration were characterized by small, pale fronds, and decreased root growth.

2.4.3 Daphnia Acute Toxicity Tests

Adult survival in both species was significantly affected ($p < 0.05$) at PFOS concentrations > 125 mg/L. *Daphnia magna* showed a LC50 of 130 mg/L, whereas *D. pulicaria* had a LC50 of 169 mg/L (**Table 2.2**). *Daphnia magna* exhibited greater sensitivity towards PFOS than *D. pulicaria*, exhibiting an immobility EC50 at 67.2 mg/L, which was 50% lower than *D. pulicaria* (134 mg/L). At 125 mg/L, organisms in the *D. magna* test displayed a significantly higher ($p = 0.005$) incidence of immobility than *D. pulicaria*. Both species of daphnid experienced immobility to a similar degree in 250 mg/L, the next highest concentration.

2.4.4 Daphnia magna Life-cycle Testing

A 21 d LC50 of 42.3 mg/L was determined for adult survival. The results are summarised in **Table 2.3**. No organisms survived past 6 days in 100 mg/L. The observed death of *D. magna* at 100 mg/L was substantially lower than the 48 h acute test result. Life-cycle tests can also be used (as well as for reproductive endpoints) to investigate effects at lower PFOS concentrations and a longer period of time typically not seen during acute tests. At 50 mg/L there was a significant reduction ($p = 0.031$) in mean number of young produced by adults and only a 50% survival rate. After 15 days, 20% of the surviving adults at 50 mg/L appeared white in colour, indicating unhealthy organisms. *Daphnia magna* exposed to 50 mg/L experienced a significant ($p = 0.010$) delay in time to first brood (~ 4 d) when compared to the controls.

Table 2.3 Endpoint effect values (mg/L) for PFOS 21-day life-cycle laboratory tests.

PFOS Conc. (mg/L)	Days to 1st Brood Mean ± Std	Adult Survival (%) (mortalities/ total)	Young per Adult[a] Mean ± Std	Young per Brood[b] Mean ± Std
control	8.5 ± 0.5	100 (0/12)	77.7 ± 10.0	5.2 ± 0.6
6	9.0 ± 0.8	92 (1/12)	66.3 ± 8.6	4.6 ± 0.9
13	9.3 ± 0.5	100 (0/12)	57.7 ± 3.8	4.3 ± 0.5
25	9.3 ± 0.5	75 (3/12)	75.7 ± 15.6	4.9 ± 1.0
50	11.7 * ± 0.6	50 * (6/12)	27.3 * ± 11.6	2.9 * ± 0.3
100	N/A[c]	0 * (12/12)	N/A[c]	N/A[c]

[a] Mean taken from total young produced over 21 days by three organisms in each of the 4 replicates.

[b] Corresponds to average number of neonates produced by each adult at each subsequent brood over the 21day test.

[c] Organisms were found dead between 3 to 6 days post exposure.

* denotes a significant difference compared to the control ($p \leq 0.05$).

2.5 DISCUSSION

In algal testing, cell density appeared to be the most sensitive endpoint, giving IC50 values 18.6% and 7.4% lower than chlorophyll(a) for *S. capricornutum* and *C. vulgaris*, respectively. This difference was not significant. However, cell density ultimately influences chlorophyll(a) abundance and both responded in a concentration-dependant fashion to treatments. Dose-dependent increases in membrane fluidity of fish leukocytes exposed to PFOS has been observed (Hu *et al.* 2000). However, fish leukocytes are distinctly different from single-celled algae therefore nothing can be inferred regarding a possible mode of PFOS action in algae cells. Response based on all endpoints for both algae

species showed IC50 values between 48.2 and 88.1 mg/L. This falls within the range of 10 to 100 mg/L for tests performed on *S. capricornutum* with a variety of similar anionic hydrocarbon (alkyl chain lengths of 8 to 18) surfactants (Yamane *et al.* 1984). Studies by 3M (2000) on *S. capricornutum* found a growth inhibition NOEC value of 44.0 mg/L, which is higher than that determined in our study (5.3 mg/L). Differences in NOEC values could be due to different algal culturing and test protocols, different endpoint measurement techniques, or different NOEC calculation methods. In comparison, Berends *et al.* (1999) found a NOEC, based on growth rate, for *S. capricornutum* exposed to trifluoroacetic acid (TFA) to be 0.1 mg/L. Thus, TFA, the shortest perfluorinated surfactant, is 34 times more toxic than PFOS to *S. capricornutum*. In contrast, PFOS was found to be more toxic to *C. vulgaris* (IC50 = 81.6 mg/L) than TFA (IC50 > 100 mg/L). The differences in relative sensitivities to various fluorinated acids may be due to different species sensitivity or the different mode of action for different compounds. To date, little research has been conducted regarding mode of action for PFOS in autotrophs.

Growth inhibition using *L. gibba* followed a concentration-dependant response. The concentration that inhibited 50% of plant growth, based on wet weight, was 31.1 mg/L (**Table 2.2**), which was 52.5% more sensitive than frond number (IC50 = 59.1 mg/L). A NOEC of 6.6 mg/L was calculated for wet weight in duckweed, whereas experiments with TFA by Berends *et al.* (1999) reported a NOEC of 300 mg/L for this organism, based on dry weight. Exposed to threshold concentrations of PFOS, wet weight was a better measure of adverse affects on duckweed than frond number because it quantified the reduction in growth better than simple physical appearance. Measuring division of fronds, regardless of frond

size and root length, may not identify an adverse effect whereas weight captured reductions in frond size and root propagation. Wet weight measurements of *L. gibba* exposed to concentrations over 40 mg/L confirmed the visual decrease in mean frond size and root length and number. Moreover, *L. gibba* fronds exposed to > 40 mg/L experienced loss in pigments (chlorosis) and cell death (necrosis). Overall, the three autotroph species had NOEC values ranging from 5.3 to 8.2 mg/L. These similar results indicate that there may be a similar mechanism of action of PFOS in cells of plants. This warrants further investigation.

Based on NOEC values, *Daphnia magna* was the most sensitive organism tested. However, based on 50% inhibition/ lethality, test results revealed that *Daphnia* spp. had relatively higher toxicity values than the plant species. The 48 h survival NOEC was 33.1 and 46.9 mg/L for *D. magna* and *D. pulicaria*, respectively. There was no significant difference in acute survival between these two daphnid species, however, there was a large difference for acute immobility. A high percentage (~ 47%) of *D. magna* experienced immobility at 31.3 mg/L. In comparison, only 5.5% of *D. pulicaria* experienced immobility in either of the 31.3 and 62.5 mg/L exposure treatments. The significant differences witnessed in immobility between like species at similar concentrations poses the question of how PFOS effects these invertebrates. Since the 48 h immobility NOEC falls below the test range, more work should be conducted with a lower concentration range. Immobility was not observed in our 21-day reproduction test with *D. magna*, which had a maximum concentration of 100 mg/L versus 450 mg/L for the 48 h test. This observation corresponds with chronic study results using the standards of the Organization for Economic Cooperation

and Development with *D. magna* by others (The Environment Agency 2000). Furthermore, chronic studies reviewed by The Environmental Agency (2000) reported a 21 d NOEC for adult survival of 12.0 mg/L, which is ~ 56% higher than our life-cycle NOEC of 5.3 mg/L. In this study, the life-cycle test revealed a 54.5% reduction in mean number of young produced at each brood at 50 mg/L compared to controls. Comparative chronic studies on the effects of exposing *D. pulicaria* to PFOS would be beneficial.

All test organisms were adversely affected by acute exposure to PFOS concentrations between 31.1 and 169 mg/L. The most sensitive NOEC values for all test organisms ranged between 0.8 and 13.6 mg/L, however, laboratory test conditions do not typically convey those seen in the environment. Generally, *S. capricornutum* is often the most sensitive organism in comparative laboratory tests with surfactants (Yamane *et al.* 1984; Berends *et al.* 1999), yet, in our study *L. gibba* and *D. magna* were more sensitive based on adverse effects and NOEC values, respectively. These laboratory organisms represent lower trophic levels in aquatic ecosystems that play important functions in freshwater communities such as nutrient cycling and energy transfer up the foodchain (Newman 1998). In a 35-day laboratory study using freshwater microcosms to simulate a pond ecosystem, Sanderson *et al.* (2002) observed a 90 - 100% reduction in relative zooplankton populations (*D. magna* included) at 10 mg/L of PFOS after 14 days. This is much more sensitive than the results of our 21-day *D. magna* test (LC50 = 42.9 mg/L). Point-source exposures of PFOS such as accidental spills from fire-training sites or production plants may introduce PFOS into aquatic environments at concentrations close to effect levels. Moody *et al.* (2002) calculated bioaccumulation factors for a point-source exposure of PFOS in the range of 6300 - 125000, based on fish liver and

surface water concentrations. Furthermore, plankton (specifically single-celled algae) have been seen to be more likely to accumulate hydrophobic organic compounds from water (Strange and Swackhamer 1994; Koelmans *et al.* 1999) because they have a high surface area to volume ratio (DeLorenzo *et al.* 2002). However, PFOS is a lipophobic compound thus accumulation into lipid-rich organisms (i.e. *Daphnia*) is unlikely. Others have observed different routes of uptake, retention times, and routes of elimination for PFOS in higher organisms (Hanhijarvi *et al.* 1982; 3M 2000; Martin *et al.* 2002c). The difference involves the uptake of PFOS through either the diet or environment then accumulation in the blood and liver by entrohepatic recirculation. Clearly more research on mode of action in freshwater biota of these unique (hydrophobic and lipophobic) compounds is needed.

2.6 CONCLUSION

The results of our aquatic toxicity tests combined with current environmental concentrations of PFOS, which typically occur in the low ng/L to low µg/L range, indicates a small likelihood of causing adverse effects to freshwater plants and crustaceans. However, persistence, preference for aqueous partitioning, and continued release may allow PFOS to accumulate to potentially toxic concentrations especially in locations such as vernal pools subject to high evaporation and no outflow (Benesch *et al.* 2002). On the other hand, isolated spill events such as that documented by Moody *et al.* (2002) resulted in short-term, high concentrations (maximum of 2210 µg/L) in aquatic environments. In light of the physical properties of PFOS as well as the limited chronic testing, further investigation of long-term effects in autotrophs and secondary consumers should be conducted.

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2.7 REFERENCES

3M (2000) 3M fluorochemical EPA submissions: 3M Company Ltd. Report # CD No. 1 St. Paul, MN, USA

American Society for Testing Materials (1999a) Standard practices for conducting static 96-h toxicity tests with microalgae: Designation E 1218 - 97a. In: Annual Book of ASTM Standards: Water and environmental technology. ASTM 11 ed. West Conshohocken, PA, USA: 537-550

ASTM (1999b) Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians: Designation E 729 - 96. In: Annual Book of ASTM Standards: Water and environmental technology. ASTM 11 ed. West Conshohocken, PA, USA: 224-244

ASTM (1999c) Standard practices for conducting *Daphnia magna* life-cycle toxicity tests: Designation E 1193 - 97. In: Annual Book of ASTM Standards: Water and environmental technology. ASTM 11 ed. West Conshohocken, PA, USA: 476-493

Banks RE (1979) Organofluorine chemicals and their industrial applications. Banks RE, (ed.) Ellis Harwood Ltd., West Sussex, UK, p 255

Benesch JA, Gustin MS, Cramer GR, and Cahill TM (2002) Investigation of effects of trifluoroacetate on vernal pool ecosystems. Environ Toxicol Chem 21:640-647

Berends AG, Boutonnet JC, Rooij CG, and Thompson RS (1999) Toxicity of trifluoroacetate to aquatic organisms. Environ Toxicol Chem 18:1053-1059

Boudreau TM, Wilson CJ, Cheong WJ, Sibley PK, Mabury SA, Muir DCG and Solomon KR (2002) Response of the zooplankton community and environmental fate of perfluorooctane sulfonic acid (PFOS) in aquatic microcosms. *Accepted*: Environ Toxicol Chem

Boutonnet JC (ed), Bingham P, Calamari D, de Rooij CG, Franklin J, Kawano T, Libre JM, McCulloch A, Malinverno G, Odom JM, Rusch GM, Smythe K, Sobolev I, Thompson R, and Tiedje JM (1999) Environmental risk assessment of trifluoroacetic acid. Hum Ecol Risk Assess 5:59-124

Brown D, and Mayer CE (2000) 3M to pare Scotchgard products. The Washington Post, May 2000, Washington, DC

Clesceri LS, Greenberg AE, and Trussell RR (1989) Chlorophyll. In: Standard methods for the examination of water and wastewater, 17th edition, American Public Health Association, New York, NY, USA

DeLorenzo ME, Taylor LA, Lund SA, Pennington PL, Strozier ED, and Fulton MH (2002) Toxicity and bioconcentration potential of the agricultural pesticide Endosulfan in phytoplankton and zooplankton. Arch Environ Contam Toxicol 42:173-181

Ellis DA, Hanson ML, Sibley PK, Shahid T, Fineberg NA, Solomon KR, Muir DCG, and Mabury SA (2001) The fate and persistence of trifluoroacetic and chloroacetic acids in pond waters. Chemosphere 42:309-318

Geis SW, Fleming KL, Korthals ET, Searle G, Reynolds L, and Karner DA (2000) Modifications to the algal growth inhibition test for use as a regulatory assay. Environ Toxicol Chem 19:36-41

Giesy JP, and Kannan K (2001) Global distribution of perfluorooctane sulfonate and related compounds in wildlife. Environ Sci Technol 35:1339-1342

Greenberg BM, Huang X-D, and Dixon DG (1992) Applications of the higher aquatic plant *Lemna gibba* for ecotoxicological risk assessment. J Aquat Ecosyst Health 1:147-155

Hanhijarvi H, Ophaug RH, and Singer L (1982) The sex-related difference in perfluorooctanoate excretion in the rat. Proc Soc Exp Biol Med 171:50-55

Hansen K J, Johnson H O, Eldridge J S, Butenhoff J L, and Dick L A (2002) Quantitative

characterization of trace levels of PFOS and PFOA in the Tennessee River. Environ Sci Technol 36:1681-1685

Hu W, King LE, Jones PD, and Giesy JP (2000) Measurements of effects of perfluorinated compounds on cell membrane fluidity by flow cytometry. In Book of abstracts 2000, 21st Annual Meeting of SETAC in Nashville, TN, USA 12-16 November 2000

Jandel, SigmaStat (1995) Jandel Corporation. Version 2.0. San Rafel, CA, USA

Kannan K, Koistenen J, Beckman K, Evans T, Gorzelany J, Jones PD, and Giesy JP (2001) Perfluorooctane sulfonate and related fluorinated organic chemicals in marine mammals. Environ Sci Technol 35:1593-1598

Karsa DR, ed. (1989) Industrial applications of surfactants II: Whitstable Litho Printers Ltd., Whitstable, Kent, UK, p 401

Key BD, Howell RD, and Criddle CS (1997) Fluorinated organics in the biosphere. Environ Sci Technol 31:2445-2454

Key BD, Howell RD, and Criddle CS (1998) Defluorination of organofluorine sulfur compounds by *Pseudomonas sp.* strain D2. Environ Sci Technol 32:2283-2287

Koelmans AA, Van Der Woude H, Hattink J, and Niesten JM (1999) Long-term bioconcentration kinetics of hydrophobic chemicals in *Selenastrum capricornutum* and *Microcystis aeruginosa*. Environ Toxicol Chem 18:1164-1172

Martin JW, Franklin J, Hanson ML, Solomon KR, Ellis DA, Mabury SA, and Muir DCG (2001) Detection of chlorodifluoroacetic acid in precipitation: a possible product of fluorocarbon degradation. Environ Sci Technol 43:274-281

Martin JW, Muir DCG, Moody CA, Ellis DA, Kwan WC, Solomon KR, and Mabury SA (2002a) Collection of airborne fluorinated organics and analysis by gas chromatography/ chemical ionization mass spectrometry. Anal Chem 74:584-590

Martin JW, Mabury SA, Solomon K, and Muir DCG (2002b) Bioconcentration and tissue distribution of perfluoroinated acids in rainbow trout (*Oncorhynchus mykiss*). Environ Toxicol Chem 22:196-204

(2002c) Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ Toxicol Chem 22:189-195

Marwood CA, Solomon KR, and Greenberg BM (2001) Chlorophyll fluorescence as a bioindicator of effects on growth in aquatic macrophytes from mixtures of polycyclic

aromatic hydrocarbons. Environ Toxicol Chem 20:890-898

Moody CA, and Field JA (1999) Determination of perfluorocarboxylates in groundwater impacted by fire-fighting activity. Environ Sci Technol 33:2800-2806

Moody CA, Martin JW, Kwan WC, Muir DCG, and Mabury SA (2002) Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. Environ Sci Technol 36:545-551

Newman, MC (1998) Bioaccumulation from food and trophic transfer. In: Fundamentals of ecotoxicology. Sleeping Bear/ Ann Arbor Press Inc., Chelsea, MI, USA: 71-86

Norberg-King TJ (1993) A linear interpolation method for sublethal toxcity: the inhibition concentration (ICp) approach. Technical report: National Effluent Toxicity Assessment Center: Environmental Protection Agency, 03-93, July 1993, Duluth, MN, USA

Olsen GW, Burris JM, Mandel JH, and Zobel LR (1999) Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production emplyees. J Occup Environ Medicine 41:799-806

Remde A, and Debus R (1996) Biodegradability of fluorinated surfactants under aerobic and anaerobic conditions. Chemosphere 32:1563-1574

Renner R (2001) Scotchgard scotched. Scientific America: March 2001, p 18

Sanderson H, Boudreau TM, Cheong WJ, Mabury SA, and Solomon KR (2002) Ecological impact and environmental fate of perfluorooctane sulfonate on the zooplankton community in indoor microcosms. Environ Toxicol Chem 21:1490-1496

Sharpe AG (1971) The physical properties of the carbon-fluorine bond. In: Carbon-fluorine compounds: chemistry, biochemistry and biological activity. Ciba foundation (ed.), Associated Scientific Publishers, London, UK: 33-54

Strange K, and Swackhamer DL (1994) Factors affecting phytoplankton species-specific differences in accumulation of 40 polychlorinated biphenyls (PCBs). Environ Toxicol Chem 13:1849-1860

The Environment Agency (UK) (2000) Review of environmental effects data of perfluorooctanyl sulfonate (PFOS)-based substances. Chemicals Assessment Section: National Centre for Ecotoxicology and Hazardous Substances, October 2000, Wallingford, Oxfordshire, UK

Ubel FA, Sorenson SD, and Roach DE (1980) Health status of plant workers exposed to fluorochemicals - a preliminary report. Am Ind Hyg Assoc J 41:584-590

Unites States Environmental Protection Agency (2000a) Perfluorooctyl sulfonates; proposed significant new use rule. In: Federal Register Vol. 65, No. 202: 40 CFR Part 721; OPPTS-50639; FRL-6745-5, October 18, 2000, Washington, DC: 62319-62333

USEPA (2000b) SIDS draft initial assessment report for perfluorooctane sulfonic acid and its salts. AR226-0978; 3M Co., St. Paul, MN, USA: 1-73

USEPA (2000c) Forty-sixth report of the TSCA interagency testing committee to the administrator, US Environmental Protection Agency. In: Toxic Substances Control Act, Interagency Testing Committee. Report No. 46, May 2000, Washington, DC: 1-16

Van Der Hoeven N (1997) How to measure no effect. Part III: statistical aspects of NOEC, ECx and NEC estimates. Environmetrics 8:255-261

Weber J (2000) 3M' s big cleanup. Businessweek. McGraw-Hill, New York, NY, USA

Wujcik C, Cahill TM, and Seiber JN (1998) Extraction and analysis of trifluoroacetic acid in environmental waters. Anal Chem 70:4074-4080

Yamane AN, Okada M, and Sudu R (1984) The growth inhibition of planktonic algae due to surfactants used in washing agents. Water Research 18:1101-1105

3 TOXICOLOGY OF PERFLUOROALKYL CARBOXYLIC ACIDS (PFCAs) IN RELATION TO CARBON-CHAIN LENGTH

3.1 ABSTRACT

Little toxicological data exists for perfluoroalkyl carboxylates (PFCAs) on freshwater organisms. Many physical and chemical properties of PFCAs relate to the chain length of the fluorinated tail. This paper reports the relative sensitivity of several species to PFCAs of three to ten carbons in length. There were different relationships between toxicity and chain length between freshwater plants and invertebrates. Plant species were sensitive to the shortest chain length (C3) and PFCAs > C7 in contrast to both invertebrate species, which were sensitive only to PFCAs > C7 in length, suggesting a different mode of action in plants and crustaceans. Low toxicity was observed for chain lengths between C4 and C7 for all species tested. *Lemna gibba* responded similarly to perfluorooctanoic acid (PFOA) in the laboratory and in 35-day microcosm studies, however, invertebrates were 19 times more sensitive in 35-day microcosm studies than 48 h laboratory tests.

3.2 INTRODUCTION

Perfluoralkyl carboxylic acids (PFCAs) are anthropogenic, surface-active compounds analogous to endogenous fatty acids (ex. octanoic acid) except that the aliphatic hydrogens are replaced with fluorine. PFCAs are superior to their hydrocarbon counterparts in reducing surface tension among other properties. PFCAs are widely used for precursors during the manufacturing of wetting agents, lubricants, corrosion inhibitors and aqueous fire fighting

foams [1]. PFCAs are typically not found in consumer products. PFCAs are almost exclusively synthesized by 3M Co. (St. Paul, Minnesota) and Dupont (Wilmington, Delaware) through a electrochemical fluorination or telomerization process, respectively. However, recent governmental regulations have focused on eliminating any new production, exportation, and use of PFCAs, especially perfluorooctanoic acid (PFOA). PFCAs exhibit hydrophobic and lipophobic properties making them capable of repelling both water and oil constituents and resistent to most forms of degradation, either thermal, chemical [2,3], or biological [4]. Particular concern with inert, polar molecules, like PFOA, is the high accumulation potential in aquatic environments. Recent reports have detected PFCAs in many samples of precipitation and surface waters [5,6]. PFOA has been found in precipitation from Canadian sites ranging from 5 to 73 ng/L [6] and perfluorobutyric acid (PFBA) was present in all samples taken from Lake Ontario, Canada [6]. This suggests that PFCAs have the potential to accumulate in aquatic environments, and perhaps become bioavailable (and some longer-chained moieties may ultimately bioaccumulate) in foodwebs.

