PERFLUOROCARBOXYLATE ISOMER ANALYSIS AS A TOOL FOR SOURCE ELUCIDATION

by

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ABSTRACT

Perfluorocarboxylates (PFCAs) are a class of anthropogenic compounds ubiquitously found in the environment. PFCAs and their precursors are largely manufactured by electrochemical fluorination (ECF) or telomerization. ECF products are mixtures of isomers with linear (70-80%) and branched perfluoroalkyl moiety. Telomerization does not produce isomer mixtures and is predominantly *n*-perfluorocarbons. This thesis examined the environmental fate and disposition of PFCAs from a relevant and novel perspective of industrial isomer signature. Potential influences of physical and biological properties of isomers on the environmental PFCA isomer pattern were investigated. Branched isomers were more water soluble than *n*-isomer, however, K_{OW} did not indicate any appreciable differences among isomers. It is possible that the similarity in K_{OW} is due to a balancing effect between elevated activity coefficients in both water and *n*-octanol. In fish and rats, the major branched isomers of ECF PFOA were eliminated faster than *n*-isomer. In comparison, PFOS isomer pharmacokinetics were indistinguishable. These findings highlight the need to understand underlying mechanisms mediating PFCA and PFOS isomer pharmacokinetics which may constrain extrapolation from animal-based models to humans. Environmental monitoring revealed PFCA isomers in both abiotic and biotic environment, in temperate regions and remote Arctic. Branched PFOA isomers were consistent with ECF production. In temperate regions, industrially produced ECF PFOA was expected to be a major source of these isomers, given its legacy and volume of production. In the Arctic, PFOA isomers consistent with an ECF signature were attributed to ECF perfluorooctylsulfonamides which likely undergo long range atmospheric transport and atmospheric reactions. The major difference in ECF signature between remote and temperate regions is the presence of ECF PFNA isomers compared to their absence in the Arctic. ECF PFNA is an impurity in ECF PFOA, comprising 0.2%. Input from a linear source, such as fluorotelomer compounds, was also suggestive as both PFOA and PFNA were >95% linear, much more than in technical ECF. Furthermore, longer chain ECF impurities do not account for the PFNA, PFDA, PFUnA, etc. in the Arctic.

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PREFACE

This thesis is organized as a series of manuscripts that have been published or are in preparation for submission to be published in peer-reviewed scientific journals. As such, repetition of introductory materials and methodology was inevitable. All manuscripts were written by Amila O. De Silva with critical comments provided by Scott Mabury. Contributions of all co-authors are provided in detail below.

Chapter One – Overview of Perfluorinated Acids and their Precursors
Contributions – Prepared by Amila O. De Silva with editorial comments provided by Scott Mabury

Chapter Two – Water Solubility and Octanol-Water Partition Coefficient of Perfluorooctylsulfonamides and Fluorotelomer Alcohols

To be submitted to – Environ. Toxicol. and Chem.
Author List – Amila O. De Silva, Naomi L. Stock, Julia Bonin, Grace W.-Y. Wong, Cora J. Young, Scott A. Mabury
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Chapter Three – Isolating Isomers of Perfluorocarboxylates in Polar Bears (*Ursus maritimus*) From Two Geographical Locations.

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Author List – Amila O. De Silva and Scott A. Mabury
Contributions – Amila De Silva conducted method development, data collection, interpretation, and manuscript preparation under the guidance of Scott Mabury.

Chapter Four – Isomer Distribution of Perfluorocarboxylates in Human Blood: Potential Correlation to Source

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Chapter Five – Disposition of Perfluorinated Acid Isomers in Rats; Part II Subchronic Dose

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Contributions - Amila De Silva was responsible for care and handling of animal subjects, preparation of the administered dose, sample acquisition, analysis of perfluorocarboxylate isomers and data interpretation. Jonathan Benskin conducted PFOS isomer determination in samples. Gilles Arsenault, Robert McCrindle, and Nicole Riddell prepared and provided purified isomers of PFOS and PFOA for identification of ECF composition. Each author contributed to the design of the study and provided critical comments to the manuscript prepared by Amila De Silva.

Chapter Six – Uptake, Accumulation and Elimination of Perfluorocarboxylate Isomers in Rainbow Trout

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Author List – Amila O. De Silva, Pablo J. Tseng, and Scott A. Mabury
Contributions – Care of animal subjects, sample collection, and preparation for analysis was by undergraduate student Pablo Tseng, under the guidance of Amila De Silva. Amila De Silva was also involved in sample preparation, analysis, and interpretation. All versions of the manuscript were prepared by Amila De Silva with critical comments provided by Pablo Tseng and Scott Mabury.

Chapter Seven – Distribution of Perfluorinated Carboxylate Isomers in the North American Environment

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Contributions – Amila De Silva was responsible for data collection, interpretation, and manuscript preparation with guidance and provision of critical comments by Derek Muir and Scott Mabury. Derek Muir arranged for and directed samples used in this study.

Chapter Eight – Summary, Conclusions and Future Work
Contributions – Prepared by Amila De Silva with additional comments provided by Scott Mabury.

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CHAPTER ONE

Overview of Perfluorinated Acids and their Precursors

Chapter One – Overview of Perfluoroalkyl Acids and their Precursors

1.1 Perfluoroalkyl Derived Compounds

Industrially produced perfluoroalkyl-containing compounds are the subject of this body of work. The perfluoroalkyl moiety refers to $F(CF_2)_x\boldsymbol{R}$ where ***R*** represents a variety of functionalities. This thesis focuses on perfluorinated acids (**PFAs**) and their precursors (perfluoroalkylsulfonamides, fluorotelomer alcohols and fluorotelomer olefin). These compounds are distinguished based on the identity of ***R*** as follows: perfluorocarboxylates (***R*** = $C(O)O^-$, **PFCA**), perfluoroalkylsulfonates (***R*** = SO_3^-), perfluoroalkylsulfonamides (***R*** = $SO_2NR'R''$, where R' and R'' correspond to an ethanol, hydrogen, and/or alkane moiety), fluorotelomer alcohols (***R*** = CH_2CH_2OH, *x*:2 **FTOH**), and fluorotelomer olefins (***R*** = $CH{=}CH_2$, *x*:2 **FTO**). Perfluoroalkylsulfonates and PFCAs are collectively referred to as PFAs. Perfluoroalkylsulfonamides are herein referred to by the acronym PFSAm. Table 1.1 lists the various perfluoroalkyl-containing compounds referred to in this thesis along with their abbreviations. Compounds in Table 1.1 have been industrially manufactured and/or are environmental degradation products of other fluorinated organics.

1.1.1 Industrial Production and Use of Perfluorinated Acids (PFAs) and Their Precursors

Major production of perfluoroalkyl compounds has largely proceeded by two separate industrial synthetic pathways: electrochemical fluorination (ECF) and telomerization. The dominant fluorinated organics from ECF were perfluorooctanoate ($C_7F_{15}COO$-, PFOA), perfluorooctanesulfonate ($C_8F_{17}SO_3$-, PFOS), and perfluorooctylsulfonamides (PFSAm). Telomerization is used to produce a perfluoroalkyl iodide ($F(CF_2)_xI$) from which a number of industrially-relevant compounds may be synthesized such as FTOHs, FTOs, PFOA and perfluorononanoate ($F(CF_2)_8C(O)O$-, PFNA). Some of these compounds are considered intermediates and raw materials which are further subjected to reactions in preparation of final sales products.

Table 1.1. Summary of perfluoroalkyl-containing compounds and acronyms.

Name	Formula	Acronym
Perfluorinated Acids (PFAs)		
Trifluoroacetic acid	$F(CF_2)C(O)O^-$	TFA
Perfluoropropianoate	$F(CF_2)_2C(O)O^-$	PFPrA
Perfluorobutanoate	$F(CF_2)_3C(O)O^-$	PFBA
Perfluoropentanoate	$F(CF_2)_4C(O)O^-$	PFPeA
Perfluorohexanoate	$F(CF_2)_5C(O)O^-$	PFHxA
Perfluoroheptanoate	$F(CF_2)_6C(O)O^-$	PFHpA
Perfluorooctanoate	$F(CF_2)_7C(O)O^-$	PFOA
Perfluorononanoate	$F(CF_2)_8C(O)O^-$	PFNA
Perfluorodecanoate	$F(CF_2)_9C(O)O^-$	PFDA
Perfluoroundecanoate	$F(CF_2)_{10}C(O)O^-$	PFUnA
Pefluorododecanoate	$F(CF_2)_{11}C(O)O^-$	PFDoA
Perfluorotridecanoate	$F(CF_2)_{12}C(O)O^-$	PFTriA
Perfluorotetradecanoate	$F(CF_2)_{13}C(O)O^-$	PFTA
Perfluoropentane sulfonate	$F(CF_2)_5SO_3^-$	PFPeS
Perfluorohexane sulfonate	$F(CF_2)_6SO_3^-$	PFHxS
Perfluoroheptane sulfonate	$F(CF_2)_7SO_3^-$	PFHpS
Perfluorooctane sulfonate	$F(CF_2)_8SO_3^-$	PFOS
Perfluorodecane sulfonate	$F(CF_2)_{10}SO_3^-$	PFDS
Fluorotelomer carboxylate		
8:2 Fluorotelomer acid	$F(CF_2)_8CH_2C(O)O^-$	8:2 FTCA
8:2 Fluorotelomer unsaturated acid	$F(CF_2)_7CF{=}CHC(O)O^-$	8:2 FTUCA
7:3 fluorotelomer acid	$F(CF_2)_7CH_2CH_2C(O)O^-$	7:3 FTCA
7:3 fluorotelomer unsaturated acid	$F(CF_2)_6CF{=}CHC(O)O^-$	7:3 FTUCA
Perfluoroalkylsulfonamides (PFSAm)		
N-methyl perfluorobutanesulfonamidoethanol	$F(CF_2)_4SO_2N(CH_3)(CH_2CH_2OH)$	N-MeFBSE
N-ethyl perfluorobutanesulfonamidoethanol	$F(CF_2)_4SO_2N(CH_2CH_3)(CH_2CH_2OH)$	N-EtFBSE
N-methyl perfluorooctanesulfonamidoethanol	$F(CF_2)_8SO_2N(CH_3)(CH_2CH_2OH)$	N-MeFOSE
N-ethyl perfluorooctanesulfonamidoethanol	$F(CF_2)_8SO_2N(CH_2CH_3)(CH_2CH_2OH)$	N-EtFOSE
Perfluorooctanesulfonamide	$F(CF_2)_8SO_2NH_2$	PFOSA
N-ethyl perfluorooctanesulfonamide	$F(CF_2)_8SO_2N(CH_2CH_3)H$	N-EtFOSA
N-methyl perfluorooctanesulfonamide	$F(CF_2)_8SO_2N(CH_3)H$	N-MeFOSA
Perfluorooctanesulfonamidoethanol	$F(CF_2)_8SO_2NH(CH_2CH_2OH)$	FOSE
Perfluorooctanesulfonamidoacetate	$F(CF_2)_8SO_2NH(CH_2C(O)OH)$	FOSAA
N-ethyl perfluorooctanesulfonamidoacetate	$F(CF_2)_8SO_2N(CH_2CH_3)(CH_2C(O)OH)$	N-EtFOSAA
N-methyl perfluorooctanesulfonamidoacetate	$F(CF_2)_8SO_2N(CH_3)(CH_2C(O)OH)$	N-MeFOSAA