Organofluorine chemistry is responsible for the effective functionality of PFCA surfactants in industrial applications. Bond strength, and hence, stability, increases with increased fluorination along the carbon tail [7]. Conversely, PFCA solubility decreases with increased chain length [8]. For example, PFCAs < PFOA are soluble in water at all proportions. However, PFCAs > PFOA are only slightly soluble in water [2,9]. PFOA is soluble even at 1 g/L.

Additionally, investigators suggest that chain length is important in the tissue distribution [10,11], excretion [12], and ultimate toxicity [12,13] of these PFAs. However,

retention and elimination rates for different PFCAs in rodents are different [14,15] and thus, ultimate exposure periods may vary. Generally, elimination of PFCAs follows the format: urinary and/or fecal excretion for PFCAs ≤ eight carbons in length (i.e. PFOA) and enterohepatic re-circulation > eight carbons in length. PFOA is transported and eliminated via both enterohepatic re-circulation and urinary excretion.

Some PFCAs, like PFOA and perfluorodecanoic acid (PFDA), are known peroxisome proliferators [16-18]. At low doses, PFOA can increase microsomal laurate hydroxylase which can lead to hepatotoxicity and tumor production [15,19-22]. Lethality was seen in laboratory animals at relatively high daily doses; ranging from 250 - 500 mg/kg [15,18,23,24]. Since environmental concentrations of PFCAs in wildlife range from low ng/ml to high μg/ml, there appears to be no immediate risk to mammals or human health. However, toxicity to aquatic organisms has not been extensively researched. Since freshwater environments could be a potential sink for PFCA accumulation [25] there is potential to reach concentrations that may be toxic to freshwater organisms.

This paper investigates the relationship between the relative toxicity of PFCAs with various chain lengths and toxicity to several freshwater organisms. The goal was to compare responses between similar species as well as between trophic levels, while providing some indication of mode of action. The potential chronic toxicity to *L. gibba* and the zooplankton community of lower concentrations of PFOA under semi-field conditions was also investigated.

3.3 MATERIALS AND METHODS

3.3.1 Laboratory Studies

3.3.1.1 Test compounds

Seven perfluoroalkyl carboxylates (PFCAs) of different chain lengths were chosen for a comparative toxicological study. All seven PFCAs used in toxicity testing were lab-grade chemicals obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and were ≥ 97% pure. Pentafluoropropionic acid (PFPrA) ($(CF_3)CF_2CHO_2$, CAS # 422-64-0; heptafluorobutyric acid (PFBA) ($(CF_3)C_2F_4CHO_2$, CAS # 375-22-4); nonafluoropentanoic acid (PFPeA) ($(CF_3)C_3F_6CHO_2$, CAS # 2706-90-3); tridecafluoroheptanoic acid (PFHpA) ($(CF_3)C_5F_{10}CHO_2$, CAS # 375-85-9); pentadecafluorooctanoic acid (PFOA) ($(CF_3)C_6F_{12}CHO_2$, CAS # 335-67-1); heptadecafluorononanoic acid (PFNA) ($(CF_3)C_7F_{14}CHO_2$, CAS # 375-95-1); nonadecafluorodecanoic acid (PFDA) ($(CF_3)C_8F_{16}CHO_2$, CAS # 335-76-2). Refer to Table 1.1 for molecular structure and acronyms.

3.3.1.2 Organisms

Five common aquatic test species were chosen for the evaluation. The invertebrates *Daphnia magna* and *Daphnia pulicaria*, and the green algae, *Selenastrum capricornutum*, (UTCC 37) and *Chlorella vulgaris* (UTCC 266) were cultured for more than three months prior to testing in the laboratory according to a combination of ASTM guidelines and SOPs developed by the Centre for Toxicology. For a detailed description of culture methods, refer to Boudreau *et al* [25](see Chapter 2). Axenic cultures of *Lemna gibba* G3 were maintained

according to Marwood *et al* [26]. *Lemna gibba* was also used in laboratory tests to compare different exposure routes with relative toxicity between algae and higher plant species. PFCAs are surface active agents and associate with interfacial boundary layers, thus the use of *L. gibba*, a floating macrophyte, was a logical choice.

3.3.1.3 Test procedure

All toxicity testing consisted of three or four replicates per treatment plus controls. Polyethylene containers were used for all stock and test vessels. Test concentrations depended upon overall solubility of the test compound; maximum concentration did not exceed the critical micelle concentration for each compound. Experimental testing followed a tiered regime where every test organism was exposed to each PFCA in a range-finder test followed by at least two definitive, with a geometric concentration series, unless stated otherwise. Non-linear regression was performed with acute test endpoints to relate toxicity to carbon chain length. All toxicity test designs, methods, and measured endpoints have been outlined previously in Chapter 2 [25].

3.3.1.4 Acute toxicity testing

Seven PFCAs were tested with common laboratory test species. Laboratory test durations, test species and concentration ranges used for each PFCA are shown in **Table 3.1.** Every compound was tested twice on each of five organisms except, that no test was performed with PFNA on *S. capricornutum* or *C. vulgaris*, and only one definitive test was conducted with PFDA on each of *S. capricornutum* and *C. vulgaris*. Tests conducted with

PFNA using *D. pulicaria* were not used due to lack of statistical power (i.e. power < 0.80).

Endpoints for the three primary producer species were based on inhibition of growth (i.e. IC50 and IC25). Green algae endpoints were cell density, measured spectrophotometrically at 430 nm, and chlorophyll(a) content calculated from spectrophotometric measurements [27,28]. Frond production and wet mass were endpoints for *L. gibba*.

Endpoints for invertebrate testing were based on lethality (i.e. LC50 and LC25) and immobilization effect (i.e. EC50), which was characterized by inferior appendage movement visible after gentle agitation. If no appendage movement was evident, visual inspection under a binocular microscope for organism heartbeat was conducted [25].

The no observable effect concentrations (NOEC) were based on a more statistically sound point estimation, where no-effect was replaced by the IC10 (or EC10) of the most sensitive endpoint for each species [29].

Table 3.1 Laboratory acute toxicity test concentrations.

Compound	Test Organism	Test Period	Concentration Range (mg/L)
PFPrA (C3)	*S. capricornutum*	96 h	0, 7.8, 15.6, 31.3, 62.6, 125, 250, and 500
	C. vulgaris	96 h	0, 7.8, 15.6, 31.3, 62.6, 125, 250, and 500
	L. gibba	7 d	0, 12.5, 25, 50, 100, 200, and, 400
	D. magna	48 h	0, 62.5, 125, 250, 500, and 1000
	D. pulicaria	48 h	0, 62.5, 125, 250, 500, and 1000
PFBA (C4)	*S. capricornutum*	96 h	0, 31.3, 62.5, 125, 250, 500, and 1000
	C. vulgaris	96 h	0, 31.3, 62.5, 125, 250, 500, and 1000

Compound	Test Organism	Test Period	Concentration Range (mg/L)
PFBA (C4)	*L. gibba*	7 d	0, 62.5, 125, 250, 500, and 1000
	D. magna	48 h	0, 62.5, 125, 250, 500, and 1000
	D. pulicaria	48 h	0, 62.5, 125, 250, 500, and 1000
PFPeA (C5)	*S. capricornutum*	96 h	0, 62.5, 125, 250, 500, and 1000
	C. vulgaris	96 h	0, 62.5, 125, 250, 500, and 1000
	L. gibba	7 d	0, 62.5, 125, 250, 500, and 1000
	D. magna	48 h	0, 62.5, 125, 250, 500, and 1000
	D. pulicaria	48 h	0, 62.5, 125, 250, 500, and 1000
PFHpA (C7)	*S. capricornutum*	96 h	0, 62.5, 125, 250, 500, and 1000
	C. vulgaris	96 h	0, 62.5, 125, 250, 500, and 1000
	L. gibba	7 d	0, 62.5, 125, 250, 500, and 1000
	D. magna	48 h	0, 62.5, 125, 250, 500, and 1000
	D. pulicaria	48 h	0, 62.5, 125, 250, 500, and 1000
PFOA (C8)	*S. capricornutum*	96 h	0, 6.7, 12.5, 25, 50, 100, 200, and 400
	C. vulgaris	96 h	0, 6.7, 12.5, 25, 50, 100, 200, and 400
	L. gibba	7 d	0, 10, 30, 50, 100, 300, and 500
	D. magna	48 h	0, 26.3, 52.6, 105, 210, and 420
	D. pulicaria	48 h	0, 26.3, 52.6, 105, 210, and 420
PFNA (C9)	*L gibba*	7 d	0, 10, 20, 30, 50, 70, and 100
	D. magna	48 h	0, 18, 37.5, 75, 150, and 300
	D. pulicaria	48 h	0, 18, 37.5, 75, 150, and 300
PFDA (C10)	*S. capricornutum*	96 h	0, 6.55, 13.2, 26.3, 52.5, 105, 210, and 420
	C. vulgaris	96 h	0, 6.55, 13.2, 26.3, 52.5, 105, 210, and 420
	L. gibba	7 d	0, 25, 50, 100, 150, 200, 275, and 350
	D. magna	48 h	0, 26.3, 52.6, 105, 210, and 420
	D. pulicaria	48 h	0, 26.3, 52.6, 105, 210, and 420

3.3.1.5 Chronic *Daphnia* testing

Two invertebrates, *D. magna* and *D. pulicaria*, were used in life-cycle tests that followed the methods and experimental design outlined in Chapter 2 [25]. Only PFNA was examined in *Daphnia* 21-d life-cycle tests for comparison with PFOS, a structurally similar PFA. Endpoints measured were adult survival, average number of young produced, and mean time to first brood over 21 d. Time restrictions did not allow for testing on other PFCAs.

3.3.1.6 Statistical analysis

All data were characterized for normality and homogeneity of variance by One Way Analysis of Variance (ANOVA). If tests failed, data were square root or log transformed to fit assumptions. Acute and chronic test data were analyzed as per methods outlined in Chapter 2 [25]. To determine if the differences between treatment groups were statistically significant, Dunnett's Method or Tukey's test were performed in ANOVA. All endpoints were compared to controls for significant differences via the Dunnett's procedure. Acute NOEC values were calculated using surrogate IC10 values [29] generated by the linear interpolation method [30]. Chronic NOEC values were represented by the highest test concentration not significantly different from controls [31]. Chronic LC50 values were generated using the Trimmed Spearman-Karber method (version 1.5) [32].

Linear regression analysis of carbon chain length versus single-species 50% effect concentrations were conducted in Sigmaplot 6.0 [33]. If linear regression was not possible, the best non-linear regression equation was chosen for the relation. Endpoint parameters had

to pass all tolerance criteria. Primary producer endpoints were related to carbon chain length with a non-linear peak (Gaussian) curve equation. Secondary consumer endpoints were fit with a linear curve equation. Regression curves, using effect endpoints for PFCAs showing no toxicity (Ex. PFHpA) were generated by molar conversion of the maximum test concentration even though actual effect concentrations remained unknown, and likely much higher. Higher test concentrations were never used because they were not ecologically realistic.

3.3.2 Microcosm Study of PFOA

3.3.2.1 Test compound

Sodium perfluorooctanoate was used in the microcosm evaluation. 3M Co. donated their fluorochemical emulsifier product, FC-1090 (CAS # 335-95-5), which states 20% PFOA wt/wt in water. It also contains Na-PFHpA, Na-PFHxA, and Na-PFDA as impurities with a total concentration of 1% wet weight per volume. The PFOA anion, without sodium, was volume corrected for all treatment concentrations.

3.3.2.2 Study design

The focus of the field evaluation was to investigate the potential toxicological impact of PFOA exposure to the zooplankton community and the floating macrophyte, *L. gibba* utilizing semi-natural microcosms, and to determine the aquatic persistence of PFOA under environmental conditions over a 35 d period. The microcosm evaluation was conducted at the University of Guelph's Microcosm Research Facility in Guelph, ON, Canada (**Appendix**

I). Study design, methodology, measured endpoints, and fate analysis (conducted at the University of Toronto, Toronto, ON) were previously described by Boudreau *et al* [34] using PFOS (Chapter 4) and the microcosm facility has been described by others [35,36].

Fifteen 12,000-L microcosms (**Appendix IIa**) were randomly divided into five triplicate groups for the study (**Appendix III**). The treatment regime consisted of nominal concentrations at 0.0, 0.3, 1.0, 30, 100 mg/L PFOA. The treatment regime was based on analytical detection limits and known environmental concentrations (0.3 mg/L) as well as single-species laboratory results (100 mg/L). Treatment with PFOA began on June 13, 2000. Water samples for PFOA residue analysis, water quality parameters (**Appendix IV**), and chlorophyll(a) (**Appendix V**) concentration were sampled at regular intervals over the respective test periods. Biological sampling of the zooplankton community occurred on days -1, 1, 2, 4, 7, 14, 21, 28, and 35 post-treatment. Two zooplankton traps (**Appendix VI**), constructed following [37], were deployed in each microcosm 24 h before sampling to provide ample collection during their daily vertical migration. Samples were combined and filtered through a 30 μm Nitex® screen then pooled into a 125-mL glass bottle with control water. Enumeration, identification, and analysis of community structure and effects followed Boudreau *et al* [34] (Chapter 4). A total of 82 organisms were identified from three main categories of zooplankton: Rotifera, Cladocera, and Copepoda.

Lemna gibba plants were monitored (**Appendix II b**) for growth endpoints on days 7, 14, 21, 28, and 35 post-treatment. Inhibition of growth was measured by number of viable plant colonies as well as number of fronds produced over test duration and compared to controls.

3.3.2.3 Statistical analysis

Monte Carlo permutations of the first canonical axis (summary of principal response curve data) were used to test significant effects caused by the treatment regime on the zooplankton community. A $NOEC_{community}$ for the zooplankton component was calculated following the procedure of Van Den Brink *et al* [38] for large complex data sets in microcosm studies. At each sample period the entire unconstrained variance of the data set is taken into account (principal component analysis) and used to determine the first principal component. The first principal components for each sample period represent the treatment regime and are tested with a William's test for significant deviations from controls. All taxa and species abundance data were analyzed following methods outlined by Boudreau *et al* [34] in Chapter 4. To determine if differences between treatment groups was statistically significant a t-test was performed for each sampling period by ANOVA in Sigmastat 2.0[39].

A 50% inhibition of growth for *L. gibba* was determined using the ICp 2.0 method in EPASTATS [30]. Treatment means were compared with controls using the Dunnett's test determined by ANOVA. A statistically sound NOEC value was determined by calculating a point estimate, IC10 and IC25, for growth endpoints following Van Der Hoeven [29].

3.4 RESULTS

Only significant test results are reported. Since, chemical analysis for all laboratory concentrations have not been completed to date, only selected test solution concentrations were analysed. Therefore, all laboratory endpoint values were based on nominal concentrations, and converted to molar units for chain length comparison. Microcosm results

were based on measured concentrations then converted to molar units for comparison between laboratory and field.

3.4.1 Single-Species Laboratory Results

3.4.1.1 Acute algal testing

The toxicity of each perfluoroalkyl carboxylate towards *C. vulgaris* and *S. capricornutum* as determined by their IC50, IC25, and IC10 values are summarised in **Table 3.2.** Comparison of molar IC50 values relating toxicity to carbon-chain length for PFCAs can be seen in **Figure 3.1** Three PFCAs of chain length C4, C5, and C7 exhibited significant ($p \leq 0.038$) effects on growth at their highest concentration, yet an LC50 could not be calculated from the data. Significant effects were seen in both algal species for PFPrA, PFOA, and PFDA (p values ≤ 0.05), corresponding to chain lengths of C3, C8, and C10, respectively. At concentrations of 0.307 M for PFPrA and 0.121 M for PFOA, there were significant effects (p-value = 0.010 and 0.023, respectively) on *C. vulgaris*. In experiments where effects were observed, *C. vulgaris* was more sensitive to PFCAs than *S. capricornutum*. *Chlorella vulgaris* IC50 values for chlorophyll(a) content were consistently more sensitive than cell density. A similar trend was not evident for *S. capricornutum*. *Chlorella vulgaris* chlorophyll(a) content IC10 ($IC10_{chl}$) ranged from 0.014 to 0.057 M (**Table 3.2**). In comparison, *S. capricornutum*, $IC10_{chl}$ values ranged from 0.107 to 0.133 M.

Table 3.2 Most sensitive effect concentrations (M) for seven PFCAs in plants.

Compound	Organism	IC50 (95% C.I.)[a]	IC25 (95% C.I.)[a]	IC10 (95% C.I.)[a]
PFPrA (C3)	*S. capricornutum*	0.304 (0.266, 0.337)	0.215 (0.144, 0.265)	0.107 (0.056, 0.222)
	C. vulgaris	0.285 (0.266, 0.306)	0.207 (0.129, 0.233)	0.088 (0.018, 0.153)
	L. gibba	0.250 (0.183, 0.333)	0.102 (0.079, 0.191)	0.041 (0.032, 0.072)
PFBA (C4)	*S. capricornutum*	2.90 (NA)[b]	NA	NA
	C. vulgaris	3.40 (NA)[b]	NA	NA
	L. gibba	> 4.7	NA	NA
PFPeA (C5)	*S. capricornutum*	> 3.8	NA	NA
	C. vulgaris	> 3.8	NA	NA
	L. gibba	> 3.8	NA	NA
PFHpA(C7)	*S.capricornutum*	> 2.8	NA	NA
	C. vulgaris	> 2.8	NA	NA
	L. gibba	> 2.8	NA	NA
PFOA (C8)	*S. capricornutum*	0.298 (0.274, 0.317)	0.197 (0.166, 0.231)	0.130 (0.020, 0.162)
	C. vulgaris	0.279 (0.249, 0.320)	0.034 (0.032, 0.040)	0.014 (0.013, 0.016)
	L. gibba	0.193 (0.142, 0.210)	0.127 (0.117, 0.146)	0.052 (0.042, 0.065)
PFNA (C9)	*S. capricornutum*	---------[c]	----------[c]	--------[c]
	C. vulgaris	---------[c]	----------[c]	--------[c]
	L. gibba	0.192 (0.180, 0.206)	0.155 (0.139, 0.165)	0.127 (0.105, 0.145)
PFDA(C10)	*S. capricornutum*[d]	0.425 (0.384, 0.470)	0.287 (0.234, 0.324)	0.133, 0.081, 0.253)
	C. vulgaris[d]	0.386 (0.333, 0.454)	0.197 (0.121, 0.257)	0.023 (0.012, 0.076)
	L. gibba[d]	0.193 (0.175, 0.206)	0.108 (0.075, 0.140)	0.045 (0.033, 0.077)

[a] Molar units.

[b] IC50 value extrapolated from single-species effect vs. concentration regression curve, therefore no confidence interval could be calculated.

[c] Data not available.

[d] Only one definitive test performed.

NA not available.

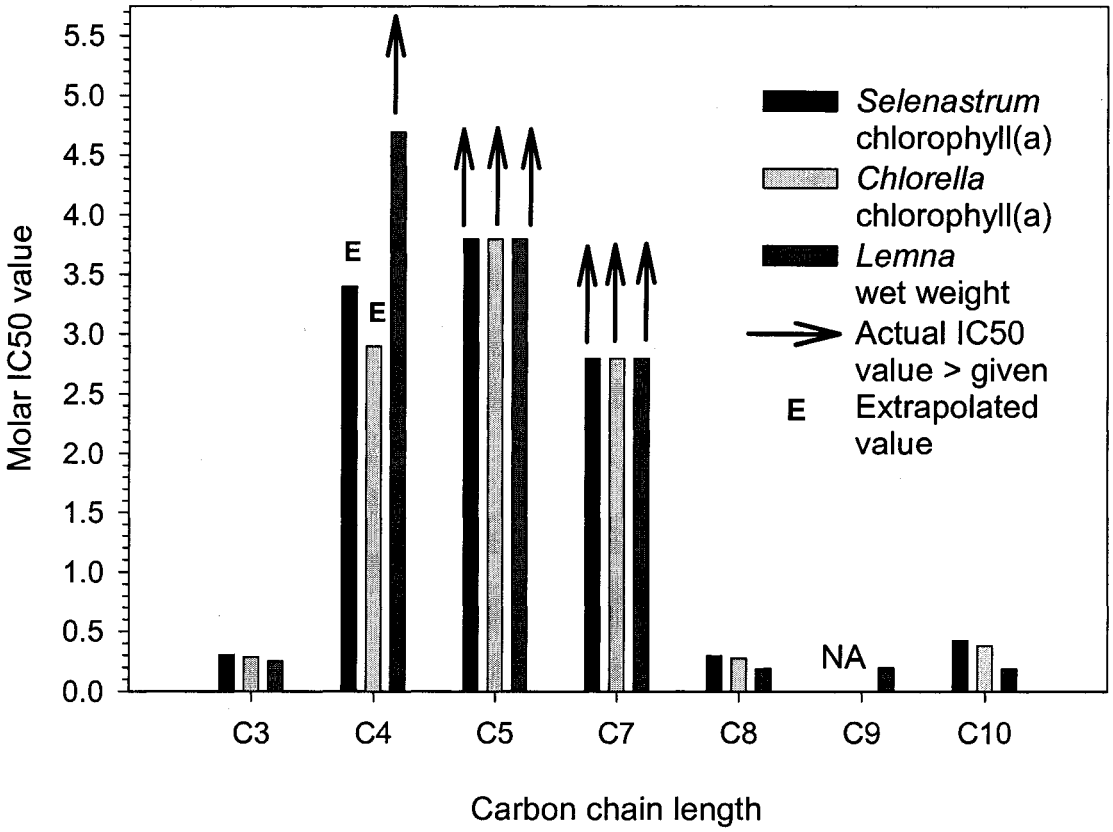

Figure 3.1 Most sensitive endpoint IC50 values (M) for plant species.

Molar IC50 values revealed the rank order for algal toxicity related to chain length as; PFOA ≥PFPrA > PFDA >> PFBA = PFPeA = PFHpA. Regression analysis for algal species revealed no significant relationship between IC50 values and carbon chain length.

3.4.1.2 Acute *Lemna gibba* testing

No toxic effects for any endpoint were evident with PFBA, PFPeA, or PFHpA. These PFCAs showed no significant change from controls beyond 2.8 M nominal concentration, which was deemed ecologically unrealistic. PFCAs displaying toxicity to *L. gibba* were PFPrA, PFOA, PFNA, and PFDA.

Wet weight was the most sensitive endpoint measured. For toxic PFCAs, wet weight was 50 ± 21% more sensitive than frond number. Wet weight IC values for all PFCAs tested on *L. gibba* can be seen in **Table 3.2**. PFOA, PFNA, and PFDA had the lowest wet weight IC50 values of 0.193, 0.192, and 0.193 M, respectively. These PFCAs showed significant differences ($p \leq 0.027$) at concentrations ≥ 0.195 M (100 mg/L) when compared to controls. PFPrA and PFDA were the most toxic compounds tested when comparing IC10 values, which were 0.41 and 0.45 M, respectively.

The rank order for toxicity from greatest to least is; PFNA ≈ PFDA ≈ PFOA > PFPrA >> PFBA = PFPeA = PFHpA. No significant relationship for *L. gibba* could be determined from regression analysis between IC50 values and carbon chain length.

3.4.1.3 Acute *Daphnia* testing

No toxic effects were evident with endpoints measured for PFCAs ≤ 7 carbons in length (PFPrA, PFBA, PFPeA, or PFHpA). These PFCAs showed no significant change from controls up to 2.8 M. Significant differences were only observed for PFCAs > 7 carbons in length (**Figure 3.2**). The longest PFCA tested, PFDA, was the most toxic compound to either daphnid species, however, no data is available for PFNA on *D. pulicaria* due to complications with testing. Molar EC10 values for PFDA were 0.107 and 0.190 for *D. magna* and *D. pulicaria*, respectively (**Table 3.3**). In comparison, PFOA had molar EC10 values of 0.358 and 0.236 for *D. magna* and *D. pulicaria*, respectively. *Daphnia magna* was more sensitive than *D. pulicaria* for all PFCAs > 7C, except for PFOA endpoints based on immobility (EC50 and EC10).

The rank order for daphnid toxicity from greatest to least is; PFDA > PFNA (*D. magna* only) > PFOA >> PFPrA = PFBA = PFPeA = PFHpA. Regression analysis for *Daphnia* spp. could not determine a significant relationship between LC50 values and carbon chain length. As a general trend, the IC50 effect concentration decreased as carbon chain length increased (**Figure 3.2**).

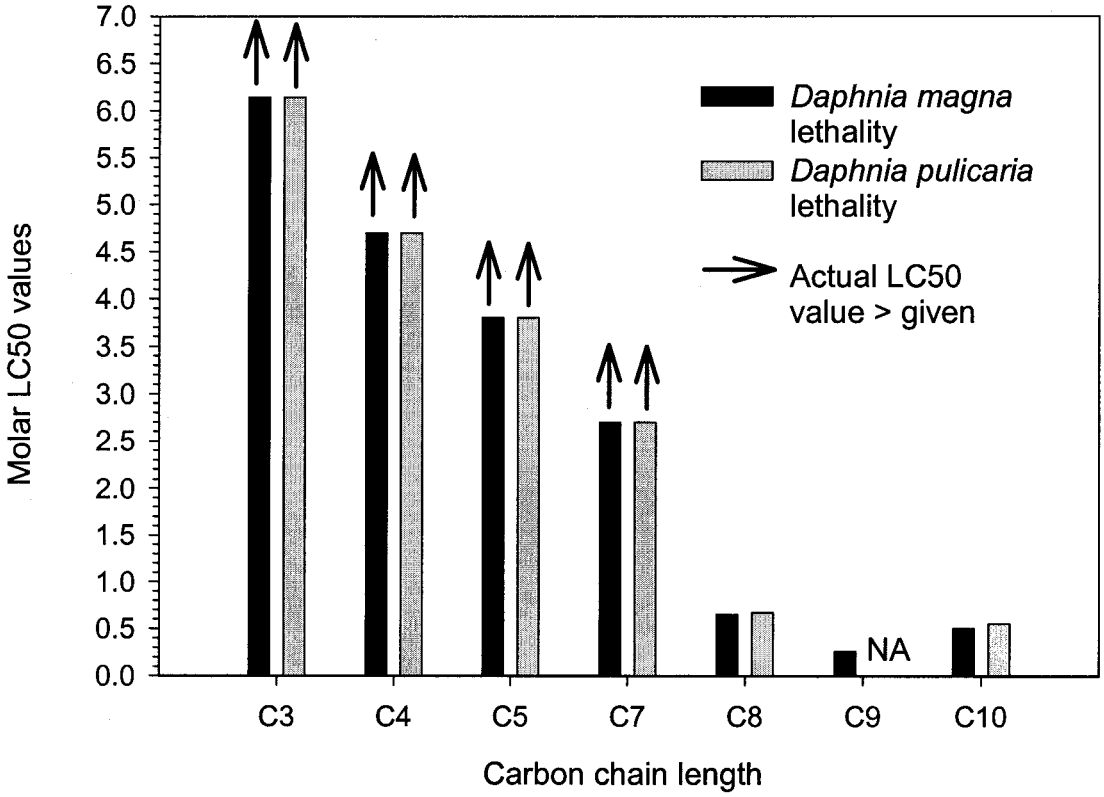

Figure 3.2 Lethality endpoint values (M) for *D. magna* and *D. pulicaria*..

Table 3.3 Effect concentrations (M) for seven PFCAs in *Daphnia* spp.

Compound	Organism	LC50[a] (95% C.I.)	EC50[a] (95% C.I.)	EC10[a] (95% C.I.)
PFPrA (C3)	*D. magna*	> 6.1	> 6.1	> 6.1
	D. pulicaria	> 6.1	> 6.1	> 6.1
PFBA (C4)	*D. magna*	> 4.7	> 4.7	> 4.7
	D. pulicaria	> 4.7	> 4.7	> 4.7
PFPeA (C5)	*D. magna*	> 3.8	> 3.8	> 3.8
	D. pulicaria	> 3.8	> 3.8	> 3.8
PFHpA (C7)	*D. magna*	> 2.8	> 2.8	> 2.8
	D. pulicaria	> 2.8	> 2.8	> 2.8
PFOA (C8)	*D. magna*	0.649 (0.545, 0.756)	0.540 (0.455, 0.639)	0.358 (0.354, 0.378)
	D. pulicaria	0.668 (0.540, 0.828)	0.492 (0.390, 0.618)	0.236 (0.227, 0.249)
PFNA (C9)	*D. magna*	0.259 (0.218, 0.311)	0.198 (0.166, 0.236)	0.161 (0.108, 0.184)
	D. pulicaria	NA	NA	NA
PFDA (C10)	*D. magna*	0.503 (0.437, 0.581)	0.252 (0.218, 0.291)	0.107 (0.101, 0.118)
	D. pulicaria	0.554 (0.505, 0.604)	0.291 (0.255, 0.330)	0.190 (0.183, 0.201)

[a] Effects values are given in molar units.

> indicates the molar conversion of the highest test concentration of 1000 mg/L for particular PFCA.

3.4.1.4 Chronic *Daphnia* testing

Due to time restrictions, PFNA was the only PFCA tested in the laboratory for chronic effects using daphnids. There was a difference in relative sensitivities between species. *Daphnia pulicaria* was more sensitive to PFNA than *D. magna* for each endpoint measured. *Daphnia pulicaria* showed similar relative sensitivity to acute tests with PFOA when compared to *D. magna*. Tests performed on *D. pulicaria* revealed 0% adult survival

by day 21 for concentrations ≥ 0.053 M and only 42% survival at 0.027 M. The 21-d LC50 for *D. pulicaria* survival was 0.019 M (with 95% confidence intervals of 0.014, 0.025 M). There was 50% adult survival for *D. magna* at 0.107 M and the 21-d LC50 was calculated to be 0.085 M (with 95% confidence intervals of 0.064, 0.113 M). Adults from either species at 0.214 M failed to survive beyond 8 d. The 21-d NOEC value for *D. pulicaria* was 0.013 M for all endpoints and the 21-d LOEC was 0.027 M. The 21-d NOEC for *D. magna* was 0.053 M for all endpoints except mean number of young produced per brood, which was 0.027 M. The 21-d LOEC values for *D. magna* were 0.107 M for all endpoints, except for mean number of young per brood 21-d LOEC was 0.053 M. There was a significant delay to first brood in concentrations > 0.013 M for *D. pulicaria* and 0.053 M for *D. magna* ($p = 0.015$ and 0.039, respectively).

3.4.2 Microcosm Results

3.4.2.1 Fate of PFOA

Microcosm water residue analysis indicated that initial concentrations were 78.6 ± 10.1% of nominal concentrations. At 35 d, only the highest treatment (0.18 M) had a significant reduction from the initial concentrations (13.8%), and no half-life could be calculated (**Appendix VII**). The measured TWA for the treatments was 0.27, 0.66, 23.9, and 74.2 mg/L (**Appendix VIII**), which corresponds to 0.3, 1, 30, and 100 mg/L, respectively (or 0.001, 0.002, 0.058, and 0.180 M, respectively). Measured concentrations were used for all analyses, however molar units were used for comparison of semi-field and laboratory study results.

3.4.2.2 Zooplankton community

From the principal response curve (PRC), there was a clear treatment-related deviation in species composition of the zooplankton community from controls in the 0.058 and 0.180 M concentrations (**Figure 3.3A**). Zooplankton communities present at 0.058 and 0.180 M increased in overall abundance relative to the controls. Communities from both 0.27 and 0.66 mg/L exposure treatments changed little from the controls over time. Monte Carlo permutation analysis revealed that the treatment regime had an significant effect ($p = 0.001$) on the zooplankton community (**Table 3.4**). As well, the PRC diagram displayed a significant portion (52.3%) of the treatment variance (**Appendix IX**). The species weight diagram (**Figure 3.3B**) showed that, of the 82 zooplankton species identified, 22% (18) were affected (species weights < -0.5 and > 0.5) by the treatment regime. Seven out of 18 species are seen to have high positive species weights, indicating an increase in abundance or conforming to the trends of the two highest concentrations. These 7 organisms were all rotifer species. The remaining 11 species (species weights < -0.5) that significantly decreased in numbers or responded oppositely to the PRC diagram, were from cladoceran and copepod populations (**Figure 3.3B**). Overall, 42 zooplankton species had negative species weights, but showed insignificant response ($> -0.5, < 0$).

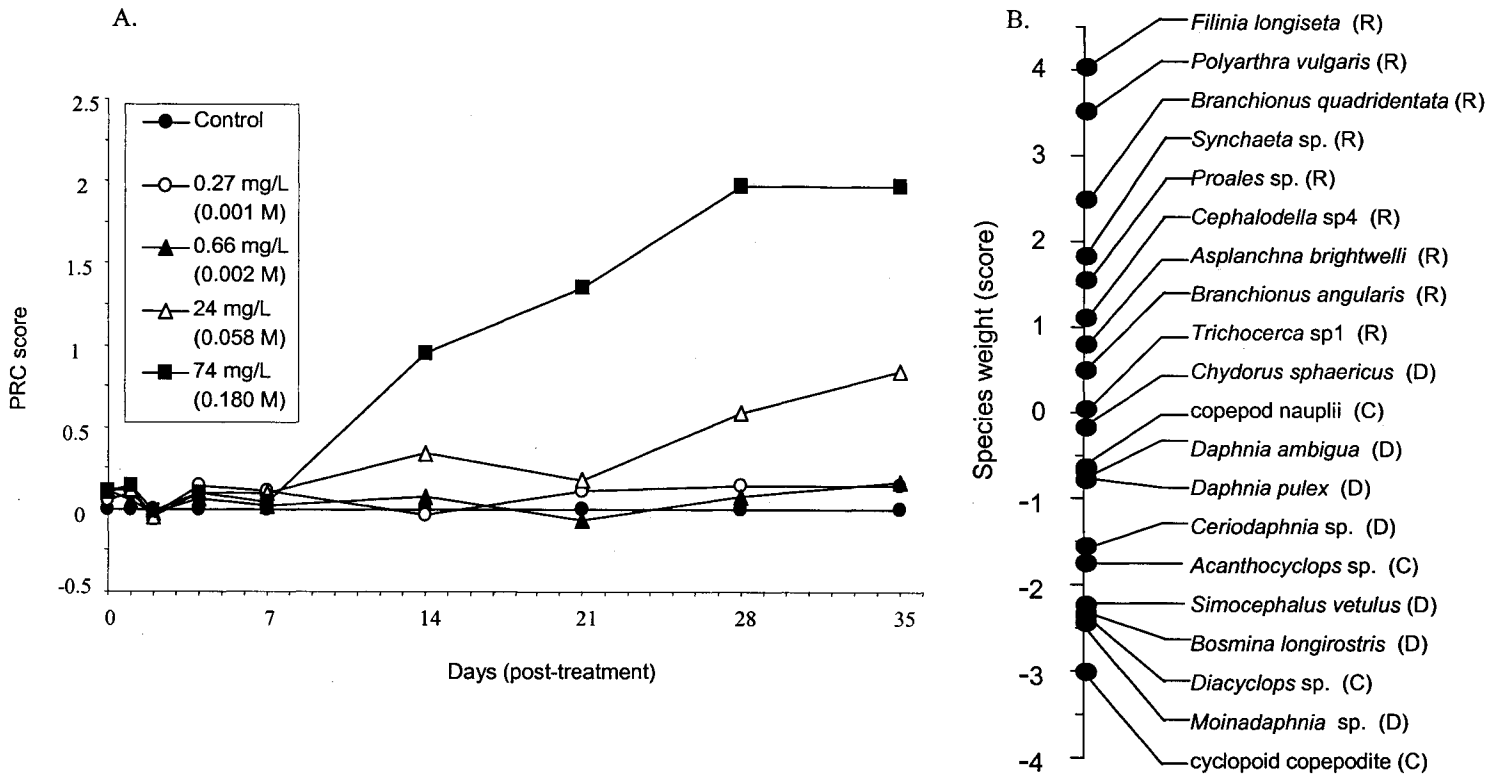

Figure 3.3 Zooplankton community response to PFOA shown by Principal Response Curves (A). Community response to each treatment was calculated from the first principal component of treatment effects, expressing deviations from the linear regression (control) line plotted against time. The corresponding species weight diagram (B) indicates the general affinity of each species for the overall PRC [38]. Species denoted by R = rotifers, D = cladocerans, and C = copepods.

Table 3.4 Monte Carlo permutation p-values and community no-observed effect concentrations for each sample period during PFOA microcosm study.

	Sample Day									
Statistical Analysis	-1[a]	1	2	4	7	14	21	28	35	entire study
Monte Carlo permutation (p-value)	0.001	0.21	0.257	0.004	0.001	0.001	0.001	0.001	0.001	0.001
Williams test[b] ($NOEC_{community}$)	0.002	>	>	0.058	0.058	0.002	0.002	0.002	0.002	------

[a] William's test showed a significant difference whereas, Dunnett's test did not. This was influenced by high rotifer abundance overall, in high treatment prior to treatment.

[b] All data passed Shapiro-Wilk's test for normality and homogeneity of variance.

> Indicates NOEC values greater than the highest field concentration, 0.180 M.

Total zooplankton abundance showed small but significant variations ($p = 0.041$, 0.024, and 0.005) prior to PFOA application (day -1) at ≥ 0.002 M (**Figure 3.4D**). Deviations became insignificant from controls immediately after treatment until day 4. Mean rotifer abundance at 0.180 and 0.058 M (**Figure 3.4C**) conformed to the PRC diagram, and the dominance of the community by Rotifera was clearly indicated by the relatively high, positive species weights (**Figure 3.3B**). Rotifer abundance at 0.180 M (**Figure 3.4C**) increased significantly after 14 d ($p \leq 0.021$) relative to the control rotifer population (**Figure 3.5**). This trend followed significant declines ($p \leq 0.027$) in the abundance of Copepoda and Cladocera populations > 14 d at this concentration (**Figures 3.4A-B**). These populations were reduced > 93% relative to controls on days 21, 28, and 35 at 0.180 M and as early as day 14 for copepods. A similar trend occurred for Cladocera at 0.058 M, but this reduction was not significant due to high variation. Copepoda populations at 0.058 M declined significantly

only at 35 d ($p = 0.039$). No recovery was evident for individual copepod or cladoceran populations by day 35 (**Figures 3.4A-B**). Population abundances decreased to near zero, compared to controls, by 14 d for both cladocerans and copepods.

The community no-observed effect concentration ($NOEC_{community}$) was 0.002 M > 7 d (**Table 3.4**). The $NOEC_{community}$ was 0.058 M as early as day 4 (**Table 3.4**).

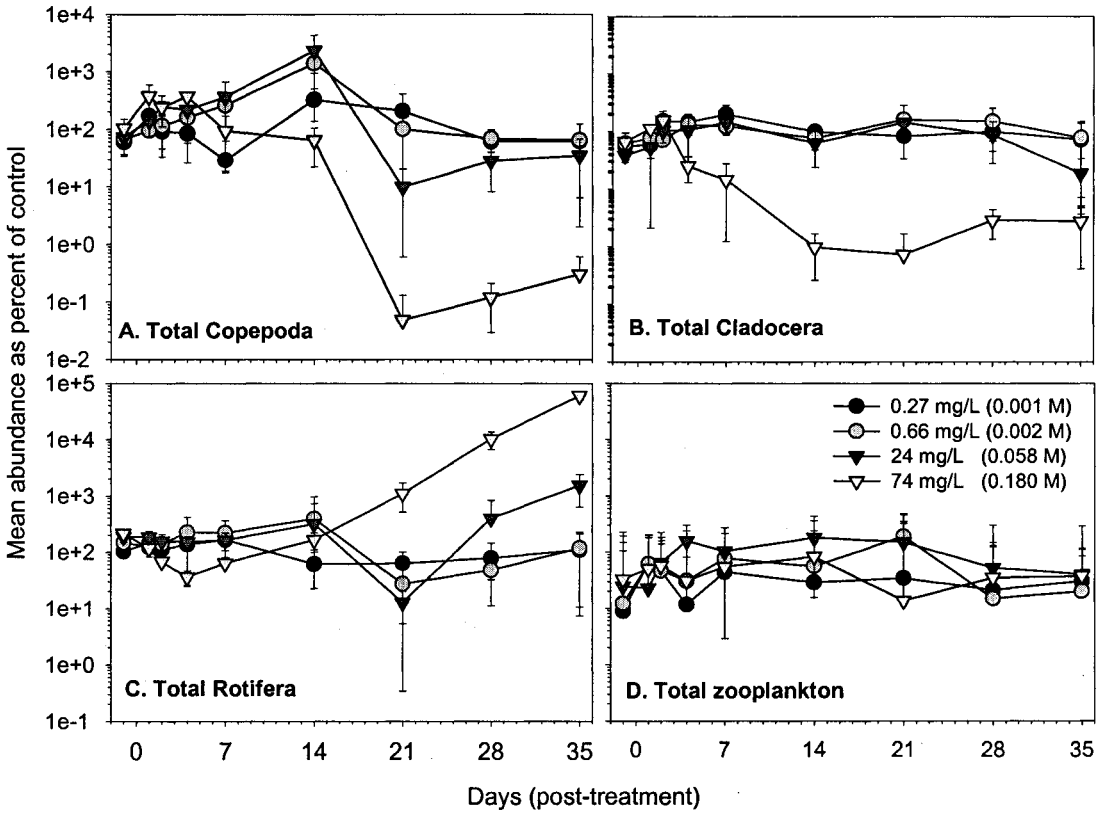

Figure 3.4 Individual zooplankton population abundance changes to PFOA over time. Graphs show mean total abundance of (A) copepods, (B) cladocerans, (C) rotifers, and (D) total zooplankton for each treatment as percent of controls. Each point represents the mean ± SE of three microcosms.

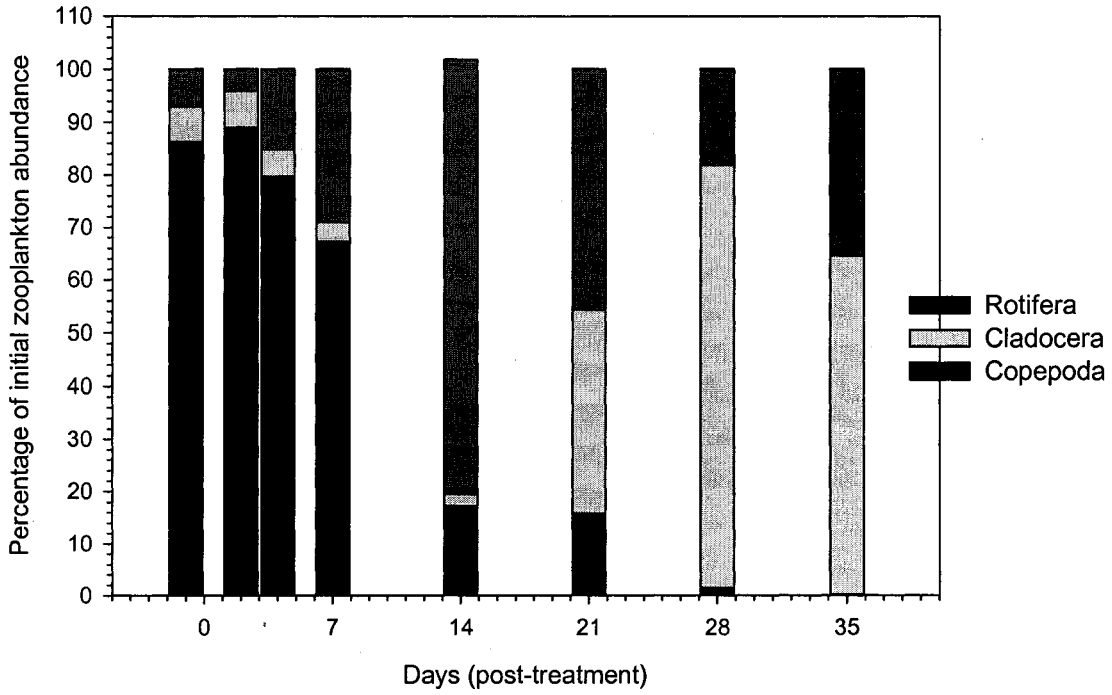

Figure 3.5 Community structure of control microcosms over time.