Name	Formula	Acronym
Fluorotelomer Alcohols		
2-(Perfluorobutyl)ethanol	$F(CF_2)_4CH_2CH_2OH$	4:2 FTOH
2-(perfluorohexyl)ethanol	$F(CF_2)_6CH_2CH_2OH$	6:2 FTOH
2-(perfluorooctyl)ethanol	$F(CF_2)_8CH_2CH_2OH$	8:2 FTOH
2-(perfluorodecyl)ethanol	$F(CF_2)_{10}CH_2CH_2OH$	10:2 FTOH
2-(perfluorododecyl)ethanol	$F(CF_2)_{12}CH_2CH_2OH$	12:2 FTOH
Fluorotelomer Olefins		
(Perfluorobutyl)ethene	$F(CF_2)_4CH{=}CH_2$	4:2 FTO
(Perfluorohexyl)ethene	$F(CF_2)_6CH{=}CH_2$	6:2 FTO
(Perfluorooctyl)ethene	$F(CF_2)_8CH{=}CH_2$	8:2 FTO
(Perfluorodecyl)ethene	$F(CF_2)_{10}CH{=}CH_2$	10:2 FTO
(Perfluorododecyl)ethene	$F(CF_2)_{12}CH{=}CH_2$	12:2 FTO
Aldehydes		
Perfluorononanal	$F(CF_2)_8C(O)H$	PFAL
8:2 fluorotelomer aldehyde	$F(CF_2)_8CH_2C(O)H$	FTAL

ECF

In ECF, a hydrocarbon feedstock chemical is dissolved in liquid anhydrous HF within a copper electrochemical cell[1]. The anode and cathode plates are made of nickel. Fluorination occurs at the anode and the electrochemical reaction was balanced with hydrogen evolving at the cathode[1]. The feedstock is perfluorinated by electrolysis via a low voltage (5 – 6 eV)[2].

In industrial synthesis, hydrocarbon analogues of PFOS, PFSAm, and PFOA were not directly fluorinated by ECF due to poor reaction yields[2]. Instead, a hydrocarbon acyl halide (or sulphonyl halide) closest in form to the desired end product was employed in the ECF reaction. Thus, the starting material *n*-octanesulfonyl halide ($H(CH_2)_8SO_2X$, where X = Cl or F) was subjected to ECF to form perfluorooctanesulfonyl fluoride ($F(CF_2)_8SO_2F$)[1] and from it PFSAm and PFOS may be obtained (Fig. 1.1). Similarly, ECF of *n*-octanoyl halide ($H(CH_2)_7C(O)X$, X = Cl or F) was implemented to form the perfluorinated analog, $F(CF_2)_7C(O)F$, which was further reacted to acquire PFOA. 3M used perfluorooctanesulfonyl fluoride to make PFOS and the following PFSAm molecules, N-methyl perfluorooctanesulfonamidoethanol (N- MeFOSE) and N-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE). N-EtFOSE and N-MeFOSE were obtained by combining perfluorooctanesulfonyl fluoride with ethyl amine or methyl amine to form the intermediates, N-ethyl perfluorooctanesulfonamide and N-methyl perfluorooctanesulfonamide (N-EtFOSA and N-MeFOSA), which reacted with ethylene glycol carbonate to make the corresponding perfluorooctanesulfonamidoethanol (Fig. 1.1).

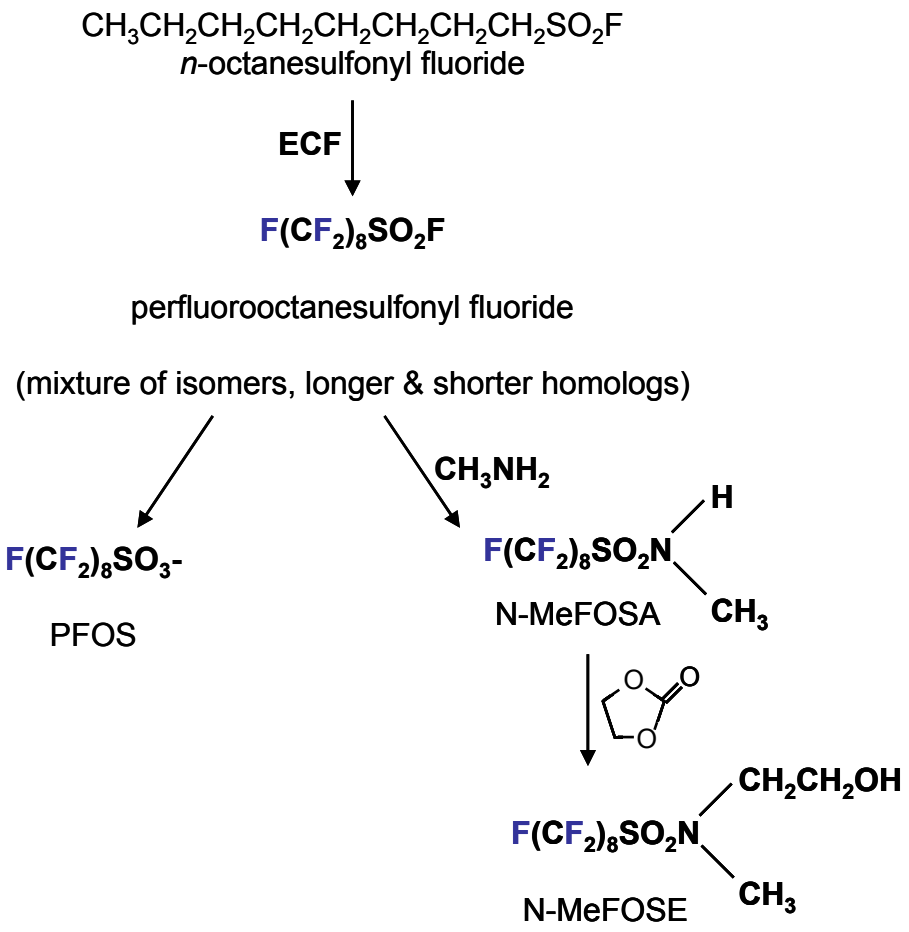

Figure 1.1. Industrial ECF Syntheses of Perfluoroalkyl Compounds by 3M (as described in [3]). Reaction of perfluorooctanesulfonyl fluoride with ethylamine produced N-EtFOSA. N-EtFOSA reacted with ethylene carbonate to form N-EtFOSE. PFOS is generated by base-catalyzed hydrolysis of perfluorooctanesulfonyl fluoride.

The mechanism of perfluorination by ECF is not well understood[4] but evidence suggests that the fluoride ion from HF undergoes oxidation at the anode forming fluorine atoms that replace hydrogens in the hydrocarbon substrate by homolytic substitution. Substitution occurs at the nickel anode and formation of a nickel fluoride film on the anode surface appears to play a crucial role as a surface upon which the organic substrate associates.

There are two theories on the nickel fluoride-organic substrate association and its involvement in the ECF mechanism. One hypothesis is that the organic substrate is oxidized to form a carbocation and C-F bonds result by inserting a fluoride ion from the anode surface layer into the hydrocarbon. This sequence repeats until complete perfluorination which is followed by

departure from the anode surface and movement into the cell bath. The overall reaction is referred to as the ECbECn mechanism in which the oxidized organic forms a radical cation (E), which loses a proton, producing the corresponding radical (Cb). The radical is oxidized to a carbocation (E) and reacts with fluoride ion in the anode surface layer. Sartori et al. refute the ECbECn mechanism and instead suggest an initial step involving formation of high valent nickel fluoride (oxidation state > +2 such as NiF_3 and NiF_4) on the surface of the anode, followed by adsorption of the organic substrate (Fig. 1.2.)[4]. They propose a free radical mechanism ensues in which the nickel fluoride layer is the actual fluorinating agent[4].