Impact on community structure was also apparent in terms of species richness expressed as total number of different species present. Species richness was greatly affected at 0.180 M for all three major zooplankton populations (**Figures 3.6A-C**), but appeared to be unaffected as a whole (**Figure 3.6D**). Cladocera were significantly reduced ($p \leq 0.03$) from controls after 14 d by 70.3 ± 13.2% (**Figure 3.6A**). Copepoda were reduced in richness by 51.8 ± 10.6% after 7 d, relative to controls (**Figure 3.6B**). Numbers of different species of Rotifera, on the other hand, increased significantly ($p \leq 0.044$) after 14 d. The mean

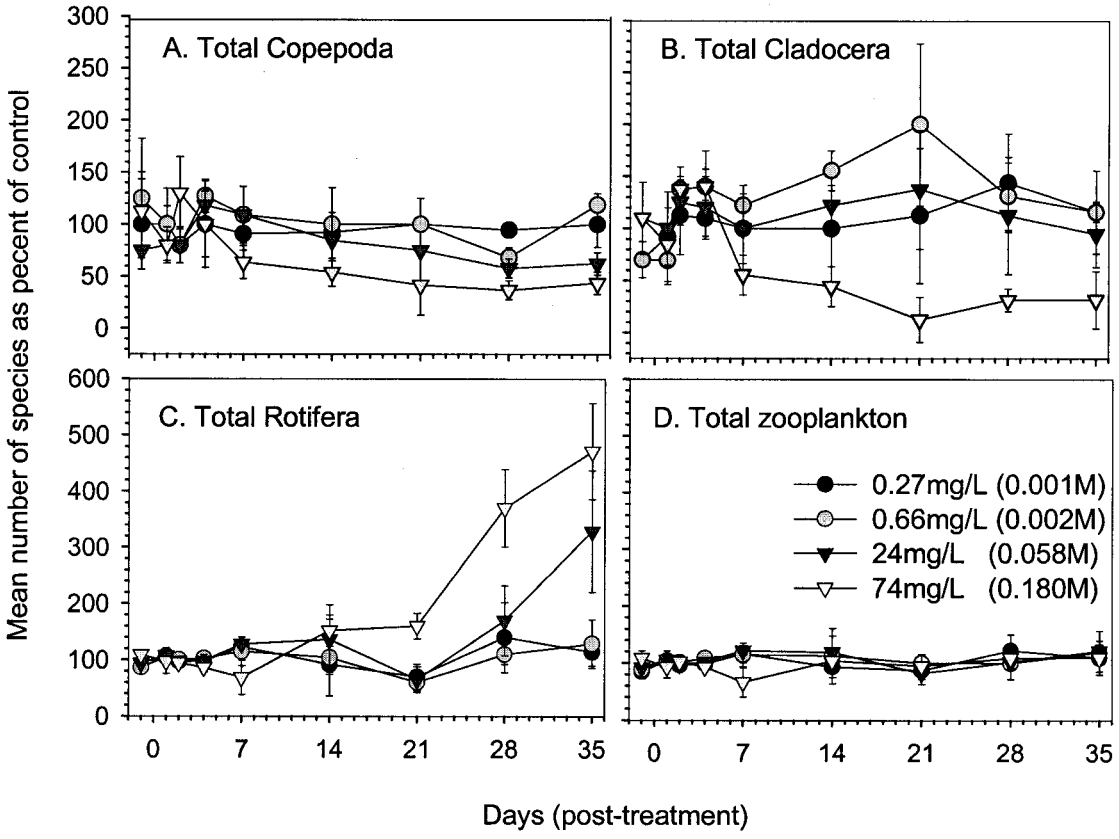

Figure 3.6 Total number of different species present for each population as a percent of control over 35 days. Each point represents the mean ± SE of three replicates at each sampling period.

increase was 2.9 ± 1.6 times greater than controls. Significant differences from controls were also observed in 0.058 M. Copepoda were reduced by 35.0 ± 8.9% after 21 d. Conversely, Rotifera significantly increased ($p = 0.036$ and 0.029) in richness by 1.7 and 3.3 times the control at days 28 and 35, respectively. No significant difference was found for cladocerans at 0.058 M.

3.4.2.3 *Lemna gibba*

Toxicity was observed only at 0.180 M (**Figure 3.7**). All other concentrations exhibited typical exponential growth ($r^2 \geq .997$) with little change from controls. Significant differences from controls were observed at 0.180 M as early as day 7 ($p = 0.002$) and continued through to test termination ($p < 0.001$). There was a > 96% reduction in frond number from controls at 0.180 M after14 d. At 14 d, fronds were characterized by pale green to yellow fronds (chlorosis), with few necrotic plants. At 35 d, frond numbers in 0.180 M PFOA were significantly different from all other treatments ($p \leq$

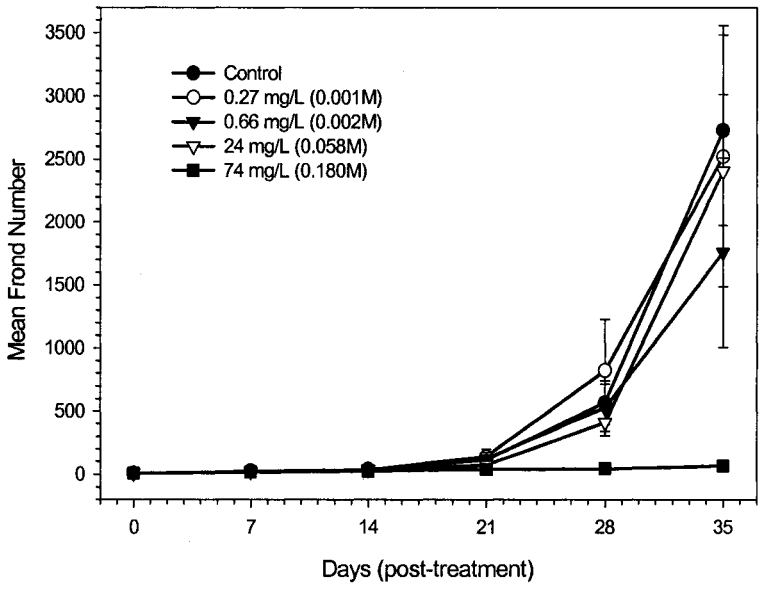

Figure 3.7 Mean number of *Lemna gibba* fronds produced over 35 days, exposed to PFOA in outdoor microcosms.

0.004). The 35-d IC50 value for mean frond number was 0.112 M (with 95% confidence interval of 0.072, 0.120 M) and the 35 d IC10 value was 0.049 M (with 95% confidence interval of 0.001, 0.070 M).

3.5 DISCUSSION

The main goal of this study was to investigate the relationship between relative toxicity and PFCA carbon chain length. Many physical and chemical properties as well as physiological properties of PFCAs proportionally change according to chain length. PFCA half-lives in trout increased as chain length increased [10,11]. Metabolism and excretion rates are smaller with increased length. As well, several toxicological affects, observed in rodents, such as peroxisome proliferation and gap junction intracellular communication inhibition typically occur with PFCAs ≥ C7. Likewise, solubility decreases with chain length. Additionally, this paper compared relative toxicity of similar species and at different levels of organization to elucidate general responses to PFCAs. The response of different trophic levels at a longer exposure period in the field was compared to acute laboratory studies.

No significant toxicity was observed in tests with PFBA, PFPeA, and PFHpA for all organisms, even at concentrations up to 1,500 mg/L (**Figures 3.1 and 3.2**). Concentrations as high as these were considered irrelevant ecologically when considering detected background concentrations. Hence, these three PFAs could be judged non-toxic to freshwater green algae, *L. gibba*, and waterfleas , at environmentally realistic concentrations. However, further investigation into mixture toxicology of low molecular weight (short chain length)

PFAs should be considered, since many products contain formulations of several PFAs.

Overall, there was a species specificity regarding PFCA toxicity. All plants were typically more sensitive to PFCAs than the invertebrates tested. Generally, toxicity was seen in all organisms tested with PFCAs containing ≥ 8 carbons. Acute toxicity only occurred at high concentrations. Previous laboratory studies with PFOS (chain length of 9) showed comparable responses in these organisms [25]. PFOS is a perfluoroalkyl sulfonate, and observed effect concentrations were consistently lower than any PFCA tested here. PFAs > 7 carbons in length are commonly tested for hepatotoxic effects in lab animals. Generally, the accepted mode by which high molecular weight PFAs express toxicity is increased cellular oxidative stress through increased production of peroxides. This occurs through induction of the fatty acid β-oxidation pathway within peroxisomes in animal cells and glyoxysomes in plant species [40]. Moreover, anionic surfactants (hydrocarbon alkanes) have shown increased cellular membrane permeability in algae leading to chlorophyll leaching [41], and Hu *et al* [42] showed that PFOS significantly increased membrane fluidity of fish leukocytes. The increased membrane permeability could be caused by a change in lipid membrane integrity through interaction with bound proteins [43], since lipophobic properties of PFCAs prohibits inclusion into cells for more direct interaction. Recent work [44] has suggested that some amphiphiles (both hydrophobic and hydrophilic molecules) reach the cell's interior by shielding the hydrophilic region with the hydrophobic portion. It is unlikely that the rigid structures of the PFAs can rearrange to shield the lipophobic head group, but perhaps enough of the hydrophobic tail gets through to elicit the start of a toxic response, such as proliferation of peroxisomes.

Toxicity occurred to all plant species exposed to PFPrA. *Lemna gibba* wet weight sensitivity was comparable to algae endpoints, even though routes of exposure vary for these different types of plants. Perhaps cellular oxidative stress also occurs within plant species exposed to PFPrA. In comparison, trifluoroacetic acid, with a carbon chain length of C2 has only been found to be toxic to *S. capricornutum* (EC50 = 0.042 M), and not in *L. gibba* or *C. vulgaris* (both EC50 > 8.8 M) [45]. This molar value of toxicity is similar to the 48 h EC50 for PFOA to *C. vulgaris* (0.034 M). Toxicity in plant species has been observed with monofluoroacetic acid (MFA). MFA toxicity occurs due to inhibition of citrate formation in the Krebs cycle. This mechanism of toxicity has not been observed with other PFAs. The reason for the difference in toxicities between plant species and structurally similar PFAs remains unresolved.

Overall, there is a similar rank order of sensitivity to PFAs for all plant species. Primary producer effect concentrations compared to chain length showed no specific relationships. However, the general trend is similar to that observed in rat hepatocytes by Upham *et al* [22] where inhibition of gap junction intercellular communication (GJIC) was significantly affected for chain lengths of 7 (PFHpA) to 10 (PFDA), but not evident for 2 to 5 carbons. Likewise, this relationship was observed for perfluorinated sulfonates in rat and dolphin hepatocytes by Hu *et al* [46]. Results presented here showed no toxicity in any plant species exposed to PFHpA, whereas Upham *et al* [22] showed a significant response even though PFHpA was the least inhibitory of GJIC. Down-regulation of GJIC has been linked to part of the process in tumor promotion of many peroxisome proliferators [22,40]. Furthermore, PFCA chain length has been linked to effects on enzymes that detoxify reactive

oxygen species and lipid peroxidation in mice liver cells [13]. Dietary treatment of PFOA and PFDA increased the activities of cytochrome P450 reductase and DT-diaphorase, yet decreased glutathion peroxidase significantly from controls. In comparison, activity of these enzymes changed little when treated with PFBA. Likewise, our results showed PFBA as having little to no effect relative to PFPeA and PFHpA on all endpoints.

Relative sensitivities between plant and invertebrate species toward PFPrA (C3) exposure was substantially different. No toxicity was observed for any concentration of PFPrA to either *Daphnia* spp. Why PFPrA toxicity varied between invertebrate and plant species remains unknown. Perhaps PFPrA toxicity is linked to inhibition of photosynthesis, whereby only plant species are sensitive. These general differences in sensitivities raises questions regarding the mechanism of PFPrA toxicity, and clearly, investigation of PFCA mode of action in aquatic organisms is required.

Invertebrates were sensitive only to PFCAs > C 7. No relationship was found when comparing effect concentrations to chain length, but the general trend indicated an increased toxicity with increased carbon chain length. Unfortunately, there is a quantitative data gap with PFNA due to tests performed on only one invertebrate, and no tests within the threshold concentration for PFCAs < C8. *Daphnia magna* was more sensitive than *D. pulicaria*. Conversely, 21 d life-cycle tests with PFNA showed that *D. pulicaria* was more sensitive than *D. magna*; similar results were found with PFOS [25].

Perhaps exposure to these compounds causes peroxisome proliferation leading to tissue damage through the production of oxygen radicals in invertebrates, as has been found in laboratory rodents [16,47,48]. Acute toxicity to fish species has been related to high

surface tension associated with high molecular weight PFAs [9]. Changes in surface tension could cause complications with movement near the surface and ingestion appendages leading to daphnid toxicity.

Measured microcosm concentrations were lower than nominals because concentrations of the PFOA anion in solution of the 3M product FC-1090 was over-stated. The environmental fate analysis of PFOA in aquatic microcosms showed little degradation over 35 days. This indicates that PFOA can persist in aquatic environments for periods equivalent to many generations for numerous species of zooplankton. Life-cycle toxicity tests would be beneficial to investigate effects to many generations spanning a longer period than the present microcosm study.

There was a significant impact of PFOA on the zooplankton community as early as day 7 given by the PRC. Rotifera communities in 0.058 and 0.180 M treatments increased relative to controls in a concentration-response fashion. Furthermore, no recovery was observed by day 35. Likewise, a microcosm study with PFOS utilizing PRC showed a increased response to the zooplankton community relative to controls with no recovery over 35 d [34]. In contrast, most studies with pesticides observe concentration-dependent reductions in the zooplankton communities followed by recovery similar to control communities [36,38,49,50]. The increase observed in the PRC was related to the large increase in Rotifera abundance at 0.180 M (**Figure 3.4C**) beyond 14 d. Shortly after treatment, individual cladoceran and copepod populations were virtually eliminated allowing for an improved competitive position, leading to the indirect effects observed by some rotifer species. However, these rotifers may have been less sensitive to PFOA allowing them to

dominate. The magnitude of the overall response to PFOA by the rotifer population (**Figures 3.4C**) was partially due to the substantial increase in densities of *Filinia longiseta*, *Polyarthra vulgaris*, and *Branchionus quadridentata* as depicted in the high positive species weights (**Figure 3.3B**). Additionally, four other species of rotifer (*Synchaeta* sp., *Proales* sp., *Cephalodella* sp4, and *Asplanchna brightwelli*) were prominent in the increase, although to a much lesser degree. The control microcosms displayed an opposite trend in community structure. Rotifers comprised > 65% of the community up to day 7 then decreased to near zero by day 35 (**Figure 3.5**). Cladoceran and Copepoda populations, on the other hand, began at abundances < 45% at day 7 and dominated beyond 14 d, comprising abundances > 85% of the total community composition.

When effects were assessed in terms of total number of species, species richness did not appear to change significantly from controls over time (**Figure 3.6D**). Total species richness was not directly evident due to contrasting trends between individual populations. Mean copepod and cladoceran species at 0.180 M showed significant reduction in richness after 7 d (**Figures 3.6A-B**), whereas, rotifers significantly increased from controls after 7 d (**Figure 3.6C**). Likewise, mean number of copepod species at 0.058 M decreased significantly in contrast to the number of rotifer species after 21 d.

The zooplankton community, at 0.180 M, became less diverse and simplified by the more resilient rotifer species. The ecological significance of this is that few species become eliminated causing alterations to the foodweb. Functional redundancy, however, indicates that an ecosystem as a whole may function normally without some sensitive populations. The zooplankton community at 0.058 M did become simplified taxonomically, but to a lesser

degree since cladoceran species were not significantly effected.

The microcosm LC50 for *Lemna gibba* frond number (0.112 M) was comparable to the laboratory wet weight LC50 (0.193 M), however, this was 62.8% lower than the laboratory frond number LC50 (**Table 3.6**). The 48 h laboratory LC50 values for either *Daphnia* spp. were nearly 20 times higher than the 35 d microcosm Cladocera population LC50 of 0.034 M (**Table 3.6**). Typically, more sensitive responses are seen in the field over longer exposure periods [51]. Ecological relevance of a concentration-response relationship may exist for a given endpoint, but at different levels of design complexity there is often a lack of knowledge regarding quantitative relationships between measured endpoints at different levels [52]. This is just one problem associated with extrapolating between laboratory and field-based microcosm studies.

3.6 CONCLUSION

It is evident that, while studying responses of various freshwater biota to PFCAs, chain length plays a significant role in expressed toxicities. Moreover, plant species seem more appropriate indicators of acute PFA stress. However, taking into account potency of PFCAs, differences in effective toxicity may also be related to route and duration of exposure, not to mention different physiology. With longer duration of exposure, this study showed that invertebrates have a more drastic adverse response compared to controlled laboratory testing than *L. gibba* (19.1x vs. 2.7x lower LC50 values, respectively). Possible synergistic effects should be considered in the future for PFCAs.

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3.7 REFERENCES

1 Banks RE. 1979. *Organofluorine chemicals and their industrial applications.* Banks RE, ed, Ellis Harwood Ltd., West Sussex, UK 255 p.

2 Kauck EA, Diesslin AR. 1951. Some properties of perfluorocarboxylic acids. *Ind Engin Chem* 43:2332-2339.

3 Karsa DR, ed. 1989. *Industrial applications of surfactants II.* Whitstable Litho Printers Ltd., Whitstable, Kent, UK, 401 p.

4 Remde A, Debus R. 1996. Biodegradability of fluorinated surfactants under aerobic and anaerobic conditions. *Chemosphere* 32:1563-1574.

5 Moody CA, Martin JW, Kwan WC, Muir DCG, Mabury SA. 2002. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. *Environ Sci Technol* 36:545-551.

6 Scott BF, Moody CA, Spencer C, Martin JW, Sargeant DB, Muir DCG, Mabury SA. 2002. Analysis for perfluoroalkanoic acids/ anions in surface waters and precipitation using GC/MS and LC/MS/MS. *To be submitted.*

7 Key BD, Howell RD, Criddle CS. 1997. Fluorinated organics in the biosphere. *Environ Sci Technol* 31:2445-2454.

8 Reo NV, Goecke CM, Narayanan L, Jarnot BM. 1994. Effects of perfluoro-n-octanoic acid, perlfuoro-n-decanoic acid, and clofibrate on hepatic phosphorus metabolism in rats and guinea pigs *in vivo. Toxicol Appl Pharm* 124:165-173.

9 Kissa E. 2001. *Fluorinated surfactants and repellents.* Hubbard AE, ed, Marcel Dekker, Inc., New York, NY, USA 615 p.

10 Martin JW, Mabury SA, Solomon KR, Muir DCG. 2002. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 22:189-195.

11 Martin JW, Mabury SA, Solomon KR, Muir DCG. 2002. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout. *Environ Toxicol Chem* 22:196-204.

12 Goecke-Flora CM, Reo NV. 1996. Influence of carbon chain length on the hepatic effects of perfluorinated fatty acids. A ^{19}F- and ^{31}P-NMR investigation. *Chem Res Toxicol* 9:689-695.

13 Permadi H, Lundgren B, Andersson K, DePierre JW. 1992. Effects of perfluoro fatty acids on xenobiotic-metabolizing enzymes which detoxify reactive forms of oxygen and lipid peroxidation in mouse liver. *Biochem Pharm* 44:1183-1191.

14 Ophaug RH, Singer L. 1980. Metabolic handling of perfluorooctanoic acid in rats. *Proceedings or the Society for Exp Biol Med* 163:19-23.

15 Hanhijarvi H, Ylinen M, Kojo A, Kosma V. 1987. Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. *Pharm Toxicol* 61:66-68.

16 Glauert HP, Srinvasan S, Tatum VL, Chen L, Saxon DM, Lay LT, Borges T, Baker M, Chen LH, Robertson LW, Chow CK. 1992. Effects of the peroxisome proliferators ciprofibrate and perfluorodecanoic acid on hepatic cellular antioxidants and lipid peroxidation in rats. *Biochem Pharm* 43:1353-1359.

17 Van Rafelghem MJ, Van Heuvel JP, Menahan LA, Peterson RE. 1988. Perfluorodecanoic acid and lipid metabolism in the rat. *lipids* 23:671-678.

18 Adinehzadeh M, Reo NV. 1998. Effects of peroxisome proliferators on rat liver phospholipids: sphingomyelin degradation may be involved in hepatotoxic mechanism of perfluorodecanoic acid. *Chem Res Toxicol* 11:428-440.

19 Ylinen M, Kojo A, Hanhijarvi H, Peura P. 1990. Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. *Bull Environ Contam Toxicol* 44:46-53.

20 Diaz MJ, Chinje E, Kentish P, Jarnot B, George M, Gibson G. 1994. Induction of cytochrome P4504A by the peroxisome proliferator perfluoro-n-octanoic acid. *Toxicology* 86:109-122.

21 Intrasuksri U, Rangwala SM, O'Brien M, Noonan DJ, Feller DR. 1998. Mechanisms of peroxisome proliferation by perfluorooctanoic acid and endogenous fatty acids. *Gen Pharm* 31:187-197.

22 Upham BL, Deocampo ND, Wurl B, Trosko JE. 1998. Inhibition of gap junction intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. *Int J Cancer* 78:491-495.

23 Olson CT, Andersen ME. 1983. The acute toxicity of perfluorooctanoic and perfluorodecanoic acids in male rats and effects on tissue fatty acids. *Toxicol Appl Pharm* 70:362-372.

24 USEPA. 2000. Perfluorooctyl sulfonates; proposed significant new use rule. Report No. No. 40 CFR 721; OPPTS-50639: USEPA. Washington DC, USA.

25 Boudreau TM, Sibley PK, Mabury SA, Muir DCG, Solomon KR. 2002. Laboratory evaluation of the toxcity of perfluorooctane sulfonic acid (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna*, and *Daphnia pulicaria*. *Arch Environ Contam Toxicol* In press.

26 Marwood CA, Dobson E, Smith REH, Solomon KR, Greenberg BM. 2001. Chlorophyll fluorescence as a bioindicator of photosynthesis inhibition from solar ultraviolet radiation in plants and algae. In: Greenberg BM, Hull RN, Roberts MH, Genemer RWJ, eds. *Environmental Toxicology and Risk Assessment: Science, Polocy, and Standardization*, American Society for Testing and Materials Philadelphia, PA, USA. p 27-39.

27 Clesceri LS, Greenberg AE, Trussell RR. 1989. Chlorophyll. In: Association APH, ed. *Standard Methods for the Examination of Water and Wastewater, 17th edition.* 17 ed, American Public Health Association New York, NY, USA. p 31-39.

28 ASTM. 1999. Standard practices for conducting static 96-h toxicity tests with microalgae - Designation: E 1218 - 97a. In: ASTM, ed. *Annual Book of ASTM Standards*. 11 ed, ASTM West Conshohocken, PA. p 537-550.

29 Van Der Hoeven N. 1997. How to measure no effect. Part III: statistical aspects of NOEC, ECx and NEC estimates. *Environmetrics* 8:255-261.

30 Norberg-King TJ. 1993. A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach. Report No.: US Environmental Protection Agency. Duluth, MN, USA.

31 Gelber RD, Lavin PT, Mehta CR, Schoenfeld DA. 1985. Statistical analysis - chapter 5. In: Rand GM, Petrocelli SR, eds. *Fundamental of Aquatic Toxicology: methods and applications*, Harper & Row Publishers Inc. New York, NY, USA. p 110-123.

32 Hamilton M. 1993. *Trimmed Spearman-Karber (TSK) program vesion 1.5* [stats]. Version 1.5: U.S. Environmental Protection Agency, Bozeman, MT, USA.

33 Jandel. 199X. *SigmaPlot ver. 6.0.* Version 5.0: Jandel Scientific, San Rafel, CA, USA.

34 Boudreau TM, Wilson CJ, Cheong WJ, Sibley PK, Mabury SA, Muir DCG, Solomon KR. 2002. Response of the zooplankton community and environmental fate of perfluorooctane sulfonic acid in aquatic microcosms. *Environ Toxicol Chem* Submitted.

35 Ellis DA, Hanson ML, Sibley PK, Shahid T, Fineberg NA, Solomon KR, Muir DCG, Mabury SA. 2001. The fate and persistence of trifluoroacetic and chloroacetic acids in pond waters. *Chemosphere* 42:309-318.

36 Sibley PK, Harris ML, Bestari JT, Steele TA, D RR, Gensemer RW, Day KE, Solomon KR. 2001. Response of zooplankton communities to liquid creosote in freshwater microcosms. *Environ Toxicol Chem* 20:394-405.

37 Whiteside MC, Williams JB. 1975. A new sampling technique for aquatic ecologists. *Limnologie* 19:1534-1539.

38 Van Den Brink PJ, Ter Braak CJF. 1999. Principle response curves: analysis of time-dependent multivariate responses of biological community to stress. *Environ Toxicol Chem* 18:138-148.

39 Jandel. 1995. *Sigmastat ver. 2.0.* Version 2.0: Jandel Corporation, San Rafel, CA, USA.

40 Vanden Heuvel JP. 1996. Perfluorodecanoic acid as a useful pharmacologic tool for the study of peroxisome proliferation. *Gen Pharm* 27:1123-1129.

41 Nalewajko C. 1992. Effects of surfactants on algae. *Algae and Water Pollution*, 195-204.

42 Hu W, King LE, Jones PD, Giesy JP. 2000. Measurements of effects of perfluorinated compounds on cell membrane fluidity by flow cytometry. Poster presentation at the SETAC 21st Annual Meeting in Nashville, TN, USA.

43 Nyberg H. 1989. Growth and ATP levels in prophyridiun purpureum (Rhodophyceae, Bangiales) cultured in the presence of surfactants. *Br Phycol J* 24:91-98.

44 Alper J. 2002 3 May, 2002. Breaching the membrane. Science:838-839.

45 Boutonnet JC, Bingham P, Calamari D, de Rooij C, Franklin J, Kawano T, Libre JM, McCulloch A, Malinverno G, Odom JM, Rusch GM, Smythe K, Sobolev I, Thompson RS, Tiedje JM. 1999. Environmental risk assessment of trifluoroacetic acid. *Hum Ecol Risk Assess* 5:59-124.

46 Hu W, Jones PD, Upham BL, Trosko JE, Lau C, Giesy JP. 2002. Inhibition of gap junction intracellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines *in vitro* and Sprague-Dawley rats *in vivo*. *Toxicol Sci* 68:429-436.

47 Harrison EH, Lane JS, Luking S, Van Rafelghem MJ, Andersen ME. 1988. Perfluor-*n*-decanoic acid: induction of peroxisomal oxidation by a fatty acid with dioxin-like toxicity. *Lipids* 23:115-119.

48 Kawashima Y, Suzuki S, Kozuka H, Sato M, Suzuki Y. 1994. Effects of prolonged administration of perfluorooctanoic acid on hepatic activities of enzymes which detoxify peroxide and xenobiotic in the rat. *Toxicol* 93:85-97.

49 Van Den Brink PJ, Van Wijngaarden RPA, Lucassen WGH. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrofos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. *Environ Toxicol Chem* 15:1143-1153.

50 Van den Brink P, Hattink J, Bransen F, Van Donk E, Brock T. 2000. Impact of the fungicide carbendazium in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquat Toxicol* 48:251-264.

51 Versteeg DJ, Belanger SE, Carr GJ. 1999. Understanding single-species and model ecosystem sensitivity: data-based comparison. *Environ Toxicol Chem* 18:1329-1346.

52 Sibley P, Chappel MJ, George TK, Solomon KR, Liber K. 2000. Intergrating effects of stressors across levels of biological organization: examples using organophosphorus insecticide mixtures in field-level exposures. *J Aquat Eco Stress & Recovery* 7:117-130.

4 RESPONSE OF THE ZOOPLANKTON COMMUNITY AND THE ENVIRONMENTAL FATE OF PERFLUOROOCTANE SULFONATE (PFOS) IN AQUATIC MICROCOSMS

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4.1 ABSTRACT

Perfluorooctane sulfonic acid (PFOS) is a surfactant used in many industrial and commercial products. Little is known of PFOS toxicity to freshwater organisms. The present evaluation examines the ecotoxicological impact associated with PFOS exposure across levels of biological organization. Triplicate 12,000 L outdoor microcosms and multivariate analyses were used to assess the response of freshwater zooplankton to four concentrations of PFOS. Zooplankton populations were significantly affected at 10 and 30 mg/L ($p < 0.05$) as indicated through Monte Carlo permutations. A community-level no observed effect concentration ($NOEC_{community}$) of 3.0 mg/L was determined over 35 d. The most sensitive taxonomic groups, Cladocera and Copepoda, were virtually eliminated at 30 mg/L by 7 d. The response of a floating macrophyte, *Lemna gibba*, was also measured over 42 d. The

IC50 for *L. gibba* (frond number) was 19.1 mg/L and the NOEC was 0.2 mg/L. Environmental persistence studies were also conducted on PFOS over 285 days under natural conditions. PFOS concentration, expressed as time weighted average, was reduced from nominal concentrations by a maximum of 6.0% (3 mg/L). A half-life could not be calculated. Our data, coupled with the current knowledge of environmental concentrations indicates little risk to these freshwater organisms.

4.2 INTRODUCTION

Perfluorooctane sulfonic acid (PFOS) is an eight-carbon, straight-chained, poly-fluorinated organic acid (PFA). The carbon-fluorine bond makes the PFOS molecule extremely rigid and stable, conferring resistance to most biotic [1, 2] and abiotic [3] breakdown processes. Perfluorooctane sulfonic acid (PFOS) is a unique substance that reduces surface tension and is thus generally known as a surfactant. PFOS has both hydrophobic and lipophobic characteristics [4]. These characteristics make PFOS, and its structurally similar derivatives, an ideal component in widely used commercial and industrial products such as; stain-repellents, insecticides, lubricants, food wrapping, and aqueous fire fighting foams [5,6]. PFOS has recently been detected in wildlife of higher trophic levels from urban and remote areas around the world [7,8]. Long range transport of PFOS may occur via volatile precursors recently measured in air from urban and rural sites [9]. These reports coupled with high bioconcentration factors for fish (1,150 - 12,5000 L/kg) [6,10] suggest a tendency for PFOS to bioaccumulate. Furthermore, PFOS production and treatment plants, as well as fire fighting training facilities, are often located in close proximity

to freshwater and groundwater systems [11]. PFOS has been detected at measurable levels (32 ± 11 ng/L) upstream from a fluorochemical manufacturing facility in Decatur, AL, USA [12]. Downstream from the same facility, PFOS was measured at 114 ± 19 ng/L. PFOS is a strong candidate for designation as a persistent organic pollutant by the UN Environmental Program [13] due to its extreme environmental persistence, high bioaccumulation potential, and potential toxicity. These factors have played a significant role in recent government and public concerns regarding the environmental activity of PFOS.

Little is known regarding toxicity and fate associated with PFOS in aquatic systems. Boudreau *et al* [4] used single-species laboratory toxicity tests to evaluate the potential toxicity to freshwater organisms associated with PFOS exposure. The 21 d lethality NOEC for *D. magna* was 5.3 mg/L [4]. The 7 d growth inhibition concentration (EC50) for *L. gibba* was 31.1 mg/L. Previous toxicological investigation with PFAs had focused on laboratory vertebrates (mostly rats) which accumulate PFAs with longer fluorinated chain lengths (e.g., PFOS) via enterohepatic recirculation [10]. Rats accumulate PFAs in the liver and blood. Lower organisms such as *D. magna* and *L. gibba* have biological systems that greatly differ from vertebrates thus, the mechanism of PFOS action in aquatic organisms needs to be addressed.

Microcosm studies examine the aquatic community or ecosystem and are a compromise between highly controlled single-species laboratory tests and the variability of actual ecosystems [14]. Microcosm studies conducted with realistic exposures provide ecologically relevant information such as direct and indirect effects, species interactions, and recovery of populations [14,15]. Therefore we examined both individual species and

zooplankton population responses over time using principle response curves (PRC), a multivariate statistical analysis specifically designed for microcosm community experiments [16]. PRC allows the effects of large and complex data sets like zooplankton populations to be expressed as a response relative to controls [17].

Aquatic primary producers play a major role in nutrient cycling and energy transfer through food webs [18]. Secondary (consumer) trophic positions play important intermediate roles as both food and potential regulators of lower trophic levels [19-20]. The objective of this research was to assess ecotoxicological responses associated with exposure of PFOS across levels of biological organization. The effects of PFOS exposure to the zooplankton community and a floating macrophyte, *L. gibba*, were investigated over a period of 35-days and 42-days, respectively. The present study also measured the persistence of PFOS over a period of 285-days. In this study, we simulated a single point-source spill of PFOS causing relatively high concentrations to elucidate potential effects on freshwater organisms. One such event occurred in Toronto, ON, Canada where the maximum PFOS concentration in surface waters was 2.2 mg/L [6].

4.3 MATERIALS AND METHODS

4.3.1 Microcosm Design

The study was conducted at the University of Guelph's Microcosm Research Facility in Guelph, ON, Canada (**Appendix I**). The microcosms each have a surface area of 11.95 m^2, a depth of 1.05 m (**Appendix II a**), and a total capacity of 12,000 L [21-23]. Water for the microcosms was provided from a spring-fed irrigation pond and was circulated among

microcosms for 10 d prior to study initiation at a rate equivalent to 11,000 L per day. This circulation period enabled establishment and equilibrium of indigenous zooplankton, periphyton, phytoplankton, benthic invertebrates, algae, and aquatic plants. The microcosms were seeded with plants (*Myriophyllum sibiricum*) and fathead minnows (*Pimephales promelas*) were contained in cages within each microcosm as part of a separate evaluation (**Appendix II a**). Microcosms were uncovered to allow aerial colonization of flying insects and were otherwise unaltered after the 10 d equilibration and PFOS treatment.

4.3.2 Experimental Design

The potassium salt of PFOS was used in the study, (donated by 3M Co., St. Paul, MN, USA, purity was 86%) as Fluorad™ fluorochemical surfactant FC-95. All treatment concentrations were based on the PFOS anion (without K+). Fifteen microcosms were randomly divided into five triplicate groups for the study (**Appendix X**). The treatment regime consisted of 0, 0.3, 3, 10, and 30 mg/L PFOS. The highest concentration was derived from single-species laboratory results [4] and chosen to produce significant effects under field conditions [24]. One day prior to treatment, circulation from the irrigation pond was terminated producing isolated systems and water and zooplankton samples were collected as a pre-treatment reference.

Stock solutions of PFOS were pre-mixed and applied (**Appendix XI**) to the microcosms using a sub-surface injection system [21]. After treatment, microcosms (including controls) were circulated for 15 min to ensure proper mixing of PFOS. At 1 h post-treatment, water residue samples were collected and designated as day 0.

4.3.3 Residue, Water Chemistry, and Chlorophyll(a) Sampling

Water samples for PFOS residue analysis, water quality parameters, and chlorophyll(a) concentration were sampled at regular intervals over the respective test periods. Polypropylene or polyethylene containers were used for all stock solutions and test vessels due to our preliminary observations that PFOS has potential to adsorb to glass and Teflon® surfaces. Sub-samples were collected from several random locations throughout each microcosm with a depth-integrating sampler [25] and pooled into 4 L containers (Nalgene®, VWR; ON, Canada). The pooled sample was then aliquoted into 125 and 500 mL containers (Nalgene®, VWR; ON, Canada) for analysis of PFOS residue and water quality, respectively. Samples were stored at 4° C in the dark before analysis. Water quality parameters included pH, temperature, hardness (as $CaCO_3$), and alkalinity. For chlorophyll(a) analyses, aliquots of 1 L were filtered through a Whatman™ glass fibre disk (Whatman International Ltd.; Kent, UK). The fibre disk plus retentate was wrapped in aluminum foil and frozen (- 28° C) until chlorophyll(a) analysis was conducted (TD-700 Laboratory Fluorometer, Turner Designs; Sunnyvale, CA, USA). For purposes of this study, total chlorophyll(a) concentration was used as a surrogate for phytoplankton abundance [20] and compared with zooplankton abundances. The unused portion of water was returned to appropriate microcosms. Maximum and minimum temperatures and dissolved oxygen within the microcosms were measured daily (YSI model 57; YSI, Yellow Springs, OH, USA).

4.3.4 PFOS Residue Analysis

Aqueous samples were analysed by ion chromatography using a DX-500 ion chromatography unit (Dionex Corporation; Sunnyvale, CA, USA). PFOS separation was detected using a Dionex IonPac NG1 4 x 35 mm guard column. Flow rate was set to 0.75 mL/min. Total run time was 14.5 minutes, with an equilibrium time of 4.5 minutes between successive runs. PFOS concentrations were calculated from linear calibration curves ($r^2 >$ 0.99). Due to limitations with analytical equipment the 0.3 mg/L treatment was below the level of detection and was not analysed.

4.3.5 Zooplankton

Zooplankton were sampled on -1 d, 1 h, and 1, 2, 4, 7, 14, 21, 28, and 35 d post-treatment between August 21 and September 26, 2000. Two zooplankton traps (**Appendix VI**) were placed in each microcosm 24 hours prior to sampling for sufficient collection of organisms during their daily vertical migration. Zooplankton traps were constructed following Whiteside and Williams [26]. The traps were suspended a few centimeters from the bottom of the microcosm by the edges of sediment trays. The traps were carefully retrieved to avoid disturbance of the sediment and quickly turned upright to prevent sample loss. Both traps from the pond were combined and filtered through a 30 μm Nitex® screen (Dynamic Aqua Supply; Surrey, BC, Canada). Filtered organisms were pooled into a 125-mL glass bottle with deionized water. Samples were preserved by first narcotizing with approximately 16% soda water followed by approximately 10 mL of 4% sucrose-formalin solution [23,27] .

Identification was accomplished with the aid of three invertebrate classification texts [27-29]. Methods for identifying zooplankton followed those described earlier [23, 30]. Zooplankton were identified to species level when possible. The zooplankton community collected from microcosms consisted of 92 species of Rotifera, Cladocera, Copepoda, macroinvertebrates, and Ostracoda. For the purpose of this study, the former three organism groups were used for analysis for a total of 68 active species. Copepods were divided into adults and nauplii and analysed as separate groups [31]. Various stages of copepod nauplii were grouped together. Number of taxa was used as a measure of diversity. Abundance and number of taxa results were presented as the mean of three replicate microcosms.

4.3.6 Analysis of Community Structure and Effects

Zooplankton community-level responses to PFOS treatments were compared by using principal response curves (PRC) generated by CANOCO [32]. PRC is based on redundancy analysis which explains community-level effects as well as determining the relative response of each species within the community [32]. Zooplankton community abundance (68 species) was compared between the treatments and controls for each sampling date. The output was a plot of the control community response, represented by a zero amplitude line, and the treatment community response at each sample date [33]. Deviations around the zero amplitude line indicate a treatment-related response by the zooplankton. A coefficient (species weight) was calculated for each taxon based on the relative effect in the community response. These species weights can be interpreted as the affinity of each species for the community response [33]. Species with high positive weights are most likely to conform to

the response trend observed, while large negative weights indicate that a species conforms strongly to the opposite response. Species with extremely small values (> -0.5, < 0.5) show a minimal change or are not likely to follow either pattern, and thus are not shown.

4.3.7 Lemna gibba

A second study using *L. gibba*, was conducted from May 21, 2001 to June 25, 2001 (42 days). Prior to microcosm exposure, duckweed was axenically cultured in the laboratory according to Marwood *et al* [22]. *Lemna gibba* were contained in the microcosms within floating wood and mesh enclosures (**Appendix II b**) divided into three equal compartments (for sub-sampling) and were fastened to the side of each microcosm. On day 0 (1 h after PFOS application), 4 healthy plants consisting of 3 fronds each were placed into each of the three compartments. *Lemna gibba* was evaluated on days 7, 14, 21, 28, 35, and 42 post-treatment. Endpoints used to determine the effects of exposure were mean plant number and mean frond number. Physical appearance (chlorosis and necrosis) of all treated plants was recorded.

4.3.8 Statistical Analysis

If homogeneity of variance and normality was not met, data were ln or square root transformed to meet assumptions. All abundance data for zooplankton was adjusted using a scaling value of 1 to eliminate zero values before ln transforming the data. Deviations within the zooplankton community were assessed using Monte Carlo permutations to test significant effects ($p \leq 0.05$) caused by the treatment regime, and to test if the PRC displayed

a significant portion of treatment variance [17]. A $NOEC_{community}$ for the zooplankton assemblage was calculated at each sampling period using the Williams test according to Van den Brink *et al* [17] using Toxstat version 3.3 [34]. Mean total zooplankton taxa were compared to controls using multivariate ANOVA Dunnett's test in Sigmastat 2.0 (Jandel Corporation, San Rafel, CA, USA).

Inhibition of growth for *L. gibba* was determined via ICpin (ver. 2.0) method of EPASTATS [35] with 50% inhibition concentration (IC50). The IC10 value was calculated as a surrogate for the NOEC as recommended by Van Der Hoeven [36].

4.4 RESULTS

4.4.1 PFOS Fate

PFOS concentration remained relatively constant over 285 d (**Figure 4.1**). The measured concentrations as calculated by the time weighted average (TWA) over 285 d changed by ≤ 6.0% from nominal concentrations (the TWA for 3 mg/L = 2.8, 10 mg/L = 9.8, and 30 mg/L = 30.1). For this reason, nominal values were used in all analyses. The average initial (1 h post-treatment) measured concentration was 14.8 ± 8.7% different from nominals. The 285-d TWA percent of initial PFOS concentration was 104.3% for 3 mg/L, 75.0% for 10 mg/L, and 85.4% for 30 mg/L (**Appendix XII**). On day 102 (December 5, 2000) one 30 mg/L replicate microcosm had a drainage-valve leak and decreased in volume. It was subsequently excluded from the fate study.

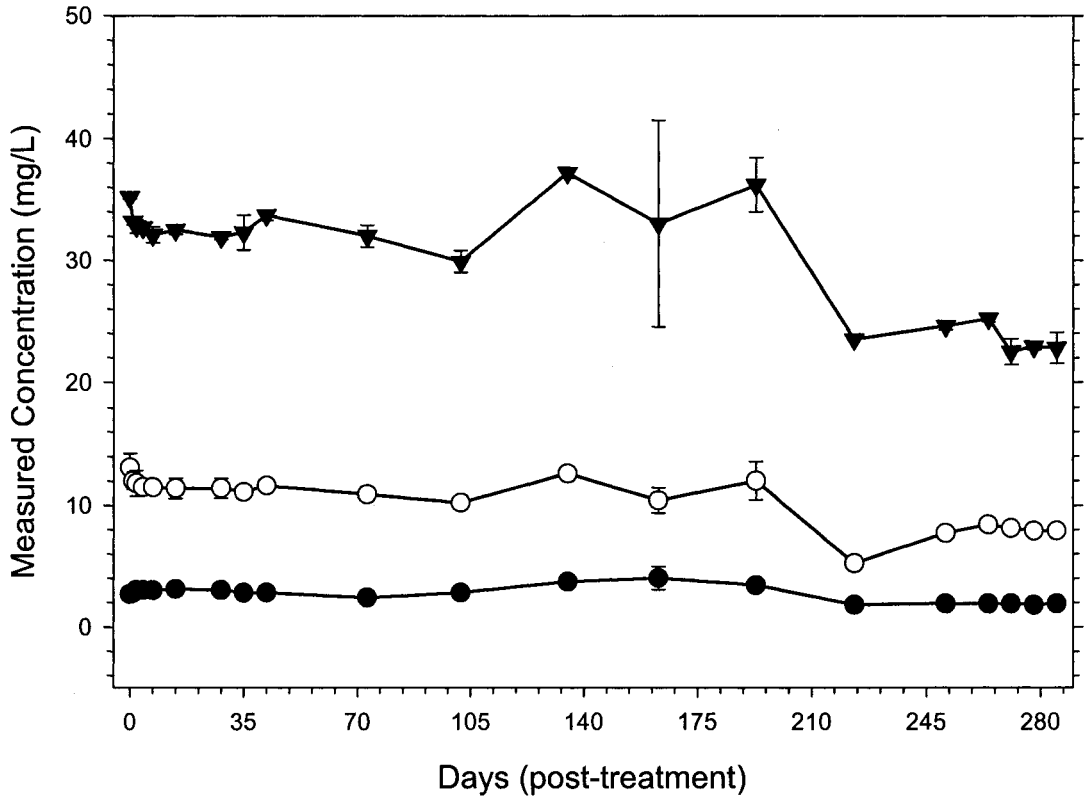

Figure 4.1 Environmental persistence of the three highest concentrations of PFOS over 285 days in outdoor microcosms. Each point represents the mean ± SE of three replicates except 30 mg/L, where one replicate was eliminated beyond day 102. The 0.3 mg/L fate analysis was not included because the concentrations was below the level of detection for our methods.