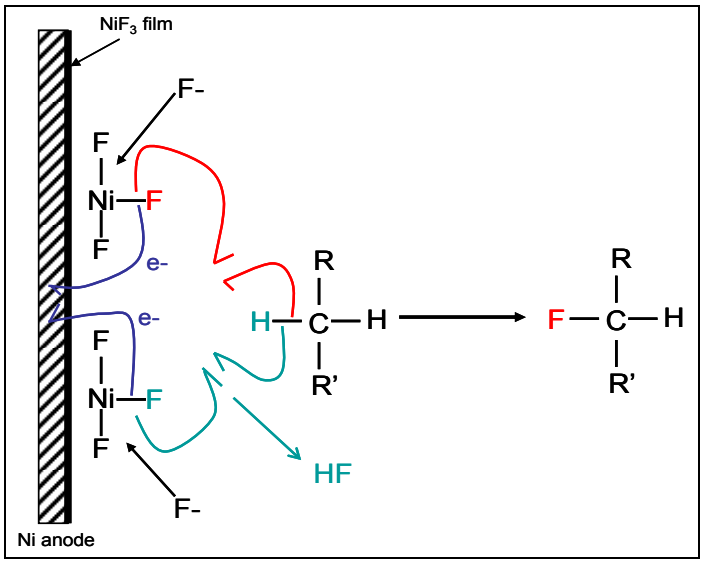

Figure 1.2. Scheme representing ECF of generic hydrocarbon via adsorption onto NiF_3 layer on anode. Adapted from Sartori et al. [4]and Ignat'ev et al.[5]

Industrial ECF-derived products are mixtures consisting of isomers, where the perfluoroalkyl tail is of linear and branched geometry, and shorter and longer chain homologues[3]. The cause of isomerization in ECF has not been extensively studied but may be due to internal migration in carbocation or bi-radical intermediates[4,5]. ECF perfluorination of *n*-octanesulfonyl halide results in a mixture of linear (*n*-isomer) and branched perfluorooctanesulfonyl fluoride isomers. In industrial synthesis, the mixture was not purified to isolate the *n*-isomer and thus further reaction generates an analogous mixture of PFOS isomers.

A recent presentation by 3M revealed isomer analysis of ECF PFOA and PFOS[6]. Numerous batches of each compound were assessed with ^{19}F-NMR (nuclear magnetic resonance spectroscopy) and the results indicated a high degree of batch-to-batch consistency in isomer

pattern, in ECF PFOA (78% *n*-isomer ±1.5% RSD, N = 18 different batches) and ECF PFOS (70% n-isomer ± 1.6% RSD, N = 8 different batches)[6]. The major classes of isomers in ECF PFOA, PFOS, and N-EtFOSE are identified in Table 1.2.

Using a commercially available standard of perfluorooctanesulfonyl fluoride, likely of ECF origin, Arsenault et al. synthesized PFOS[7]. Preparative liquid chromatography (LC) and re-crystallization were used to separate and purify individual isomers, each characterized via ^{19}F-NMR, permitting structural identification and refined composition assignment of individual isomers (Table 1.2.)[7]. Similarity in composition between N-EtFOSE and PFOS was expected because both were derived from ECF perfluorooctanesulfonyl fluoride. The contribution of individual isomers in ECF PFOA and ECF N-EtFOSE has not been fully identified. For example, in ECF PFOA, the contribution of each possible internally substituted CF_3 isomer has not been investigated. Instead all possible isomers belonging to this category were reported as a collective 12% by 3M[8]. The general isomer pattern identified in PFOS is expected to be maintained in ECF PFOA but PFOA, having one less perfluorocarbon than PFOS, is presumably less isomer-laden (i.e. lower percentage of branched isomers).

In Table 1.2., the isomer labeling system for ECF isomers follows the trend where *m* refers to methyl branch and the number preceding indicates the carbon position in the perfluoroalkyl tail on which the branch resides. Dimethyl substituted branched isomers are labeled with m_2 and two numbers referring to the carbons where the CF_3 branch resides, separated by a comma. This convenient labeling system was devised by Benskin et al.[9] based on that introduced by Langlois et al.[10].

Yields acquired with ECF improve with shorter chain length[1]. Subjecting ethylsulfonyl chloride, octylsulfonyl chloride, and decylsulfonyl chloride to ECF produces perfluorinated analogs in yields of 79%, 25% and 12%, respectively[1,11]. Similarly, ECF of acetyl chloride and octanoyl chloride have contrasting yields of 80 and 10% for perfluoroacetyl fluoride and perfluorooctanoyl fluoride[1]. By-products in ECF PFAs are predominantly shorter chain impurities and minor constituents are longer chain PFAs (Table 1.3)[6]. Longer chain PFCAs (PFNA and longer) only constitute 1% by weight of PFA homologues[6].

Table 1.2. Isomer Composition in ECF PFOA and PFOS as determined by 3M[8] and Arsenault et al.[7].

ECF PFOS Arsenault et al.[7]	**3M ECF PFOS [8] Lot 217**	**3M ECF N-EtFOSE Lot 30107[12]**	**3M ECF PFOA[8] Lot 332**
n-isomer 62.3%	*n*-isomer 70.5%	*n*-isomer 69.9%	*n*-isomer 77.9%
$CF_3CF_2CF_2CF_2CF_2CF(CF_3)CF_2SO_3$-, 2*m*-PFOS 2.2% $CF_3CF_2CF_2CF_2CF(CF_3)CF_2CF_2SO_3$-, 3*m*-PFOS 6.5% $CF_3CF_2CF_2CF(CF_3)CF_2CF_2CF_2SO_3$-, 4*m*-PFOS 5.3% $CF_3CF_2CF(CF_3)CF_2CF_2CF_2CF_2SO_3$-, 5*m*-PFOS 8.0%	Internal methyl branch, collectively 17.1%	Internal methyl branch, collectively 17.4%	Internal methyl branch, collectively 12.7%
Isopropyl 11.4%, (*iso*-PFOS), $(CF_3)_2CFCF_2CF_2CF_2CF_2CF_2SO_3$-	Isopropyl 0.4%	Isopropyl 10.7%	Isopropyl 9.0%
Alpha branch 2.4%, (1*m*-PFOS), $CF_3CF_2CF_2CF_2CF_2CF_2CF(CF_3)SO_3$-	Alpha branch 1.6%	Alpha branch 1.6%	Alpha branch 0.1%
t-butyl branch 0.4% $(CF_3)_3CCF_2CF_2CF_2CF_2SO_3$-	*t*-butyl 0.23%	*t*-butyl 0.23%	*t*-butyl 0.2%
$CF_3CF_2C(CF_3)(CF_3)CF_2CF_2CF_2SO_3$-, 4,4-$m_2$-PFOS 0.13% $(CF_3)_2CFCF(CF_3)CF_2CF_2CF_2SO_3$-, 4,5-$m_2$-PFOS 0.56% $(CF_3)_2CFCF_2CF(CF_3)CF_2CF_2SO_3$-, 3,5-$m_2$-PFOS 0.12%	Internal *gem*-dimethyl 0.15%	Internal *gem* dimethyl 0.13%	Internal *gem*-dimethyl 0.13%

Table 1.3. Perfluorinated carboxylate and sulfonate impurities in ECF PFOS (left) and ECF PFOA (right).

ECF PFOS	% impurity (wt)	% branched	ECF PFOA	% impurity (wt)	% branched
PFBS[13]	1.2	N/A*	PFHxA[14]	0.73	N/A*
PFPeS[13]	1.3	N/A*	PFHpA[14]	3.7	N/A*
PFHxS[13]	4.7	N/A*	PFNA[6]	0.2	65
PFHpS[13]	1.1	N/A*	PFDA[6]	0.0005	54
PFOA[6]	0.79	19	PFUnA[6]	0.0008	28
PFNA[6]	0.002	70	PFDoA[6]	0.0008	32
PFDA[6]	0.0005	51			
PFUnA[6]	0.0002	46			
PFDoA[6]	0.0004	33			

* N/A = data not available

Historical production volumes of PFOA by ECF are not transparent. In a letter by Dr. Larry Wendling, Vice President of Performance Materials Division of 3M, in 2003 addressed to USEPA, ECF PFOA production occurred between 1969 and 2002[15] but elsewhere it is suggested that ECF PFOA production began in 1947[16]. Early production volumes of ECF PFOA are not obvious; however, between 1992 and 2002, 3M's total PFOA production was 113 tonnes per year[15] with 1999 ECF PFOA reportedly amounting to 260 tonnes[16]. The majority (97%) of this was used by industrial customers of 3M for aid in fluoropolymer processing [15]. The remaining 3% of ECF PFOA was used in medical film coatings and to a lesser degree, printed circuit boards and electronic precision bearings[15]. In these latter electronic applications, PFOA was derivatized to an ester to make methacrylate co-polymers, which has been suggested as having minimal environmental exposure[15]. As of 2002, 3M ceased manufacture of perfluorooctane-based products for commercial sale. A subsidiary of 3M, Dyneon, continues small-scale production of ECF PFOA in Gendorf, Germany for its own use in fluoropolymer production[15]. Since 2002, major industrial manufacture of PFOA is by telomerization (discussed further below).

3M has asserted that it was the dominant producer of PFOS, N-EtFOSE and N-MeFOSE, all of which are based on ECF perfluorooctanesulfonyl fluoride as well as PFOA from 1947 to 2002[16,17]. Total production of perfluorooctanesulfonyl fluoride by 3M in 2000 was 3500 tonnes and 175 tonnes a year later[18] at plants located in Decatur (AL, USA) and Antwerp (Belgium). There were two other plants in the US with only limited production, Cordova (IL) and Cottage Grove (MN)[3]. Apart from 3M, additional ECF production of perfluorooctylsulfonyl fluoride has been confirmed by Miteni (Italy) and Dianippon Ink & Chemicals (Japan)[17] but may extend to other locations. In a survey conducted by OECD (Organisation for Economic Co-operation and Development) in 2005 amongst OECD member countries, perfluorooctanesulfonyl fluoride production was revealed in Germany, Italy and Japan[19]. Production of PFOA by 3M was conducted at plants in Antwerp, Cottage Grove and since 1999, Decateur[20]. A breakdown of PFOS, N-MeFOSE, and N-EtFOSE historical production is not available but 3M has stated that PFOS was not a major commercialized product and that its perfluorooctylsulfonyl fluoride synthesis was focused on N-EtFOSE and N-MeFOSE based product lines[3]. These statements are partially supported by an assessment of perfluoroalkyl containing compounds imported into Canada by Environment Canada. In that study it was revealed that perfluorooctanesulfonyl fluoride-based chemicals (PFOS, N-MeFOSE, N-EtFOSE) represented 43% of perfluoroalkyl-related imports. Of this, <2% was comprised by PFOS[21].

Use of Industrial ECF Products

The major use of PFOS was as a surfactant in fire fighting foams and minor uses include alkaline cleaners (as a surfactant ingredient), emulsifier in floor polish, mist suppressant, and in ant bait traps[17]. In contrast to PFOS, both N-EtFOSE and N-MeFOSE were building block intermediates in industrial synthesis of fluorochemical based sales products. N-EtFOSE was incorporated into mono-, di-, and tri-phosphate esters primarily for use in food packaging such as plates, bags, wraps, cartons and containers[17]. N-MeFOSE was used in polymers for treatment of carpet, upholstery in home furnishings, and fabric[17]. Polymerization reactants for N-MeFOSE included urethane, acrylate, and adipate[3]. By weight, the N-MeFOSE production was slightly greater than that of N-EtFOSE[3].

In many of these ECF product lines, although raw materials were subjected to esterification or polymerization, residual quantities of non-polymerized intermediates are present. 3M reported residual quantities of N-MeFOSE, N-EtFOSE, N-MeFOSA, N-EtFOSA, N-methyl perfluorooctanesulfonamidoacetate ($F(CF_2)_8SO_2N(CH_3)CH_2C(O)O^-$, N-MeFOSAA), N-ethyl perfluorooctanesulfonamidoacetate ($F(CF_2)_8SO_2N(CH_2CH_3)CH_2(O)O^-$, N-EtFOSAA), perfluorooctanesulfonamidoacetate ($F(CF_2)_8SO_2NHCH_2(O)O^-$, PFOSAA), and perfluorooctanesulfonamide ($F(CF_2)_8SO_2NH_2$, PFOSA) collectively comprising <1-2% concentration in final products[3,22]. Dinglasan-Panlilio & Mabury reported N-MeFOSE residual to represent 0.39% (dry weight) of a commercially available rug and carpet protector formulation produced by 3M in which the active ingredient was intended to be a perfluorooctyl-based copolymer[23].

Telomerization

Telomerization was originally developed by Du Pont for polymerization of ethylene[1] and is used to synthesize a number of fluorochemicals. In telomerization, a perfluoroalkyl iodide (telogen) reacts in a radical process with an unsaturated perfluoroalkene (taxogen/monomer). The taxogen and telogen most used in industrial telomerization correspond to tetrafluoroethene ($CF_2=CF_2$) and *n*-perfluoroethyl iodide (CF_3CF_2I)[1]. The initial telogen is from reaction of the taxogen with catalysts, such as IF_5 and I_2[1]. The telogen undergoes photochemical converstion to a perfluoroalkyl radical ($CF_3CF_2\cdot$) and the reaction proceeds with repetitive addition of the radical to $CF_2=CF_2$, thereby lengthening the perfluoroalkyl radical in units of CF_2CF_2. Reaction with I_2 or perfluoromethyl iodide transfers I· to the perfluoroalkyl radical, forming a perfluoroalkyl iodide. Alternatively, the perfluoroalkyl radical is combined with ethene to yield a perfluoroalkylethyl iodide ($F(CF_2)_xCH_2CH_2I$, *x*:2 fluorotelomer iodide). Synthesis of PFOA and PFNA is by oxidation and carboxylation, respectively, of perfluorooctyl iodide[16]. Reaction of fluorotelomer iodides produces FTOHs and FTOs. FTOHs are obtained by hydrolysis with a number of possible reagent combinations such as oleum, water, and $NaHSO_3$ or nonoxidizing acids[1]. FTOs are acquired from bimolecular elimination (E2) of the fluorotelomer iodide with a strong base such as KOH[24]. A schematic summarizing these reactions is shown in Figure 1.3.

Since telomerization is a radical polymerization, upon termination of perfluoroalkyl chain propagation, multiple chain lengths are formed in the reaction mixture. As the reaction is based on addition of CF_2CF_2 units to the taxogen, telomer chemicals are typically a mixture of even numbered fluorocarbons. The resulting mixture of even-numbered perfluoroalkyl chain lengths may not be subjected to clean-up processes for removal of longer and shorter chain impurities. This is evident in a study by Dinglasan-Panlilio & Mabury in which residual 6:2, 8:2, 10:2, and 12:2 FTOHs were measured in a variety of commercially available telomer based surfactants[23] as well as fluorotelomer monomers involved in polymer synthesis[25]. Collectively the residual FTOHs ranged from 0.04 to 3.80% per dry weight of these surfactants[23].

Use of Telomer Fluorochemicals

Fluorotelomer-based production emerged in the early 1970s[16]. Global production of perfluoroalkyl iodide by telomerization was estimated to be 5000 – 6000 t/y between 2000 and 2002[16]. Approximately 80% of fluoropolymer products center around the acrylate monomer[16]. Historical ECF PFOA and current telomer PFOA production was mainly for use as a polymerization aid for the manufacture of fluoropolymers and fluoroelastomers[26]. Specifically, PFOA solubilizes fluoromonomers to enhance their aqueous polymerization. Users of PFOA for this purpose included Asahi Glass Fluoropolymers USA, Inc., Daikin America Inc., Dyneon, LLC, and E.I. du Pont de Nemours and Company[26]. In 2002, 33 sites of industrial fluoropolymer synthesis were identified, 8 in North America, 7 in Japan, 7 in China, 7 in Europe, 2 in Russia, and 1 in India, with production amounting to 144 000 tonnes[16]. PFNA manufacture is primarily in Japan and like PFOA, is used as a polymerization aid in fluoropolymer synthesis, specifically for polyvinylidene difluoride (PVDF). There are reportedly two locations using PFNA for PVDF synthesis in the US[27]. PFOA production greatly exceeds that of PFNA[16]. Estimated total production of PFOA from 1951 to 2004 is 3600 – 5700 tonnes and for PFNA, 1975-2004 accounts for 800 to 2300 tonnes[16]. These time interval and production volumes corresponding to PFNA are very rough estimates. The accuracy of these numbers have yet to be ascertained considering they were based on the year of the first patent listing and suspected PVDF production volumes[16]. These PFCAs are residuals in some fluoropolymers, and are estimated to be <2000 to 7000 μg/g PFOA or 100 to 200 μg/g PFNA[16]. A 2005 OECD survey reported current PFOA production in Germany, Japan,

Sweden, and the US[19]. However, apart from Miteni located in Italy, it is unknown whether any of PFOA production post 2002 is via ECF[19].