4.4.2 Zooplankton Community and Population Responses

Prior to PFOS application, mean zooplankton abundance displayed no significant differences ($p > 0.05$) between treatments and control. Following PFOS application, there was a treatment-related (> 3 mg/L) deviation in species composition relative to controls as displayed in the PRC (**Figure 4.2A**). Monte Carlo permutations conducted for each sample date verified that the treatment regime significantly impacted ($p = 0.001$) the

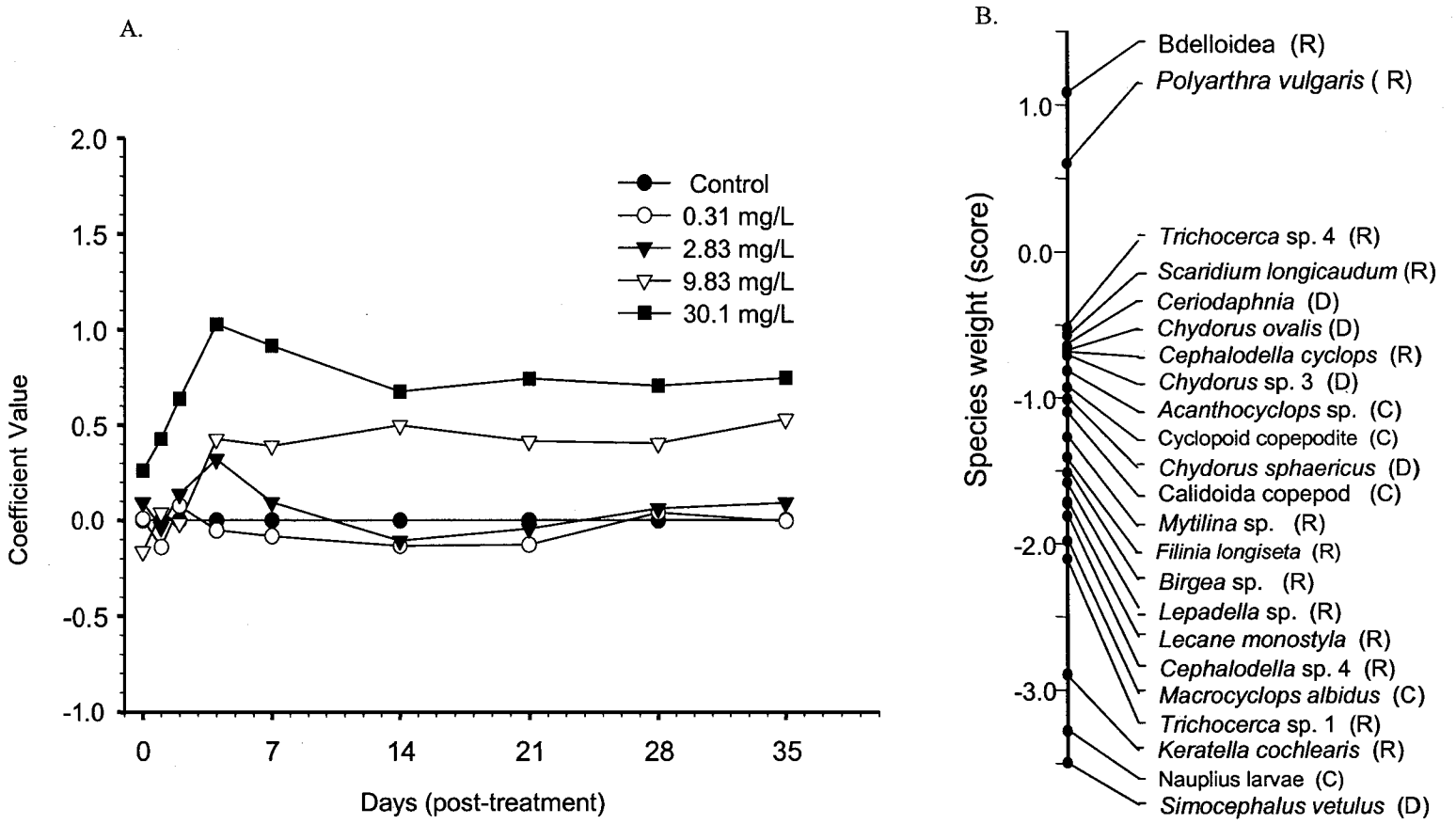

Figure 4.2 Zooplankton response to PFOS over 35 d shown by Principal Response Curves (A). Species weight diagram (B) indicates the affinity of each species has for the treatment curves in (A). Species denoted by R = rotifers, D = cladocerans, and C = copepods.

zooplankton community response. The PRC showed that the zooplankton community increased in abundance in 10 and 30 mg/L exposure treatments, relative to controls. The communities from both 0.3 and 3 mg/L treatments showed little change from the control over time. The species weight diagram (**Figure 4.2B**) showed that 23 out of 68 total zooplankton contributed the most to the community response seen in the PRC. The other 45 individual zooplankton changed little in abundance thus had less effect on the community response over the duration of the study. The $NOEC_{community}$ was 3 mg/L from 4 d to 28 d except 14 d, where a NOEC could not be calculated due to low power (**Table 4.1**). The lowest $NOEC_{community}$ was 0.3 mg/L at day 35 (**Table 4.1**).

Table 4.1 Monte Carlo permutation p-values and community no-observed effect concentration values for zooplankton data at each sampling period over 35 d.

	Sample Day									
Statistical Analysis	-1	1	2	4	7	14	21	28	35	overall
Monte Carlo permutation (p-value)	0	0.001	0.001	0.001	0.002	0.042	0.001	0.001	0.001	0.0011
Williams test $NOEC_{community}$	> 30	10	10	3	3	NA[a]	3	3	0.3	----

[a] indicates NOEC value could not be calculated due to low power, therefore not available.

The most prevalent effect on the community was demonstrated by two species of rotifers. The rotifers, *Bdelloidea* sp. and *Polyarthra vulgaris* increased in abundance at 10 and 30 mg/L, relative to controls. Indeed, these two rotifer species were largely responsible for the trend seen in the PRC, as indicated by species weights ≥ 0.6. Likewise, a 100-fold increase was observed in the 10 mg/L treatment for total rotifer abundance, which was also

largely due to *P. vulgaris* (**Figure 4.3C**). Of the total zooplankton population, *Bdelloidea* sp. comprised up to 22.8% at 30 mg/L and 1.4% at 10 mg/L compared to only 0.6% in the controls. Moreover, *P. vulgaris* comprised up to 21.6%of the total zooplankton community at 30 mg/L and 87.7% at 10 mg/L compared to only 5.0% in the controls. In contrast, there were 21 rotifers with species weights < - 0.6, indicating a response contrary to that described by the PRC. Impacts on total rotifer abundance were most notable at 30 mg/L, in which a ≥ 89% reduction occurred between day 1 and day 21 compared to the controls (**Figure 4.3C**). Only three sample days (4, 21, and 35) yielded significant reductions ($p \leq 0.05$) for rotifers relative to controls.

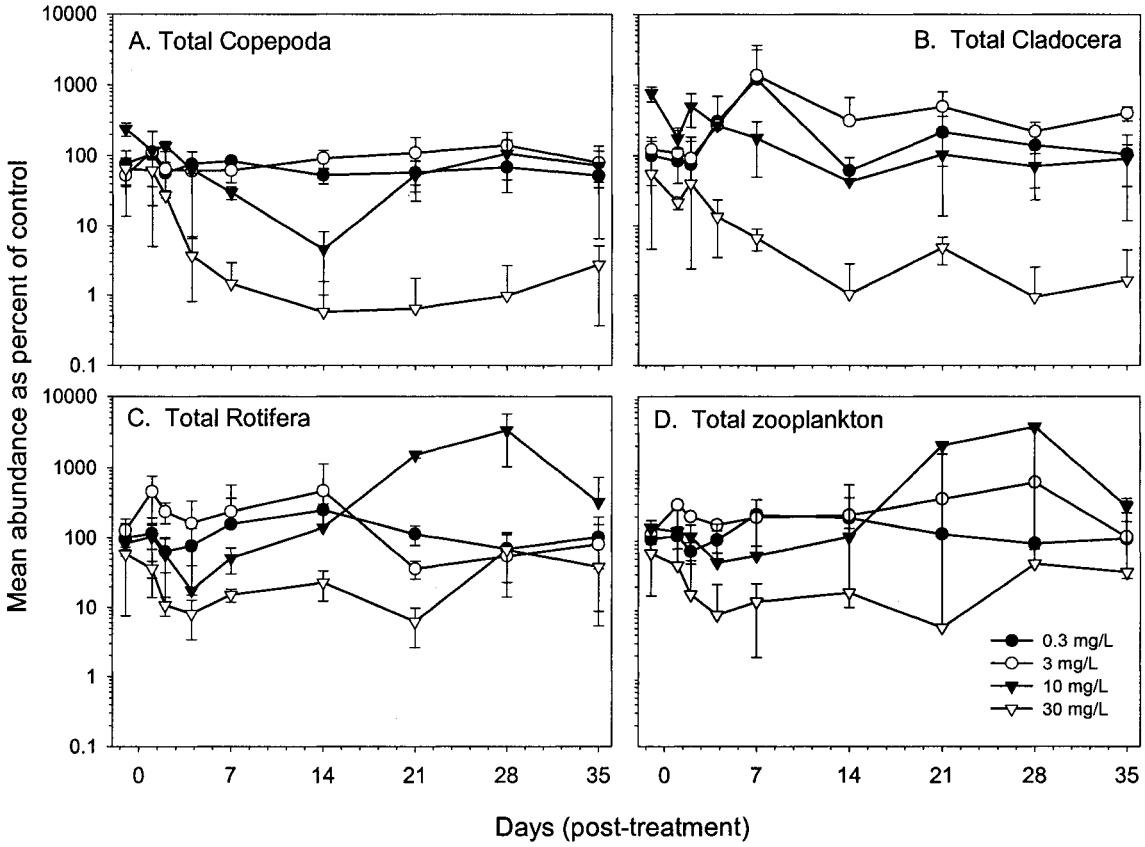

Figure 4.3 Abundance of zooplankton populations over 35 d. A = total Copepoda, B = total Cladocera, C = total Rotifera, and D = total zooplankton. Each point represents the mean ± SE of three replicates as a percent of controls.

Populations of Cladocera and Copepoda were greatly impacted by PFOS exposure. The species weight diagram revealed a total of 5 copepods and 5 cladocerans with values < -0.6, indicating a trend opposite to the PRC. The cladoceran, *Simocephalus vetulus* and copepod nauplii experienced the greatest treatment-response decrease in abundance compared to all other species, as indicated by the large negative species weights of -3.5 and -3.3, respectively (**Figure 4.2B**). Similarly, total copepod and cladoceran abundance in 30 mg/L was reduced significantly ($p \leq 0.05$) from day 4 until the end of the evaluation (**Figure 4.3A-B**). Copepods in 10 mg/L microcosms experienced a maximum temporal decrease at 14 d

but increased to control levels by 28 d.

Impact on zooplankton community structure was also apparent in terms of species richness expressed as the total number of different species. The number of different taxa present in the 30 mg/L treatment was significantly reduced ($p < 0.05$) after 2 d, compared to the controls and remained so until the end of the study. Mean reduction in taxa at the highest treatment was 74.3 ± 8.7% (**Figure 4.4**). The number of taxa in the 10 mg/L treatment was significantly reduced ($p < 0.05$) between 14 d and 35 d post-treatment, with a mean reduction of 45.1 ± 4.2%. No difference in number of taxa from controls was detected for either the 0.3 or 3 mg/L treatments.

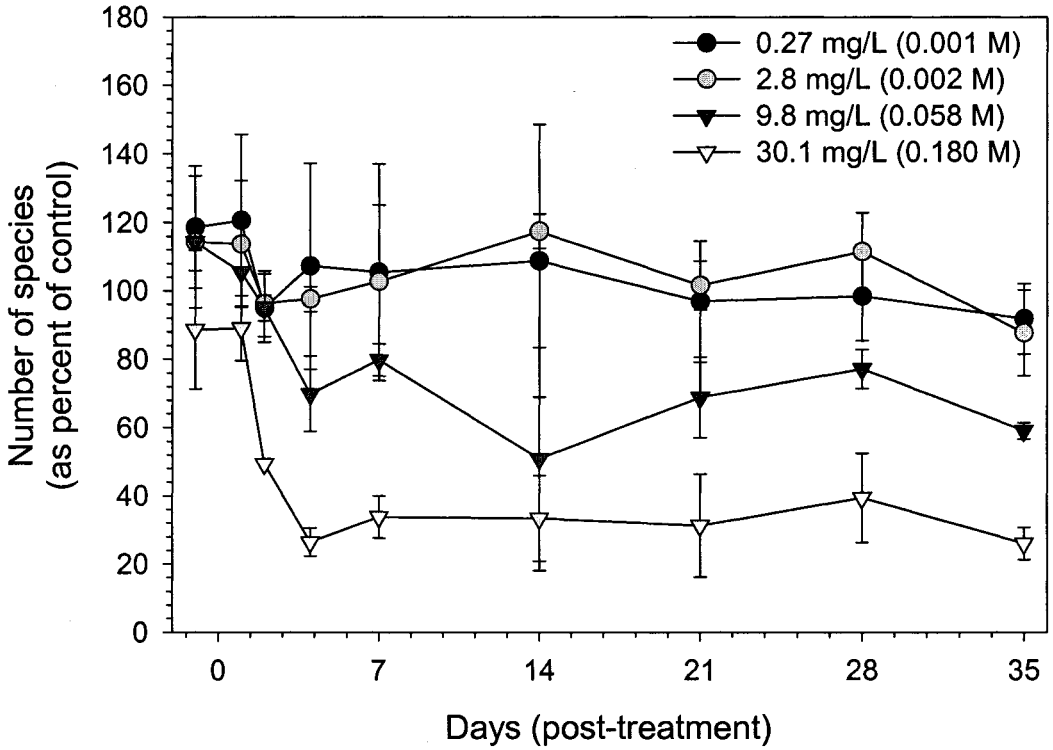

Figure 4.4 Total number of different species present at each sample period as relative to control over 35 d. Each point represents the mean ± SE of three replicates.

4.4.3 Water Parameters and Chlorophyll(a)

There were no significant differences between treatment and controls in water quality parameters over the study period (**Table 4.2**). There was little variation for these parameters among treatment microcosms.

Table 4.2 Water chemistry parameters for PFOS microcosm study from August 21 to October 3, 2000.

PFOS (mg/L)	Temperature (°C) (n =16)		Dissolved Oxygen in mg/L (n = 16)	pH (n = 10)	Alkalinity[a] (n = 10)	Hardness[a] (n = 10)
	Maximum	Minimum				
0 (control)	20.2 ± 3.1	16.3 ± 3.4	7.2 ± 2.6	8.3 ± 0.4	166 ± 15.2	296 ± 8.3
0.3	20.5 ± 3.1	16.2 ± 3.6	8.8 ± 2.8	8.8 ± 0.5	160 ± 13.8	293 ± 6.9
3	20.4 ± 3.1	15.9 ± 3.6	7.6 ± 2.5	8.4 ± 0.4	167 ± 20.9	300 ± 11.9
10	20.4 ± 3.1	16.2 ± 3.6	8.3 ± 2.5	8.6 ± 0.5	157 ± 14.9	292 ± 5.7
30	20.3 ± 2.9	16.4 ± 3.5	7.9 ± 2.5	8.5 ± 0.4	158 ± 18.6	294 ± 9.1

[a] Parameters measured in mg C_aCO_3/L.

Chlorophyll(a) concentration in treated microcosms showed elevated fluctuations during the first 21 d compared to controls (**Figure 4.5**). All treatments followed similar trends but few significant differences could be detected due to low power as a result of high within-treatment variability. All treatments returned to control levels by day 21. Significant increases ($p \leq 0.05$) in chlorophyll(a) concentration for 10 mg/L occurred at day 4 and 14. A significant increase ($p = 0.03$) for 30 mg/L was determined at day 7.

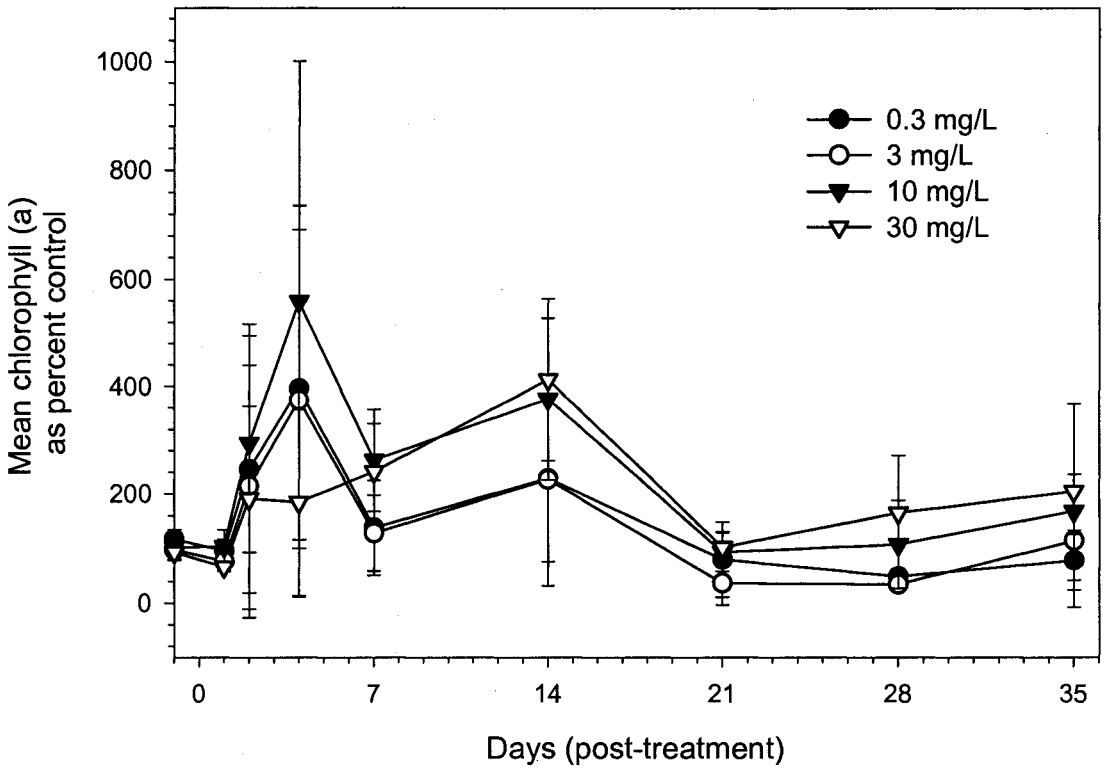

Figure 4.5 Chlorophyll(a) concentration present in water column of treated microcosms as percent of controls over 35 d. Each point represents the mean ± SE of three replicates.

4.4.4 Lemna gibba

Acute toxicity to *L. gibba* was evident only at 30 mg/L (**Figure 4.6**). Visual evidence of toxicity, such as chlorosis and necrosis, was observed as early as day 4 in the 30 mg/L treatment microcosms and by 14 d these effects were obvious. *Lemna gibba* in 30 mg/L showed significant reductions ($p < 0.01$) in frond numbers from the controls as early as day 14. By the end of the study there was a 90% reduction in frond number compared to controls. The 42 d IC50 for frond number was 19.1 mg/L and 20.9 mg/L for plant number. The NOEC for *L. gibba* frond number was 0.2 mg/L. A majority of plants in the 10 mg/L concentration displayed small, pale fronds with decreased root growth, however, the mean frond number was not statistically different from controls.

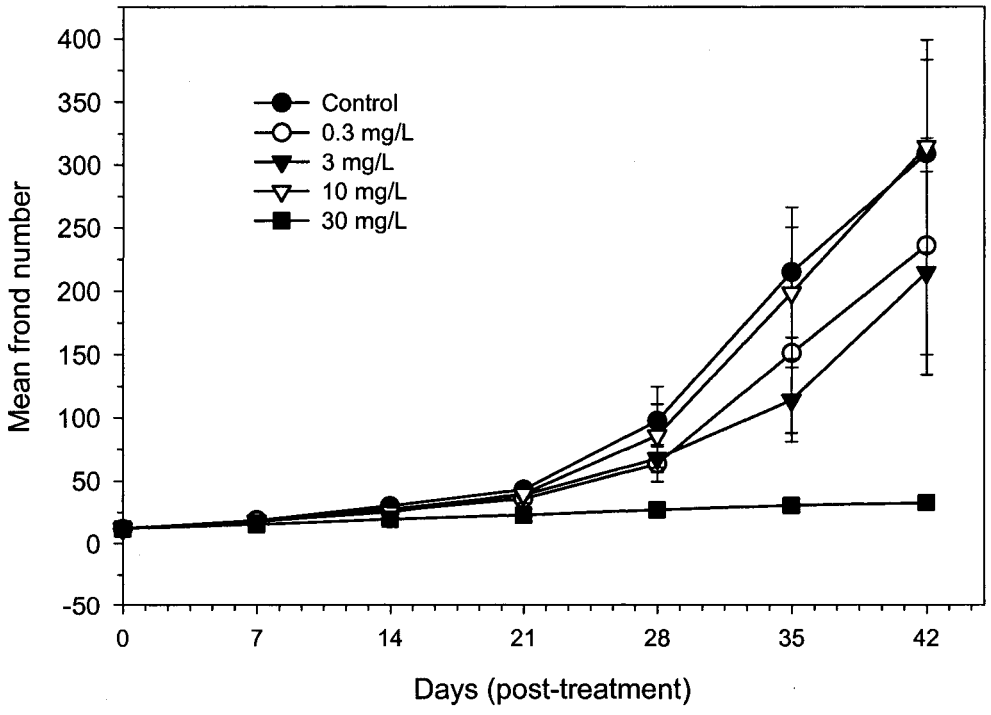

Figure 4.6 Growth of *Lemna gibba* measured as mean number of fronds produced over 42 d. Each point represents the mean ± SE of three replicates.

4.5 DISCUSSION

PFOS was persistent in aquatic microcosms for the study duration (285 d). The high variability seen in the 30 mg/L treatment at day 163 was due to sampling method whereby the sample became inadvertently diluted with ice melt-water. Although an obvious outlier, this sample was included because only two replicates remained for this sampling period. In our study, an increase in concentration for all treatments was evident between days 135 to 193, followed by a decline from day 223 to 285. These temporal deviations correspond to the winter season and were caused by ice formation, which decreased the amount of liquid water present in each microcosm and increased the relative PFOS concentration by eutectic exclusion of ice solute from the ice [37]. The subsequent spring thaw and inputs of precipitation filled the microcosms, thus diluting the PFOS concentration. Overall, mean reduction in PFOS from initial nominal concentrations over the 285-day period was 3.5% ± 3.2% (i.e. not significantly reduced).

Fluorinated compounds have been observed to persist for long periods in closed aquatic systems. Hanson *et al* [21] reported that trifluoroacetic acid, the shortest PFA, did not degrade in aquatic systems over a one year period. Similarly, Sanderson *et al* [38] observed no change in PFOS up to 35 d using 30 L freshwater microcosms. Because PFOS is persistent, there is concern that concentrations in aquatic systems have the potential to cause adverse effects, especially if inputs continue. In addition, PFOS has a low Henry's Law Constant suggesting that volatilization from water to air is not likely [39]. These data confirm that closed aquatic systems such as lakes and vernal pools have a high potential for PFOS accumulation.