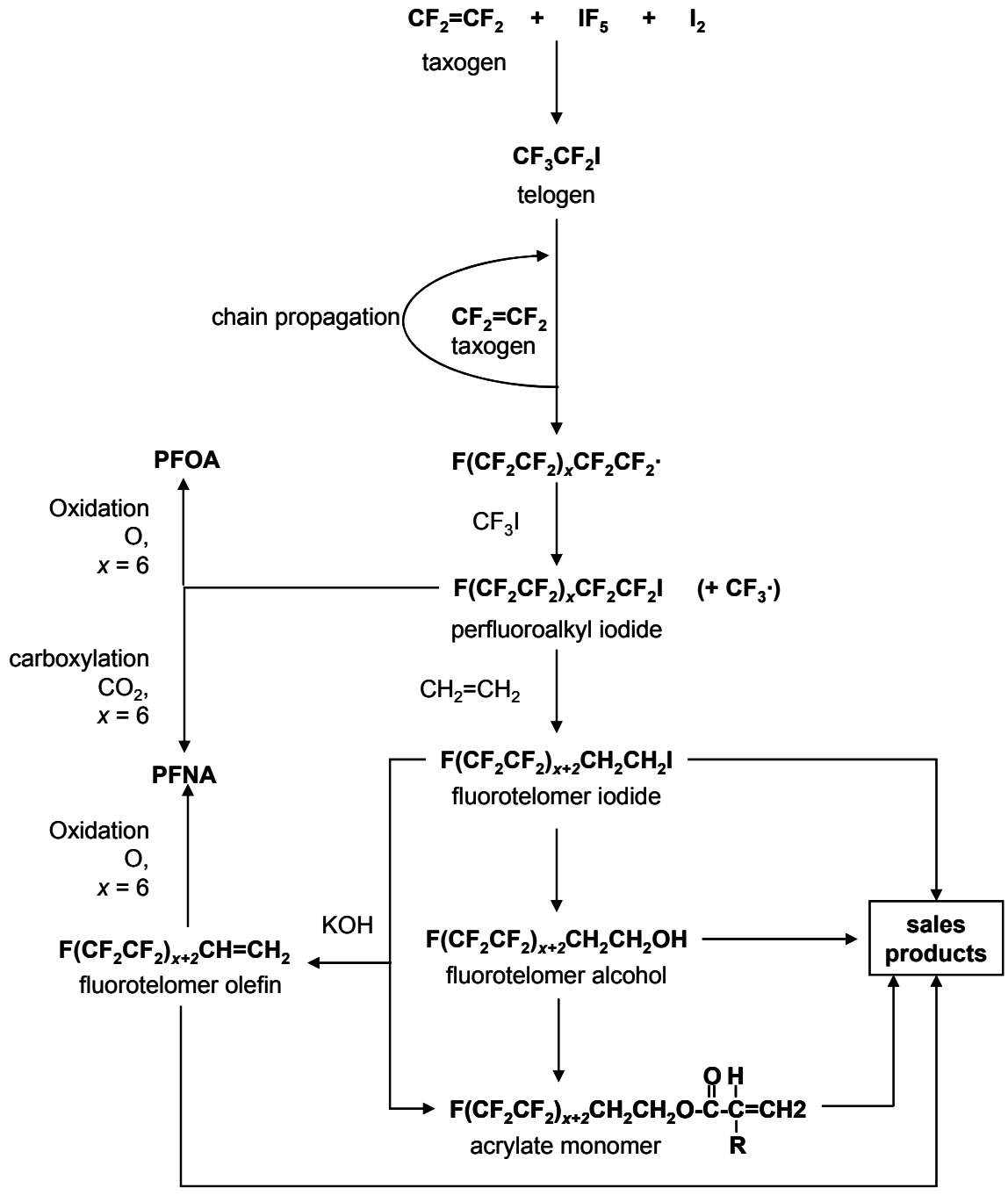

Figure 1.3. Industrial production by telomerization [1,16,24].

Due to the linear arrangement of perfluorocarbons in the taxogen and telogen, industrial fluorochemicals created via telomerization with perfluoroethyl iodide and tetrafluoroethene are

believed to also be linear. Telomerization reaction may be used to intentionally produce odd numbered perfluorocarbons as well as terminal isopropyl perfluoroalkyl geometry. For example, Haszeldine reported synthesis of odd-chain length perfluoroalkyl iodide upon reaction of perfluoromethyl iodide with tetrafluoroethene[28]. Balague et al. describes telomerization of an isopropyl telogen $(CF_3)_2CFI$ for production of an isopropyl perfluoroalkyl iodide[29]. Similar processes are described in the patent literature, in particular the production of the corresponding isopropyl perfluorocarboxylate from isopropyl perfluoroalkyl iodide[30]. Despite of reporting in patent and scientific literature of odd-perfluorocarbon or isopropyl perfluorocarbon geometry use in telomerization, their industrial significance is not currently known.

1.1.2 Characterization of Perfluoroalkyl-related Compounds in the Environment

The first suggestion of organic fluorine in non-occupationally exposed humans was by Taves et al. who determined a fluorinated organic compound bound to serum albumin in the late 1960s[31]. Using NMR the compound was assigned to PFOA but the author acknowledged the possibility that its properties could be consistent with PFOS[31]. Early analysis of PFAs was conducted by nonspecific methods such as the oxyhydrogen torch method. Lack of available standards was a further hindrance. As such, PFA monitoring was limited to analysis of serum from fluorochemical plant employees or spill-regions where detection limits were not critical[32,33]. With the advent of liquid chromatography coupled to mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS), compound-specific detection in a variety of matrices became possible[34,35].

The presence of PFOA and PFOS was subsequently confirmed in human blood sera [34] and as reported in a series of comprehensive studies by Giesy and Kannan, wildlife around the world including Arctic bears and seals, temperate North American organisms such as otters, turtles, fish-eating birds, and fish, and mammals from Europe and Asia[35-40]. These findings indicated that perfluorooctyl acid (PFOS and PFOA) pollution was extensive, reaching into areas remote from use and production. In 2002, the first detection of longer chain PFCAs from PFOA to perfluorotetradecanoate (PFTA) was reported, occurring in fish upstream from an aqueous film forming foam (AFFF) spill in Ontario[41]. This was surprising because beyond PFOA, PFCAs were not known to have direct industrial production. This research highlighted the need

to expand environmental monitoring of fluorochemicals from PFOA and PFOS to longer and shorter chain lengths as well as investigate the origin of exposure.

Consequently, numerous findings of longer chain PFCAs in a variety of biological samples[42], including human blood[43,44] and in ocean waters[45] emerged. However, observation of long-chain PFCAs and PFOS in Arctic biota[46,47] lead to a new hypothesis that PFAs could be the result of long range atmospheric transport and subsequent transformation of volatile fluorinated precursor compounds[48]. Candidates for these precursors were PFSAm and FTOHs, which had been previously detected in North American air samples[48,49]. This hypothesis is discussed in Section 1.1.3.

Since the first discovery of longer chain PFCAs by Moody et al.[41], thorough characterization of environmental PFCA and PFOS contamination has been conducted. A recent review by Houde et al. comprehensively describes the presence of PFAs in biota[50]. In addition, PFOS and PFCA contamination has been observed in precipitation[51-53], Arctic snow and ice[54], Arctic sediment and lake water[55], soil and sediment in temperate regions[56-58], marine water[45], and fresh water lakes and rivers[59].

Biota

Investigation of PFOS and PFCAs in biological organisms has focused heavily on marine and freshwater food chains. Of the PFAs, in Arctic biota PFOS occurs in the highest concentrations and the PFCA concentration profile is a characteristic even-odd pattern whereby the odd-numbered PFCA exceeds the preceding even-numbered PFCA ([PFNA]>[PFOA], [PFUnA]>[PFDA], etc.) as observed in polar bears, ringed seals, mink, loon, various fish, and birds[46,47,60-64]. Polar bears (*Ursus maritimus*) have the highest reported PFA concentrations compared to any abiotic or biotic sample, ranging from liver concentrations of 263 ng/g to 6340 ng/g in Greenland[47]. Individual PFCA concentrations are typically on the order of 10^1 to 10^2 ng/g in polar bear livers. For example, Smithwick et al., observed the following PFCA pattern in polar bears from Svalbard in eastern Greenland: 12 ng/g PFOA, 76 ng/g PFNA, 23 ng/g PFDA, 83 ng/g PFUnA, 10 ng/g PFDoA, and 17 ng/g PFTrA [47]. Polar bears are an apex predator in which PFCA concentrations are likely elevated due to biomagnification. Organisms at lower trophic levels depict a similar pattern in PFA contamination but at lower magnitudes. In brook

trout (*Salvenlinus fontinalis*) from a Canadian sub-Arctic region in northern Quebec, concentrations were 5.9 ng/g PFNA, 2.3 ng/g PFDA, 4.9 ng/g PFDUnA, 0.83 ng/g PFDoA, 1.1 ng/g PFTriA, 0.2 ng/g PFTeA and 23 ng/g for PFOS[46]. Biological samples from Antarctica are less contaminated as demonstrated by blood PFOS concentrations of elephant seals, <LOQ-3.5 ng/ml and polar skuas, <LOQ – 1.4 ng/ml[35,65].

Consistent with observations in Arctic biota, PFOS exceeds individual PFCAs in biota from temperate regions [57,66-69]reviewed in[50]. However, unlike in the Arctic, PFCA contamination profile in biota appears to vary depending on sample, species, and location. For example, fish from the Great Lakes region and beaver, fish, and birds from Poland had PFOS concentrations that were at least an order of magnitude greater than any PFCA but within the group of PFCAs, each was of the same order of magnitude[66,67,69]. In the blood plasma of dolphins (*Tursiops truncatus*) from the Florida coast, PFOS was 200-2000 ng/g and each PFCA was 1-100 ng/g[68]. One exception to the [PFOS]>[PFCA] trend was observed in sea otters (*Enhydra lutris nereis*) from the California coast where PFOA and PFOS levels were nondistinct (mean ± standard deviation 48.9 ± 31.4 ng/g PFOA and 30.9 ± 27.2 ng/g PFOS) and very little PFNA was observed (1.8 ± 1.9 ng/g)[70].

Human blood analysis reveals a consistent pattern of PFOS>PFOA>PFNA>PFDA regardless of the subject's geography[9,43,44,71-77]. PFDA and longer chain PFCAs are not as frequently observed above detection limits compared to PFOS, PFOA, and PFNA (Table 1.4.). Human blood from donors in St. Paul, MN revealed a decline in PFOS and PFOA concentrations between 2000 and 2005[78]. The decrease was attributed to the 2002 phase-out of PFOS and PFOA production by 3M including at their plant in St. Paul, MN[78]. Calafat et al. also noted a decrease in PFOS and PFOA human blood concentrations in samples collected across the US as part of the National Health and Nutrition Examination Survey (NHANES) in 2003-2004 compared to 1999-2000[79]. In that study, decreases in PFOS and PFOA corresponded to 10 and 1 ng/g, respectively but PFNA increased by 0.4 ng/ml[79].

Table 1.4. PFA profile in human blood as demonstrated by range and (mean, where available).

	Concentration in human blood sera and plasma (ng/ml)					
Location and Reference	**PFOS**	**PFOA**	**PFNA**	**PFDA**	**PFUnA**	**PFDoA**
Australia[75]	13-30	5-10	0.4-2.0	N/A	N/A	N/A
Sweden[80]	13-23	1.9-3.3	0.2-0.5	<0.1 – 0.4	N/A	N/A
Urban Sri Lanka[76]	1.5-18.2 (7.8)	0.6 – 22.8 (9.5)	0.1-1.3 (0.4)	0.01-0.7 (0.2)	0.01-1.0 (0.2)	0.01-0.9 (0.02)
Rural Sri Lanka[76]	1.9-17.5 (6.3)	1.9-23.5 (9.1)	0.05-0.2 (0.08)	0.02-0.1 (0.05)	0.02-0.06 (0.04)	0.004-0.02 (0.008)
Baltic coast of Poland[77]	5.2-84	1.2-8.7	0.3 – 3.8	0.06 – 1.4	0.03 – 1.1	4-380 pg/ml
Beijing, China[81]	12-64	0.2-2.4	0.3-0.6	0.1-0.3	0.1-0.5	<LOQ
Atlanta, GA, USA[44]	4-164 (56)	0.2-10 (5)	1-4 (2.5)	<LOD-1.4 (0.5)	<LOD-1.0 (0.7)	only in 2 samples at 1-2 ng/ml
Midwest, US (Chapter 4)[82]	N/A	0.9-8.6 (4.4)	0.17-1.2 (0.77)	0.05-0.25 (0.17)	<LOD-0.067	<LOD
U.S. 2003-2004[79]	19.2-22.3 (20.7)	3.6-4.3 (3.9)	0.8-1.1 (1.0)	N/A	N/A	N/A
U.S. 1999-2000[79]	27.1-33.9 (30.4)	4.7-5.7 (5.2)	0.5-0.7 (0.5)	N/A	N/A	N/A

*N/A = not reported in manuscript

Analysis of PFA isomers has been conducted in human blood samples from the mid-western US[82], Australia and Sweden[83], and Edmonton, AB[9]. PFOS isomer profiles in Australian and Swedish blood were acquired using LC-MS and LC-MS/MS and revealed a pattern enriched in branched isomers relative to the *n*-isomer compared to technical PFOS. The parent daughter ion transition monitored was 499 → 80 m/z [83] corresponding to $F(CF_2)_8SO_3^-$ and SO_3-, however this transition has been reported to over-estimate PFOS in some cases due to a co-eluting interference[9]. Hence, 499→99 is recommended to be a more robust transition for PFOS monitoring. Using a more sophisticated analysis consisting of 7 separate parent-to-

daughter ion transitions corresponding to different isomers in ECF PFOS, the PFOS isomer pattern in blood from Edmonton, AB closely matched that of the technical mixture[9]. PFCA isomers were also measured and corroborated previous results by De Silva and Mabury[82] in which the PFOA isomer profile was significantly enriched in the *n*-isomer (>95%) compared to ECF PFOA. This study is presented in greater detail in Chapter 4.

Precipitation

PFA monitoring in precipitation (rainwater and snow) is limited to four studies. A single rainwater sample in Winnipeg (Manitoba, Canada) was found to contain 0.59 ng/L of PFOS[53]. Saturated and unsaturated fluorotelomer carboxylic acids ($F(CF_2)_xCH_2COOH$, *x*:2 FTCA and $F(CF_2)_xCF=CHCOOH$, *x*:2 FTUCA) were also monitored. Concentrations of 8:2 FTCA, 8:2 FTUCA, 10:2 FTCA, and 10:2 FTUCA corresponded to 1.0, 0.12, 0.3, and 0.12 ng/L, respectively, but all PFCAs were below the LODs (1-7 ng/L)[53]. PFCAs in rainwater collected from rural and urban locations of North America were reported by Scott et al.[84]. At all sites, the shorter chain length PFCAs, perfluoropropionate ($F(CF_2)_2COO$, PFPrA), perfluorobutanoate ($F(CF_2)_3COO$, PFBA), perfluoropentanoate ($F(CF_2)_4COO$, PFPA), PFHxA, and PFHpA, were more abundant (0.1-120 ng/L) than PFOA (0.1 – 37 ng/L)[51]. Longer chain length PFCAs (PFNA to PFUnA) were frequently detected and ranged from 0.01 to 9.7 ng/L[51]. Regional differences in PFCA concentration in precipitation were not obvious but PFDoA was observed in 25% of the rainwater samples collected from non-urban Egbert, ON compared to 86% of the samples from urban Toronto[51]. However, concentrations between both locations for this analyte were not significantly different (0.1 – 3.3 ng/L versus 0.1 – 5.2 ng/L in Egbert and Toronto, respectively)[51]. These two areas are only separated by 100 km and as such were not expected to have significant differences in PFCA rainwater profiles. FTCAs and FTUCAs were less frequently observed and in low concentrations 0.1-1.1 ng/L[51]. This data was corroborated by a recent investigation of snow and rainwater from Albany (New York, USA)[52]. Of the PFCAs measured (PFHpA to PFDoA), PFOA was the most dominant with mean concentrations of 2.2 ng/L and 2.7 ng/L in rain and snow, followed by PFNA at 1.0 and 0.6 ng/L, respectively[52]. Longer chain PFCAs were at lower concentrations ranging from <LOD to 0.3 ng/L[52]. In biota, PFOS is typically greatest in concentration but in precipitation of Albany, it was less abundant than PFOA ranging from <0.3 to 1.5 ng/L in rain and in snow <0.3 to 1.9 ng/L[52].

PFOS and PFCAs were measured in ice collected from High Arctic ice caps[54]. In depth samples corresponding to 1996 to 2005, PFAs were present in pg/L concentrations, much less than the ng/L levels in precipitation reported by Scott et al.[51]. Ice cap contamination ranged from 3 – 86 pg/L for PFOA, 12-147 pg/L for PFOA, 5-246 pg/L for PFNA, <LOQ – 22 pg/L for PFDA, and <LOQ – 27 pg/L PFUnA[54]. This pattern was consistent with an atmospheric source based on the similarity in concentrations of PFOA with PFNA and PFDA with PFUnA. In addition, these PFAs were not correlated with sodium ion concentrations which would be anticipated in the case of oceanic transport[54].

Sediment

Sediment from remote Arctic Lakes and from temperate regions such as the Great Lakes, US coastal areas, and Japan have been assessed for PFA content. Higgins et al. observed PFCAs ranging from PFOA to PFTA in surface sediment from the US (California coast, Baltimore (MD) and Corvallis (OR))[56]. PFOA and PFDA were most prevalent with dry weight concentrations of <0.1 to 0.6 ng/g PFOA and 0.1 to 0.75 ng/g PFDA[56]. Both PFNA and PFUnA were seldom observed above LOD but PFDoA was present in 30% of the samples at concentrations (0.2 to 0.6 ng/g) comparable to PFOA and PFDA[56]. Approximately 65% of these samples contained PFOS with concentrations slightly higher than PFCAs and ranging from 0.2 to 4.0 ng/g[56]. This is dissimilar to sediment in Lake Ontario, where PFOS exceeded PFCAs in surface sediment[85]. Dry weight concentrations of PFOS ranged from 5 to 26 ng/g, PFOA was generally <1.4 to 3.5 ng/g, and longer chain PFCAs from PFNA to PFDoA did not exceed 1.7 ng/g[85]. Sediment samples from rivers of Kyoto, Japan as reported by Senthilkumar et al. (1.3 to 3.9 ng/g PFOA and <1.4 to 11 ng/g PFOS, dry weight)[57] were similar to Lake Ontario sediment concentrations. The only longer chain PFCA monitored by the authors was PFDoA which ranged from 0.7 to 2.4 ng/g[57]. In contrast, sediments on the southwestern coast of Japan from the Ariake Sea contained more PFOA (0.84 to 1.1 ng/g dry weight) than PFOS (0.09 to 0.14 ng/g dry weight)[58]. In the remote location of Cornwallis Island (NV, Canada) sediment from three separate lakes, Amituk, Char and Resolute Lake were analyzed[55]. The pattern of PFAs was variable between lakes and indicated local contamination to Resolute Lake which was comprised mostly PFOS (85 ng/g), PFOA (8 ng/g) and PFNA (3 ng/g)[55]. The most prevalent PFA in Char and Amituk Lake sediment was PFOA at 1-2 ng/g[55]. In Char Lake sediment,PFAs corresponded to 1.1 ng/g PFOS, <0.5 ng/g PFNA, and 0.6 ng/g PFDA and in

Amituk Lake, 0.06 ng/g PFOS, 0.4 ng/g PFNA, and 0.12 ng/g PFDA[55]. It is interesting to note that PFCA concentrations in Arctic sediment are on the same order of magnitude of PFCAs in sediment from mid-latitudes, suggesting global dissemination of PFCAs with potentially a common source.

Water (Marine and Freshwater)

Analysis of PFAs in groundwater and surface waters have been used to track point source contamination including an accidental fire fighting foam spill near an airport[41], use of fire fighting foam at a military base[86], and in the Tennessee River close to an ECF fluorochemical manufacturing plant[87]. High concentrations were noted in the Tennessee River with PFOS and PFOA ranging from 20-150 ng/L and 140 – 600 ng/L, respectively[87]. These concentrations appeared to be correlated to discharge of a fluorochemical plant. Waters remote from point sources, in urban and industrialized areas reveal lower concentrations with the PFA profile consisting predominantly of PFOA. Sinclair et al. observed median concentrations of 2-6 ng/L PFOS and 15-35 ng/L PFOA in surface water samples acquired from lakes and rivers around NY state[88]. In surface water samples collected near the Canadian-US border in MN state, PFHpA, PFOA, and PFOS were each in the range of 1 – 10 ng/L[89]. PFNA was frequently <LOD and did not exceed 1 ng/L [89]. Freshwater samples in Sri Lanka contained PFOA (1.1- 12 ng/L), PFOS (0.7-5 ng/L) and shorter-chain PFAs, PFHpA (0.3-2 ng/L) and PFHxS (0.1-1.4 ng/L)[90]. Low concentrations of PFNA (0.1-0.6 ng/L), PFDA (0.1 – 0.5 ng/L), and PFUnA (0.02 – 0.13 ng/L) were measured but PFCAs with more than 11 carbons were <LOD[90]. These values are similar to those obtained by Kim and Kannan in lake water from an urban area of NY for PFOA and PFOS but generally higher for other PFAs: PFHpA (1-13 ng/L, median 4 ng/L), PFOA (3-16 ng/L, median 2 ng/L), PFNA (<LOD – 3.5 ng/L, median 2 ng/L), PFDA (0.3 – 4 ng/L, median 1 ng/L), PFUnA (<LOD – 1 ng/L, median <LOD), PFDoA (<LOD), PFOS (<LOD – 13 ng/L, median 3 ng/L)[52]. The higher concentrations may be due in part to contribution of surface water runoff from storm drains.