In the present study zooplankton community response indicated acute toxicity at PFOS concentrations ≥10 mg/L for 35 days. The lowest $NOEC_{community}$ calculated over the 35-day study was 0.3 mg/L, however, a more accurate threshold level for PFOS exposure to this zooplankton community was determined to be 3 mg/L. This threshold level falls in the upper range (0.16 - 2,210.0 µg/L) of PFOS surface water concentrations documented during an aqueous fire fighting foam spill [6], although average environmental concentrations are much lower [12]. General effects of PFOS on zooplankton were evaluated by assessing raw abundance, PRC, and total number of taxa data together. A reduction in population abundances of Cladocera, Copepoda, Rotifera (**Figure 4.3A-C**) from 30 mg/L treatments and in total zooplankton (**Figure 4.3D**) were observed immediately following application. The greatest decrease in abundance from controls occurred in only 5 species of zooplankton. Species weights for these taxa showed a 2 - 3.5 fold decrease from control abundances (**Figure 4.2B**).

The PRC analysis, on the other hand, indicated that the general response of the zooplankton community at 10 and 30 mg/L was an increase in abundance relative to controls. This apparent incongruity can be explained by the species weights. Only two of 68 total zooplankton species, the rotifers *Bdelloidea* sp. and *P. vulgaris*, dominated the community PRC due to their increased abundance in treatments relative to controls. These taxa were present at densities 10-100 times those observed in controls. Initially, during the first 2 days, only *P. vulgaris* contributed to the increased response observed in both 10 and 30 mg/L treatments, and was significantly different from controls ($p = 0.004$ and 0.006, respectively). *Polyarthra vulgaris* and *Filinia longiseta* abundance at days 21 and 28 at 10 mg/L was

significantly ($p \leq 0.01$) different from control. *Filinia longiseta* had a relatively low species weight value, which most likely reflects the fact that it was only present at high densities at 10 mg/L. Over time, *P. vulgaris* disappeared from the 30 mg/L treatments. *Bdelloidea* sp. abundance began having a significant impact ($p \leq 0.05$) in both 10 and 30 mg/L treatments beginning from 21 d to 35 d. These two species may have been more resilient to PFOS toxicity and were able to become more numerous due to reduced competition by less resilient species. Increases in abundance by taxa of rotifers in an effected zooplankton community, after PFOS exposure, have been observed previously with indoor microcosms [38].

Community recovery is an ecologically significant end-point and experiments should be conducted until recovery is observed in the effect variables [24]. In pulse exposures, it is common to observe dramatic short-term declines in zooplankton populations after addition of non-persistent stressors followed by recovery to control levels [17, 20, 23]. However, with the extremely persistent compound PFOS, recovery could not be observed because the concentrations remained stable within the microcosms. The ability of an organism to resist a xenobiotic (e.g., PFOS) is referred to as resilience. In our case, a few rotifer species increased to numbers greater than the controls implying insensitivity to PFOS. In contrast, no evidence of resilience was observed in cladoceran or copepod populations, although the copepod population at 30 mg/L did increase slightly in mean abundance by day 35, but remained significantly lower than control populations.

Community structure, defined by the different number of taxa present in each treatment, was significantly affected. The number of individual species over 35 d in the controls was 23.8 ± 2.3, whereas the 30 mg/L treatment had 8.2 ± 4.7 species. In addition,

the 10 mg/L treatment showed significant reduction in number of species ($p < 0.05$) ≥ 14 d. This significant alteration to community structure could affect the function of the ecosystem as a whole. If the number of species affected increases, indirect effects can contribute to more significant disruption in the ecosystem as a whole [40].

We assessed chlorophyll(a) concentrations as a surrogate measure of the phytoplankton community. Increases in mean chlorophyll(a) concentrations were greatest in 10 and 30 mg/L treatments relative to the controls up to 14 d post-application. Chlorophyll(a) concentrations increased between 15 - 322% relative to control up to 21 d, but returned close to control levels thereafter with only minor deviations. The increase could be due to PFOS affecting selected phytoplankton species present in the community at the beginning of the study. The zooplankton component of the microcosm community was considered in conjunction with chlorophyll(a) (phytoplankton) changes. Zooplankton feed primarily on phytoplankton and can significantly influence population densities [19-20], and hence, chlorophyll(a) concentration. Although chlorophyll(a) concentrations did not follow a clear concentration-response relationship, it is interesting that peak chlorophyll(a) concentrations coincide with maximum declines in zooplankton abundances. This may have been due to the loss of many taxa within the rotifer and cladoceran populations during this time period. Initially, rotifer and cladoceran populations in 30 mg/L treatment decreased in abundance (> 72%) between days 2 and 14. Only rotifer populations in 10 mg/L decreased for the first 4 days then increased to levels much higher than controls. These trends in zooplankton abundances might have decreased the grazing pressure enough to influence the increase observed in chlorophyll(a) concentration up to day 21.

Lemna gibba growth was unaffected at all treatments except 30 mg/L displaying typical exponential growth ($r^2 > 0.96$). From 14 to 42 d, growth of *L. gibba* was significantly inhibited in the 30 mg/L treatment relative to the controls. Although all other treatments showed no change between controls, the growth inhibition NOEC value of 0.2 mg/L was less than the smallest concentration tested. Long term studies using a narrower concentration range might help elucidate effect concentrations.

4.6 CONCLUSIONS

The environmental persistence of PFOS was evaluated up to 285 days in aquatic systems. Due to the lack of knowledge for the behavior of PFOS in aquatic systems, it is prudent to investigate the potential effects this compound has on freshwater organisms. From what little data there is, aqueous environments could be potential sinks for PFOS accumulation, and thus contribute to xenobiotic stress on freshwater ecosystems. Based on low µg/L to low mg/L concentrations of PFOS in water following accidental release, our data indicates that freshwater communities may be sensitive to point-source inputs of PFOS [6]. Adverse effects could manifest themselves on certain freshwater organisms under current regulations that allow continued input and environmental concentrations to remain unchecked. However, freshwater ecosystems do not appear to be in jeopardy at current, observed background concentrations.

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4.7 REFERENCES

1. Remde A, Debus R. 1996. Biodegradability of fluorinated surfactants under aerobic and anaerobic conditions. *Chemosphere* 32:1563-1574.

2. Key BD, Howell RD, Criddle CS. 1998. Defluorination of organofluorine sulfur compounds by *Pseudomonas* sp. strain D2. *Environ Sci Technol* 32:2283-2287.

3. Sharpe AG. 1971. The physical properties of the carbon-fluorine bond. In Ciba foundation, ed, *Carbon-Fluorine Compounds: Chemistry, Biochemistry and Biological Activities*. Associated Scientific Publishers, London, UK, pp 33-54.

4. Boudreau TM, Sibley PK, Mabury SA, Muir DCG, Solomon KR. 2002. Laboratory evaluation of the toxicity of perfluorooctane sulfonic acid (PFOS) on *Selenastrum capricornutum*, *Chlorella vulgaris*, *Lemna gibba*, *Daphnia magna*, and *Daphnia pulicaria*. *Arch Environ Contamin Toxicol* In press.

5. Karsa DR. 1989. *Industrial Applications of Surfactants II.* Whitstable Litho Printers Ltd., Whitstable, Kent, UK.

6. Moody CA, Martin JW, Kwan WC, Muir DCG, Mabury SA. 2002. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. *Environ Sci Technol* 36:545-551.

7. Giesy JP, Kannan K. 2001. Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* 35:1339-1342.

8. Kannan K, Franson JC, Bowerman WW, Hansen KJ, Jones PD, Giesy JP. 2001. Perfluorooctane sulfonate in fish-eating birds including bald eagles and albatrosses. *Environ Sci Technol* 35:3065-3070.

9. Martin JW, Muir DCG, Moody CA, Ellis DA, Kwan WC, Solomon KR, Mabury SA. 2002. Collection of airborne fluorinated organics and analysis by gas chromatography/ chemical ionization mass spectrometry. *Anal Chem* 74:584-590

10. Martin JW, Mabury SA, Solomon KR, Muir DCG. 2002. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout. *Environ Toxicol Chem* 22:196-204.

11. Moody CA, Field JA. 1999. Determination of perfluorocarboxylates in groundwater impacted by fire-fighting activity. *Environ Sci Technol* 33:2800-2806.

12. Hansen KJ, Johnson HO, Eldridge JS, Butenhoff JL, Dick LA. 2002. Quantitative characterization of trace levels of PFOS and PFOA in the Tennessee River. *Environ Sci Technol* 36:1681-1685

13. Kaiser J, Enserink M. 2000. Treaty takes a POP at the dirty dozen. Science pp 2053.

14. Shaw J, Kennedy J. 1996. The use of aquatic field mesocosm studies in risk assessment. *Environ Toxicol Chem* 15:605-607.

15. Shaw JL, Manning JP. 1996. Evaluating macroinvertebrate population and community level effects in outdoor microcosms: use of *in situ* bioassays and multivariate analysis. *Environ Toxicol Chem* 15:608-617.

16. Van den Brink P, Hattink J, Bransen F, Van Donk E, Brock T. 2000. Impact of the fungicide carbendazium in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquat Toxicol* 48:251-264.

17. Van Den Brink PJ, Van Wijngaarden RPA, Lucassen WGH. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. *Environ Toxicol Chem* 15:1143-1153.

18. Odum EP. 1993. *Ecology and Our Endangered Life-Support Systems*. Sinauer Associates, Inc., Sunderland, MA, USA, 38-47.

19. Williamson C. 1991. Copepoda. In Thorp JH, Covich AP, eds, *Ecology and Classification of North American Freshwater Invertebrates*. Academic Press, San Diego, CA, USA, pp 783-807.

20. Cottingham KL, Knight SE, Carpenter SR, Cole JJ, Pace ML, Wagner AE. 1997. Response of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment. *J Plank Res* 19:995-1010.

21. Hanson ML, Sibley PK, Ellis DA, Fineberg NA, Solomon KR, Mabury SA, Muir DCG. 2001. Trichloroacetic acid (TCA) fate and toxicity to the macrophytes *Myriophyllum spicatum*, and *Myriophyllum sibiricum* in aquatic microcosms. *Aquat Toxicol* 56:241-255.

22. Marwood CA, Solomon KR, Greenberg BM. 2001. Chlorophyll fluorescence as a bioindicator of effects on growth in aquatic macrophytes from mixtures of polycyclic aromatic hydrocarbons. *Environ Toxicol Chem* 20:890-898.

23. Sibley PK, Harris ML, Bestari JT, Steele TA, D RR, Gensemer RW, Day KE, Solomon KR. 2001. Response of zooplankton communities to liquid creosote in freshwater microcosms. *Environ Toxicol Chem* 20:394-405.

24. Sanderson H. 2001. Replication of micro/mesocosm studies. *Environ Sci Pollut Res* 8:1-7.

25. Solomon KR, Smith K, Stephenson GR. 1982. Depth integrated samplers for use in limnocorals. *Hydrobiologia* 94:71-75.

26. Whiteside MC, Williams JB. 1975. A new sampling technique for aquatic ecologists. *Limnologie* 19:1534-1539.

27. Pennak RW. 1989. *Freshwater Invertebrates of the United States*. John Wiley & Sons, Inc., New York, NY.

28. Balcer MD, Korda NL, Dodson SI. 1984. *Zooplankton of the Great Lakes: A Guide to the Identification and Ecology of the Common Crustacean Species*. The University of Wisconsin Press, Madison, WC, USA.

29. Thorp JH, Covich AP. 1991. *Ecology and Classification of North American Freshwater Invertebrates*. Academic, San Diego, CA, USA.

30. Faber MJ, Thompson DG, Stephenson GR, Kreutzweiser DP. 1998. Impact of glufosinate-ammonium and bialaphos on the zooplankton community of a small eutrophic northern lake. *Environ Toxicol Chem* 17:1291-1299.

31. Rand GM, Petrocelli SR. 1985. *Fundamentals of Aquatic Toxicology*: Methods and Applications. Harper & Row Inc., New York, NY, USA.

32. ter Braak CJF, Smilauer P. 1998. CANOCO for Windows, version 4. Centre for Biometry - Microcomputer Power, Wageningen, The Netherlands.

33. Van Den Brink PJ, Ter Braak CJF. 1999. Principle response curves: analysis of time-dependent multivariate responses of biological community to stress. *Environ Toxicol Chem* 18:138-148.

34. Gulley DD, Boelter AM, Bergman HL. 1991. Toxstat, version 3.3. Department of Zoology and Physiology, University of Wyoming, Laramie, WY, USA.

35. Norberg-King TJ. 1993. A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach. Technical report 03-93. US Environmental Protection Agency, Duluth, MN, USA.

36. Van Der Hoeven N. 1997. How to measure no effect. Part III: statistical aspects of NOEC, ECx and NEC estimates. *Environmetrics* 8:255-261.

37. Hanson ML, Sibley PK, Mabury SA, Muir DCG, Solomon KR. 2001. Chlorodifluoroacetic acid fate and toxicity to the macrophytes *Lemna gibba*, *Myriophyllum spicatum*, and *Myriophyllum sibiricum* in aquatic microcosms. *Environ Toxicol Chem* 20:2758-2767.

38. Sanderson H, Boudreau TM, Mabury SA, Cheong WJ, Solomon KR. 2002. Ecological impact and environmental fate of perfluorooctane sulfonate on the zooplankton community in indoor microcosms. *Environ Toxicol Chem* 21:1490-1496.

39. USEPA. 2000. Perfluorooctyl sulfonates; proposed significant new use rule. Federal Register Vol. 65, No. 40 CFR 721. U.S. Environmental Protection Agency, Washington DC.

40. Solomon KR. 2001. Ecotoxicological Risk Assessment of Pesticides in the Environment. In *Handbook of Pesticide Toxicology; Principles*, Academic Press, New York, NY, USA.

5 GENERAL DISCUSSION AND CONCLUSION

This thesis evaluated the general toxicity of PFAs to selected freshwater primary producers and secondary consumers through both laboratory and field microcosm studies. Several single-species laboratory studies and two separate outdoor microcosm studies were conducted to investigate the relative toxicity of PFAs within and between trophic levels. Organisms within trophic levels responded similarly to PFA exposure, but sensitivity between trophic levels was different, depending on chain length of the PFA. Chain length of PFAs has been reported to cause differences in physiological properties as well. In accumulation studies by Martin *et al* [1,2], trout depuration rates and tissue half-lives increased with increased chain length for both PFSAs and PFCAs. Compound specificity has an implication when considering the best organism(s) for regulatory testing. As chain length of PFAs increases, the most sensitive organism varies. Overall, PFOS was the most toxic PFA tested, more so than its PFCA homologues, represented by PFNA (laboratory tests) and PFOA (field study). Furthermore, since many of these compounds are manufactured into mixtures, more research on the toxicity and bioconcentration/ bioaccumulation potential of PFCA mixtures in the environment would be prudent.

The mechanism of toxic action for PFAs is commonly believed to be caused by oxidative stress induced by peroxisome proliferation, especially with PFAs ≥ C7. Down-regulation of gap junction intracellular communication (GJIC) has been coupled to tumor promotion by many peroxisome proliferators [3], but whether down-regulation alone can lead to a mitogenic event or not remains to be determined. Therefore, there appears to be varying mechanisms by which PFAs elicit toxic responses. The mechanism for which PFAs ≥ C7

inhibit GJIC has yet to be determined, but it is believed to be activated by phosphorylation of gap junction proteins [3]. Moreover, inhibition of GJIC did depend on chain length of the PFA [3].

Comparing results from plant and invertebrate studies with PFPrA indicates that toxicity for this particular PFA is perhaps related to chloroplasts or photosynthesis in general. PFA interaction with membrane-bound proteins has also been hypothesized to cause membrane leakage [4,5]. In the studies with algae, chlorophyll(a) measurements were sometimes not as sensitive as cell density, which might be due to the fact that there was free chlorophyll(a) in the medium due to loss of membrane function. This indicates that perhaps chlorophyll became liberated by compromised cells, resulting in lower IC50 values compared to cell density. On the other hand, toxic action for PFA $\geq$ C7 may be promoted by proliferation of fatty acid β-oxidation pathway in organelles leading to oxidative stress due to free radicals.

Trophic level interactions play a major role in freshwater foodweb dynamics. Elimination of too many species from a community can lead to loss of structure and ultimately affect the functionality of the ecosystem as a whole [6]. The microcosm research showed a simplification of the zooplankton community through both direct and indirect effects as well as no sign of recovery for affected species exposed to relatively high concentrations of PFOS as well as PFOA. Hydrophobic organic compounds can bind to the surface of algae cells [7-9], but the same does not occur with PFAs. The potential result of binding to algal cells could be the alteration of the availability of PFAs into foodwebs by algae-consuming zooplankton in aquatic ecosystems [7,8]. The availability of hydrophobic

organic compounds to biota is driven by their partitioning out of water into lipid tissues [10]. PFAs do not behave like classic hydrophobic organic compounds because they are lipophobic as well, thus partitioning into lipid tissues is unlikely. Due to the unique physical chemical properties of PFAs and how they interact with biomolecules, more research on the bioconcentration and bioaccumulation potential of PFAs is required.

The PRC method is specifically designed to analyze large data sets, especially from microcosm studies, and provide a clear output of both direct and indirect treatment effects at the community level [11]. This method compares the development of the treatment community in comparison to the controls over time, thus, in conjunction with species weights, it can be used to discriminate responses of individual species as well as populations [11]. This thesis used the PRC method to determine the impact of selected PFAs on the zooplankton community. In both studies, PRC showed that populations of copepods and cladocerans were eliminated at the highest concentrations, whereas few rotifer species appeared to overcome the direct effects of PFA exposure and dominate the community. Recovery studies were not possible due to the persistence of PFOS and PFOA in the aquatic environment (half-lives could not be calculated either).

Microcosms offer risk assessors a sound and realistic approach to study effects of anthropogenic stressors across levels of biological organization under environmental conditions rather than controlled laboratory conditions [12,13]. Additionally, more emphasis is being placed on populations as well as the community as a whole for evidence of adverse effects on aquatic ecosystems [14,15]. PRC and Monte Carlo permutation testing is a novel method that allows researchers to quantify deviations in the community from controls.

Extrapolating effects seen in laboratory or micro/mesocosm studies to that in natural assemblages might not be meaningful. In order to produce more accurate predictions of effects on natural assemblages, assessors should analyse results from both laboratory tests and semi-field studies [16]. However, there are problems associated with interpreting results of laboratory and micro/mesocosm tests and relating them to effects seen on populations in the field. The use of the no-observed effect concentration derived from laboratory studies has been criticized and micro/mesocosm studies, because of low replication, are associated with high variability [17].

The toxicity data from this research shows that PFAs, particularly ≥ C7, can be toxic to freshwater ecosystems, but that these toxic concentrations are many orders of magnitude higher than reported environmental concentrations. However, point-source PFA contamination, especially in a spill situation, has the potential to reach adverse concentrations. Until recently, regulatory agencies had little to no toxicological data to use in risk assessments on PFAs. This research should add significantly to the available toxicity data for these persistent compounds, particularly PFOS and PFCAs. The relationship of toxicity and length of the carbon chain may aid researchers in the investigation of the mechanism of PFA action.

5.1 REFERENCES

1 Martin JW, Mabury SA, Solomon KR, Muir DCG. 2002. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 22:189-195.

2 Martin JW, Mabury SA, Solomon KR, Muir DCG. 2002. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout. *Environ Toxicol Chem* 22:196-204.

3 Upham BL, Deocampo ND, Wurl B, Trosko JE. 1998. Inhibition of gap junction intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. *Int J Cancer* 78:491-495.

4 Nyberg H. 1989. Growth and ATP levels in *Prophyridiun purpureum* (Rhodophyceae, Bangiales) cultured in the presence of surfactants. *Br Phycol J* 24:91-98.

5 Hu W, Jones PD, Upham BL, Trosko JE, Lau C, Giesy JP. 2002. Inhibition of gap junction intracellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines *in vitro* and Sprague-Dawley rats *in vivo*. *Toxicol Sci* 68:429-436.

4 Solomon KR. 2001. Ecotoxicological Risk Assessment of Pesticides in the Environment. *Handbook of pesticide toxicology; principles*, Academic Press New York, NY, USA. p.

5 Swackhamer DL, Skoglund RS. 1993. Bioaccumulation of PCBs by algae: kinetics versus equilibrium. *Environ Toxicol Chem* 12:831-838.

6 Taylor G, Baird DJ, Soares A. 1998. Surface binding of contaminants by algae: consequences for lethal toxicity and feeding to daphnia magna straus. *Environ Toxicol Chem* 17:412-419.

7 Koelmans AA, Van Der Woude H, Hattink J, Niesten JM. 1999. Long-term bioconcentration kinetics of hydrophobic chemicals in *Selenastrum capricornutum* and *Microcystis aeruginosa*. *Environ Toxicol Chem* 18:1164-1172.

8 Russell RW, Gobas FAPC, Haffner GD. 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. *Environ Toxicol Chem* 18:1250-1257.

11 Van Den Brink PJ, Ter Braak CJF. 1999. Principle response curves: analysis of time-

dependent multivariate responses of biological community to stress. *Environ Toxicol Chem* 18:138-148.

12 Shaw J, Kennedy J. 1996. The use of aquatic field mesocosm studies in risk assessment. *Environ Toxicol Chem* 15:605-607.

10 Rand GM, Petrocelli SR. 1985. *Fundamentals of aquatic toxicology: methods and applications.* Harper & Row Inc., New York, NY, USA 660 p.

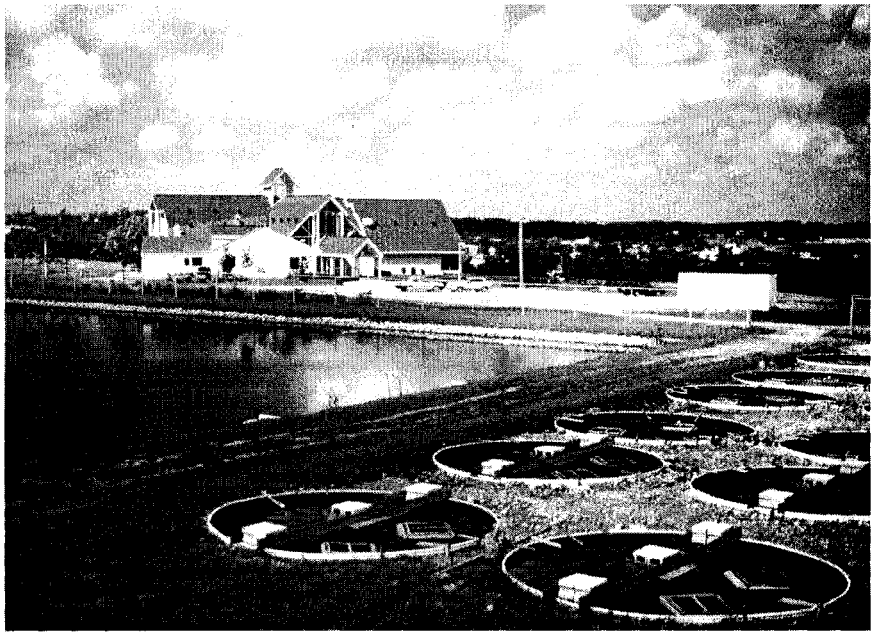
11 Pratt JR, Bowers NJ. 1992. Variability of community metrics: detecting changes in structure and function. *Environ Toxicol Chem* 11:451-457.