In Asia, certain hotspots of industrialization appear to have resulted in elevated PFA water concentrations. Waters in highly industrialized Osaka, Japan [91] and industrialized areas of Korea and Hong Kong[92-94] had higher concentrations of PFAs than in water collected from remote areas in Asia[92]. For example PFOS, PFOA and PFNA had mean water concentrations

of 90, 19, and 3 ng/L in an industrialized area compared to 5, 2 and 0.7 ng/L in a region far from the industrialized zone[92].

Remote ocean water is considerably less contaminated with PFAs. Only PFOA and PFOS are > 1 pg/L detection limits[94,95]. As demonstrated by Yamashita et al., in the southern hemisphere, central Pacific Ocean contained 15- 60 pg/L PFOA, 1- 16 pg/L PFNA and 1-20 pg/L PFOS compared to 100-400 pg/L PFOA and 40-70 pg/L PFOS in equatorial central Atlantic Ocean (PFNA not analyzed in Atlantic Ocean sample) [94]. South of Australia PFOS ranged from 6-20 pg/L, PFOA 6-11 pg/L and longer and shorter chain PFAs were <LOD[95]. Near Antarctica, only PFOS was significant in seawater samples ranging from 5 to 23 pg/L[95].

Air

Atmospheric FTOHs and PFSAm were first reported by Martin et al. at pg/m^3 levels of N-MeFOSE, N-EtFOSE, N-EtFOSA, 6:2, 8:2, and 10:2 FTOHs in two sites in ON, urban Toronto and rural Long Point[48]. This gave rise to a North American air sampling campaign to monitor these analytes in six cities[49]. In each city airborne fluorinated organic compounds were observed but rural locations were less contaminated than urban areas and some sites demonstrated evidence of point sources such as Griffin, GA, which is near carpet manufacturing plants[49]. Air sampling in Germany revealed higher concentrations of FTOHs and lower concentrations of PFSAm compared to North America[96]. In that study, urban and rural concentrations were not significantly different[96]. Marine air samples were acquired from aboard a cruise along an extensive latitudinal gradient from northern Germany along western Europe and Africa to Capetown, South Africa[97]. The greatest contamination of FTOHs and PFSAm occurred in Bremerhaven, Germany and generally diminished southward, although a spike in N-MeFOSE was noted near the coast of Senegal and Guinea[97]. Air sampling in northwest Europe was conducted by Barber et al.[98]. Across these northwestern European locations, 4:2 FTOH and less frequently 12:2 FTOH were also observed and also the first detection of FTOs, namely 6,8,10, and 12:2 FTO[98]. The presence of FTOs was scattered and not frequently above limits of quantitation (LOQ) but of the group of FTOs monitored, 10:2 FTO was the most widespread. Where >LOQ, FTO concentrations ranged from 0.2 – 20 pg/m^3[98]. Piekarz et al. detected FTOHs and PFSAm in the Cascade region of OR and on the northwest coast of Okinawa, Japan at 2700 and 60 m above sea level, respectively[99]. PFSAm were less

frequently detected than FTOHs and the OR air samples were more contaminated with FTOHs than those from Japan [99]. Consistent with other studies, 10:2 and 12:2 FTOs were sporadically detected[99].

Air samples in the Canadian Arctic Archipelago and in the Atlantic Ocean near 60 N, contained FTOHs and PFSAm and were of the same order of magnitude as samples collected from Toronto[100]. N-MeFOSE (2.6 – 31 pg/m^3) and 8:2 FTOH (5.8-26 pg/m^3) were the most dominant in all locations[100]. The same profile was observed in air samples from Cornwallis Island in the Canadian Arctic[55].

In order to assess human exposure, indoor air concentrations are important and two investigations to date have demonstrated that FTOHs and PFSAm are much higher in indoor than outdoor air in homes of Ottawa, ON and in Tromsø, Norway [98,101,102]. For example, in Tromsø homes, individual FTOH and PFSAm concentrations were 100-1000x greater than outdoor air with 6:2, 8:2, and 10:2 FTOHs each approximately 3000 pg/m^3 and N-MeFOSA, N-EtFOSA, N-MeFOSE, and N-EtFOSE each approximately 6000 pg/m^3[98].

1.1.3 Global Transport of PFAs

Numerous environmental monitoring studies of PFAs and precursors indicate their ubiquitous presence. Direct sources of PFAs consist of emissions from manufacturing, usage and waste disposal. Precursor industrial building blocks such as PFSAm, FTOHs and FTOs are considered indirect sources as they yield PFAs via reactive pathways, detailed in Section 1.2. These semi-volatile precursors may be released to the environment as residual unbound components in the industrial product and also by industrial emissions.

Two hypotheses are in circulation regarding the dominant source of PFAs in remote regions, including the Arctic. One is that the PFAs are the result of long range transport of precursors followed by atmospheric oxidation to produce the acids and subsequent deposition. The other is that the industrially-produced PFAs in temperate regions are subject to long range transport to the Arctic via oceanic currents.

Global dispersion of precursors is attributed to their associated vapour pressure and atmospheric lifetime. Air sampling reveals wide distribution of precursors in the troposphere, including the Arctic, suggesting these compounds are indeed capable of long range transport. Estimates of atmospheric lifetime of FTOHs are based on ·OH reaction rates because air-water partition coefficients (K_{AW}) imply they will be primarily in the gas phase with insignificant wet or dry deposition[103-106]. Perfluoroalkyl chain length of FTOHs had no discernible impact on reaction rates with ·OH and hence atmospheric lifetime was estimated to be 20 days for each FTOH[106]. Based on an average wind speed of 13.8 km/h, FTOHs may travel 7000 km within their atmospheric lifetime[106]. Analogous studies with PFSAm support their ability to react to form PFCAs in the Arctic[107,108]. Evidence of precursor transport and oxidation of PFAs is their presence and pattern in snow deposited in the High Arctic in which ratios of PFOA/PFNA and PFDA/PFUnA were approximately 1[54]. Further support for this hypothesis is the detection of PFAs in Arctic Lakes isolated from waterborne emissions[55], temporal PFOS declines in Arctic biota corresponding to production phase-out[61,109], and the distinct even-odd pattern of PFCA in Arctic organisms [[46] and others reviewed in previous section]. The even-odd pattern in which the odd chain length PFCA exceed the preceding even chain length PFCA, may be accounted for based on similar production of PFCAs with *x* and *x*+1 carbons from atmospheric reactions of *x*:2 FTOH coupled with greater bioaccumulation of the *x*+1 PFCA.

Based on estimated pK_a values (-0.1 to 0.7 for PFOA, -0.2 to 0.8 for PFDoA)[110], PFAs are expected to be anions at environmental pH and as such, water soluble. Large quantities of PFOA and to a lesser extent PFNA, were synthesized for use in processing of fluoropolymers and presumably readily enter waterways. PFOA emissions from the fluoropolymers industry were exemplified in a study by Emmett et al. [111]. In that study, a fluoropolymer plant established in 1951 in Washington, WV, emitted 25400 kg (56000 lbs) of PFOA into the adjacent Ohio River in 1999 but subsequently reduced its annual emissions to 22000 kg in 2000, 2300 kg in 2003 and 700 kg in 2004[111]. Residential drinking water was significantly contaminated with PFOA (mean 448 ng/ml) in the surrounding area along the Ohio-West Virginia state border which was correlated to the PFOA serum levels (mean 423 ng/ml, range 175-537 ng/ml) of residents with no occupational exposure[111]. Although air sampling reveals some PFAs on particulates, long range atmospheric transport of PFAs is not expected due to removal via wet or dry deposition within a few days[112]. Using a fugacity-based dynamic model, Globo-POP, Armitage et al. calculated long range oceanic transport of estimated direct

PFOA emissions accurately predicted current PFOA seawater concentrations[113]. In a second study using Globo-POP, the modeled PFCA Arctic contamination potential arising from atmospheric FTOH transport was comparable to direct PFCA oceanic transport[114]. Deciphering the dominant pathway using this technique is highly dependent on the accuracy of FTOH and PFOA emissions. Using reported emissions, the model estimated oceanic transport of directly emitted PFOA was dominant over FTOH movement. However, the even-odd pattern of longer chain PFCAs, which do not have reported industrial production, in Arctic biota remains to be accounted for by oceanic transport. In addition, the inclusion of other PFOA precursors, such as PFSAm and FTOs, should be considered. Few studies of FTO reactivity exist[115,116] and in order to be incorporated into models such as Globo-POP, data regarding emission and partitioning properties[104] is necessary.

1.2 Environmental Chemistry of Perfluoroalkyl Precursors

Perfluoroalkyl precursors are industrially produced and may undergo environmental reactions to form persistent PFAs. Biologically, PFSAm produce PFOS but not PFCAs; however, via atmospheric oxidation, both types of PFAs are formed. Final products of abiotic and biotic reactions of FTOHs are PFCAs although the chain lengths of PFCAs in the product depend on the type of reaction. Results of smog chamber studies suggest that PFCAs will result from atmospheric reactions involving FTOs[115,117] and that the atmospheric lifetime of FTOs corresponds to approximately 8 days rendering them candidates for long range transport[117]. It is plausible that biologically-mediated reactions may transform FTOs to PFCAs [118]but this has yet to be determined. A review of the abiotic and biotic environmental reactions of PFA precursors follows.