12 Joern A, Hoagland KD. 1996. In defense of whole-community bioassays for risk assessment. *Environ Toxicol Chem* 15:407-409.

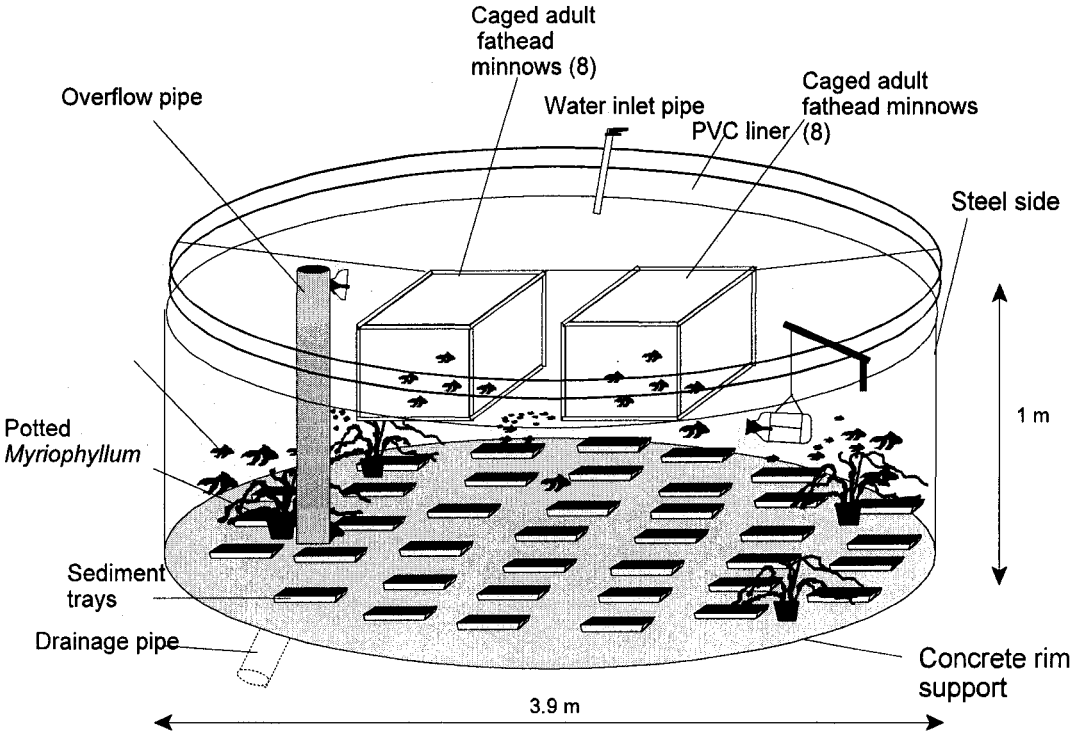
13 Shaw JL, Manning JP. 1996. Evaluating macroinvertebrate population and community level effects in outdoor microcosms: use of in situ bioassays and multivariate analysis. *Environ Toxicol Chem* 15:608-617.

14 Crane M, Grosso A. 1999. Limits to forecasts of population dynamics in ecotoxicology. *Asp Appl Biol* 52:149-155.

6 APPENDICES

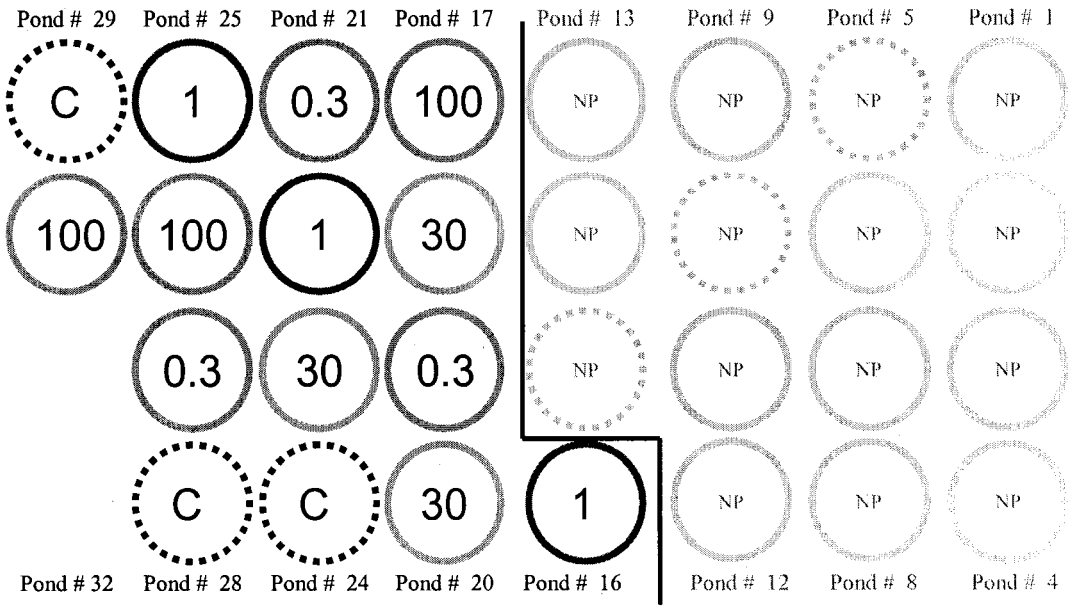


Appendix I The Guelph Microcosm Research Facility. The right side of the picture shows the replicable man-made, semi-natural microcosms under a different study. The large irrigation pond, on the left, provided water and subsequent biotic assemblages for each microcosm.



Appendix II a Illustration of one 12,000 L microcosm located at the Guelph Microcosm Research Facility used in studies to evaluate the effects of PFAs on the aquatic community (adapted from Takacs, 1999).

Appendix II b Photograph of a floating *Lemna* cage. Introduction of individual plant colonies after PFOA treatment is depicted here.

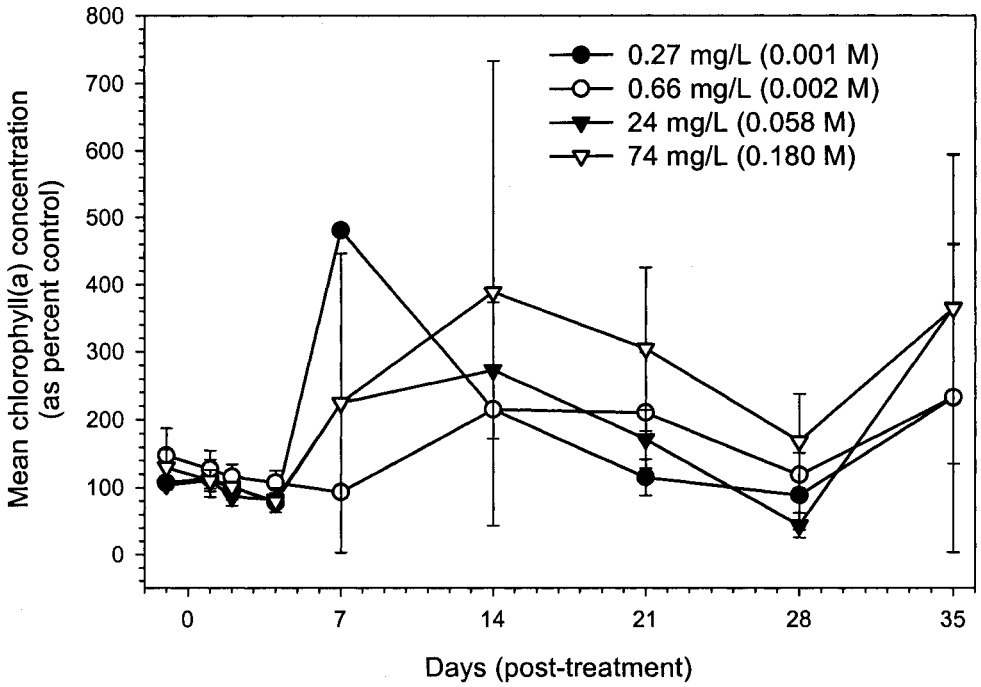


Appendix III Randomized allocation of treatments for PFOA microcosm study. Like circles indicate nominal treatment groups in mg/L; C = control.

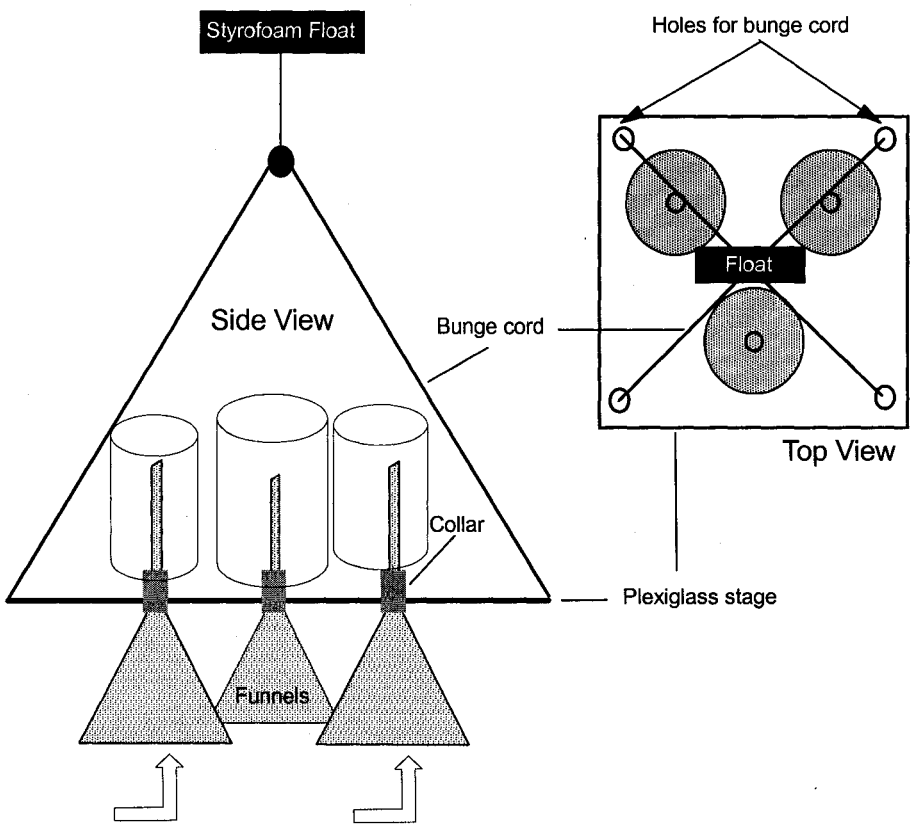
Appendix IV Water chemistry parameters for PFOA microcosm study from June 11 to July 18, 2000.

	Temperature (° C)					
PFOA (M)	**Maximum (n = 29)**	**Minimum (n = 29)**	**DO (n = 25)**	**pH (n = 10)**	**Alkalinity[a] (n = 10)**	**Hardness[a] (n = 10)**
0	21.5 ± 1.9	17.9 ± 2.1	7.3 ± 2.1	8.4 ± 0.3	104 ± 42.0	218 ± 6.8
0.001	21.9 ± 2.2	18.1 ± 2.0	7.6 ± 2.0	8.5 ± 0.3	111 ± 18.2	218 ± 7.0
0.002	21.5 ± 2.0	17.8 ± 1.9	7.5 ± 2.2	8.5 ± 0.3	110 ± 16.2	217 ± 7.5
0.058	22.0 ± 2.1	18.3 ± 1.8	7.5 ± 2.3	8.6 ± 0.3	109 ± 16.8	217 ± 7.0
0.180	21.6 ± 2.6	17.7 ± 2.3	8.4 ± 1.6	8.7 ± 0.2	110 ± 17.4	216 ± 6.2

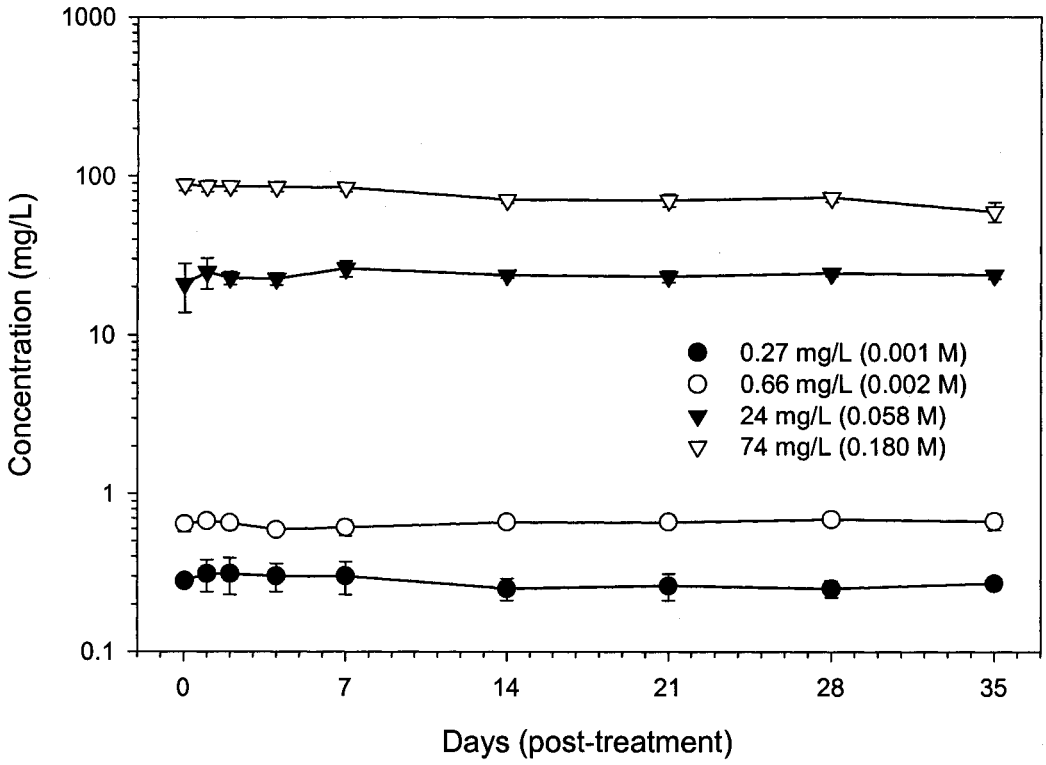
[a] Parameters are measured as mg of $CaCO_3$/L.



Appendix V Chlorophyll(a) concentration in PFOA treatment microcosms over 35 d. Each point represents the mean ± SE of three replicates as percent of controls. Chlorophyll data was used as a surrogate for phytoplankton abundance and used in conjunction with zooplankton data. The highest treatment had the highest concentration of chlorophyll(a) suggesting an indirect effect from the reduced consumption pressure of zooplankton, which were significantly decreased.



Appendix VI schematic representation of zooplankton collection traps. Zooplankton entered jars through the inverted funnels during their daily vertical migration.



Appendix VII Environmental persistence of PFOA at 4 treatments in outdoor aquatic microcosms over 35 days. Each point represents the mean ± SE of three replicates. Little if any degradation occurred, thus a half-life could not be determined.

Appendix VIII Time weighted average concentration values for each treatment in PFOA microcosm study. Measured water residue analysis was conducted at University of Toronto's ANALEST laboratory.

	Nominal PFOA Concentration (mg/L)			
	0.3	**1.0**	**30**	**100**
Time (days)	**Mean Measured Concentration (mg/L)**			
0	0.28	0.64	20.9	87.8
1	0.31	0.67	24.8	86.0
2	0.31	0.65	22.7	85.9
4	0.30	0.59	22.5	85.0
7	0.30	0.61	26.1	84.4
14	0.25	0.66	23.7	70.5
21	0.26	0.66	23.2	70.0
28	0.25	0.69	24.3	72.9
35	0.27	0.67	23.7	59.5
TWA	0.27	0.65	23.9	74.1
% of Initial	95.0	102	114	84.5
% of Nominal	90.2	65.4	79.7	74.1

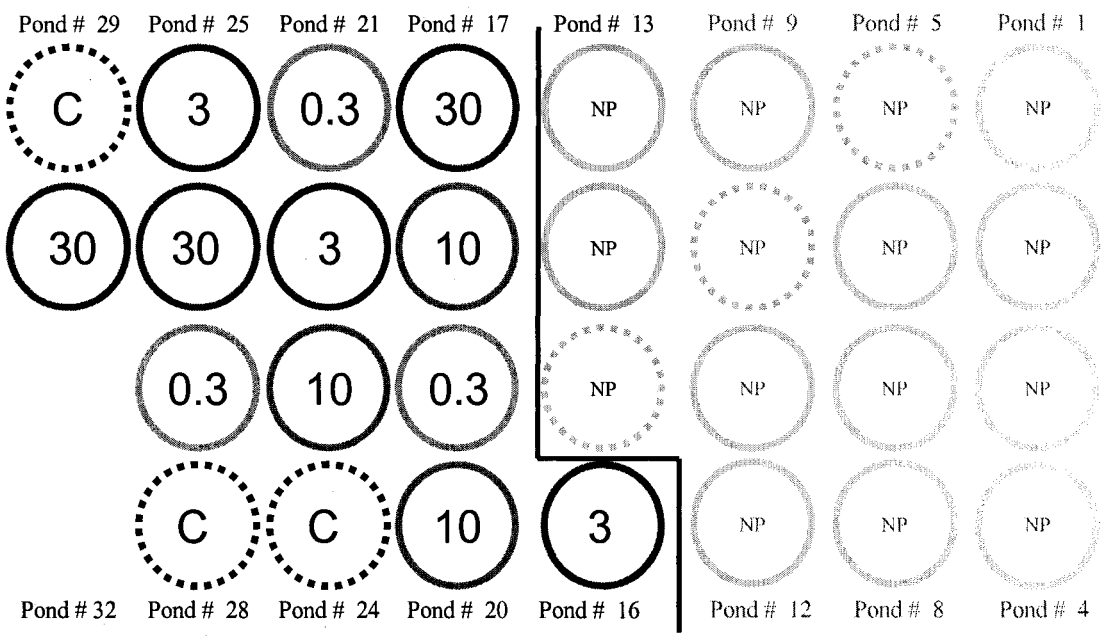
Appendix IX Interpretation of variance allocation given by principal components analysis at each sampling period.

	Principle Canonical Analysis for Sample Period									PRC
Percent Variance	-1	1	2	4	7	14	21	28	35	Entire 35 d
Between Replicates	45.3	68.6	68.6	48.8	48	33.9	22.4	28.9	26.8	15.4
Due to Treatment	54.7	31.4	31.4	51.2	52	66.1	77.6	71.1	73.2	26.4

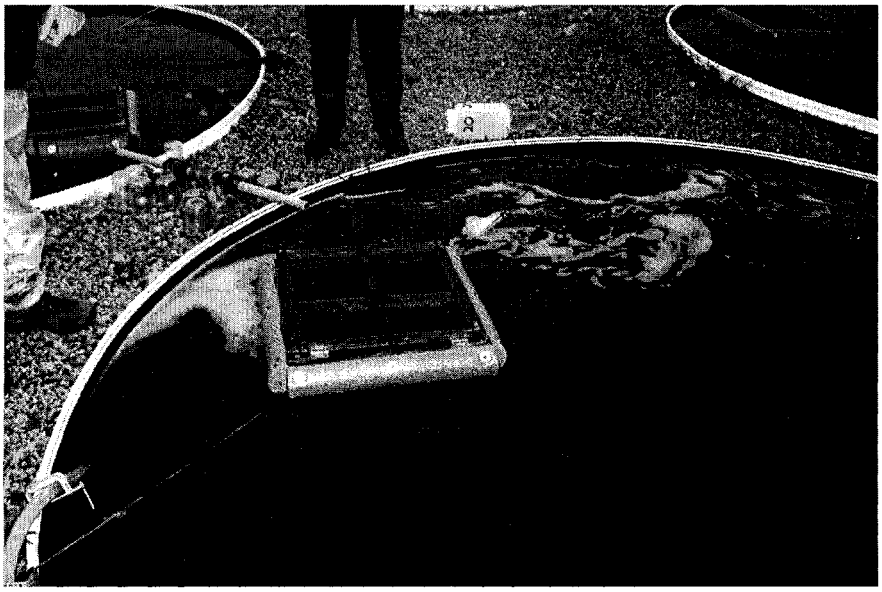
Did the PRC diagram (**Figure 3.3A**) display a significant portion of treatment variance? Yes, since Monte Carlo permutation p = 0.0010.

Did the treatment regime have a significant effect when considering all sample dates? Yes, since Monte Carlo permutation p = 0.0010.

The percent of the variance explained by treatment regime in the PRC diagram was 52.3%.



Appendix X Randomized allocation of treatments for PFOS microcosm study. Like circles indicate nominal treatment groups in mg/L; C = controls.



Appendix XI Picture of foaming attribute associated with PFOS application into a treated microcosm.

Appendix XII Time weighted average concentration values for each treatment in PFOS microcosm study. Individual events influencing measured concentrations are denoted in parentheses.

	Nominal PFOS Concentration (mg/L)		
	3.0	10	30
Time (days)	Mean Measured Concentration (mg/L)		
0	2.7	13.1	35.2
1	2.8	11.9	33.2
2	3.0	11.7	32.8
4	2.9	11.5	32.7
7	3.0	11.5	32.1
14	3.1	11.4	32.5
28	3.0	11.4	31.9
35	2.8	11.1	32.3
42	2.8	11.6	33.6
73	2.4	10.9	32.0
102	2.9	10.2	30.1
135 (freezing)	3.9	12.6	37.2
163	4.2	10.4	33.0
193	3.7	12.0	36.2
223 (thaw and rain)	1.8	5.2	23.5
251	1.9	7.7	24.6
264	1.9	8.4	25.2
271	1.9	8.1	22.5
278	1.8	7.9	22.9
285	1.9	7.9	22.8
TWA	2.82	9.83	30.1
% of Initial	104	75.0	85.4
% of Nominal	94	98	100