1.2.1 Biological transformation of FTOHs

Numerous published works describe metabolism and microbial degradation of FTOHs[118-125]. All studies agree in the formation of PFCAs by these reactions, however, the pathway is still in discussion[118,124]. Microbial activity on *x*:2 FTOH leads to PFCA containing *x* carbons (i.e. PFOA from 8:2 FTOH) and is postulated to occur by a beta oxidation pathway[120,122,126]. This pathway also appears to occur in animals such as rodents, fish and humans, whereby PFOA is the dominant PFCA generated from 8:2 FTOH exposure but a minor

pathway, possibly alpha oxidation, generates PFNA[118,123,124] (Fig. 1.4.). The production pathway and fate of some of the identified metabolites such as the 7:3 unsaturated and saturated fluorotelomer acids (7:3 FTUCA and 7:3 FTCA) are pending further investigation. Many of the intermediates undergo phase II metabolism (conjugation) as observed in urine of FTOH treated rats, including 8:2 FTUCA, 8:2 FTUAL gluatathione conjugates[118,124]. Reactivity of these intermediates with glutathione may be suggestive that these compounds could react with other endogenous nucleophiles such as amino and nucleic acids. MacDonald et al. also demonstrated the toxicity of FTOH metabolites with three common freshwater species[127]. In that study FTCAs were more toxic than the corresponding FTUCA (i.e. $EC_{50,\ 8:2FTCA} < EC_{50,\ 8:2\ FTUCA}$, $EC_{50,\ 10:2FTCA} < EC_{50,\ 10:2\ FTUCA}$, etc.) and both FTCA and FTUCA were more toxic than the final PFCA product of metabolism[127]. The toxicity of telomer intermediates reinforces the importance of deciphering the source of PFCAs in the environment.

Metabolism of fluorotelomer mono- and di-phosphate ester surfactants, $(R_FCH_2CH_2O)P(O)(OH)_2$ and $(R_FCH_2CH_2O)_2P(O)(OH)$ (where R_F is C_8F_{17}, 8:2 monoPAPs and 8:2 diPAPs), in rats formed metabolites consistent with FTOH degradation including PFOA[119]. Dephosphorylating enzymes were hypothesized to play a role, providing a pathway applicable to other organisms including humans. The biological stability of fluorotelomer monomers seems to be dependent on linkage chemistry[25]. For example, ester-linked monomers such as 2(perfluorooctyl) ethyl acrylate and 2(perfluorooctyl) ethyl methacrylate underwent microbial degradation generating 8:2 FTOH and subsequent PFOA production but a fluorotelomer monomer linked by an ether-oxetane group, did not degrade[25]. Given the likelihood of FTOH inhalation in indoor air and ingestion of fluorotelomer phosphates, precursor metabolism is presumably a relevant pathway for PFCA exposure in humans. Fluorinated monomers are intended for polymerization but to date, fluoropolymer degradation has not been substantiated mainly due to experimental uncertainty caused by the presence of residual FTOHs[128].

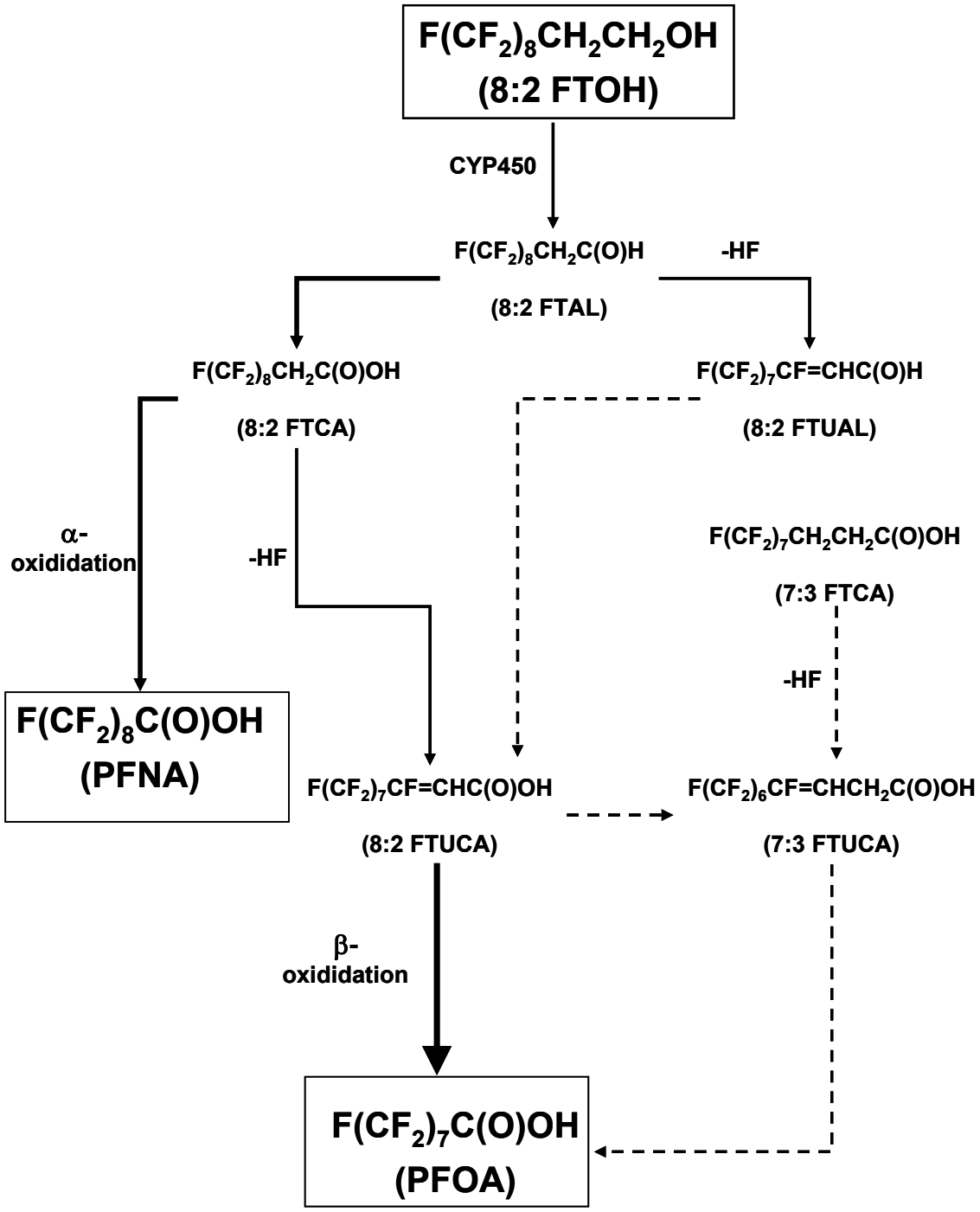

Figure 1.4. Rodent metabolism of 8:2 FTOH – observed products and proposed mechanism. Dashed arrows indicate unconfirmed pathways. Adapted from [118,124].

1.2.2 Biological transformation of PFSAm

Perfluoroalkylsulfonate appears to be the ultimate product of PFSAm metabolism. In rats receiving a subchronic dietary dose of N-EtFOSE, PFOS was the major metabolite, accumulating

in the liver[129]. Similarly, Tomy et al. observed production of the intermediate PFOSA and PFOS when fish liver microsomes were incubated with N-EtFOSA[130].

Xu et al. investigated the pathway (Fig. 1.5) of PFSAm metabolism by incubating N-EtFOSE and some of its isolatable metabolites with the following, human cytochrome P450 (CYP450), rat CYP450, rat liver microsomes, rat liver cytosol, and rat liver slices[131]. CYP450 is a super-family of proteins present throughout organisms and is involved in metabolism of endogenous and exogenous compounds usually by oxidative metabolism. Microsomes are vesicles containing CYP450 and are produced when a tissue is mechanically disrupted (homogenized). Utilization of microsomes is advantageous to study metabolism because it generates a concentrated stock of CYP450. Some portions of the pathway hypothesized by Xu et al. have not been confirmed as indicated by dashed arrows in Fig. 1.5. The dominant pathway was noted to begin with N-deethylation of N-EtFOSE and subsequent conversion to PFOSA, followed by enzymatic hydrolysis forming PFOS. The acetate metabolites FOSAA and N-EtFOSAA undergo further transformation to PFOS as evidenced by Higgins et al. who observed PFOS production in worms exposed to N-EtFOSAA[132].

In animals, N-EtFOSE, PFOSA, and perfluorooctanesulfonamidoethanol (FOSE) can undergo glucuronidation by UDP-glucuronosyltransferase enzyme (UGT) to produce hydrophilic glucuronide analogs[131]. In the shaded circle in Fig. 1.5., N-glucuronidation of PFOSA is indicated. FOSE and N-EtFOSE result in O-glucuronides where the glucuronide linkage involves the oxygen of the alcohol moiety[131]. Formation of glucuronide derivatives presumably facilitates excretion of the parent and metabolite products in animals.

With the exception of the glucuronide conjugates which are specific to animals, similar products are expected via microbial degradation. In addition, the metabolic pathway observed for N-EtFOSE is likely to occur for N-MeFOSE and in either case, perfluoroalkyl geometry would be conserved in the products. In other words, isopropyl N-EtFOSE (*iso*-N-EtFOSE) would generate *iso*-PFOS. However, geometry does appear to influence reactivity as demonstrated in a recent study by Benskin et al. in which the rate of metabolism varied in N-EtFOSA isomers[9]. Biological transformation of N-EtFOSE phosphate esters nor N-MeFOSE-based polymers has yet to be reported.

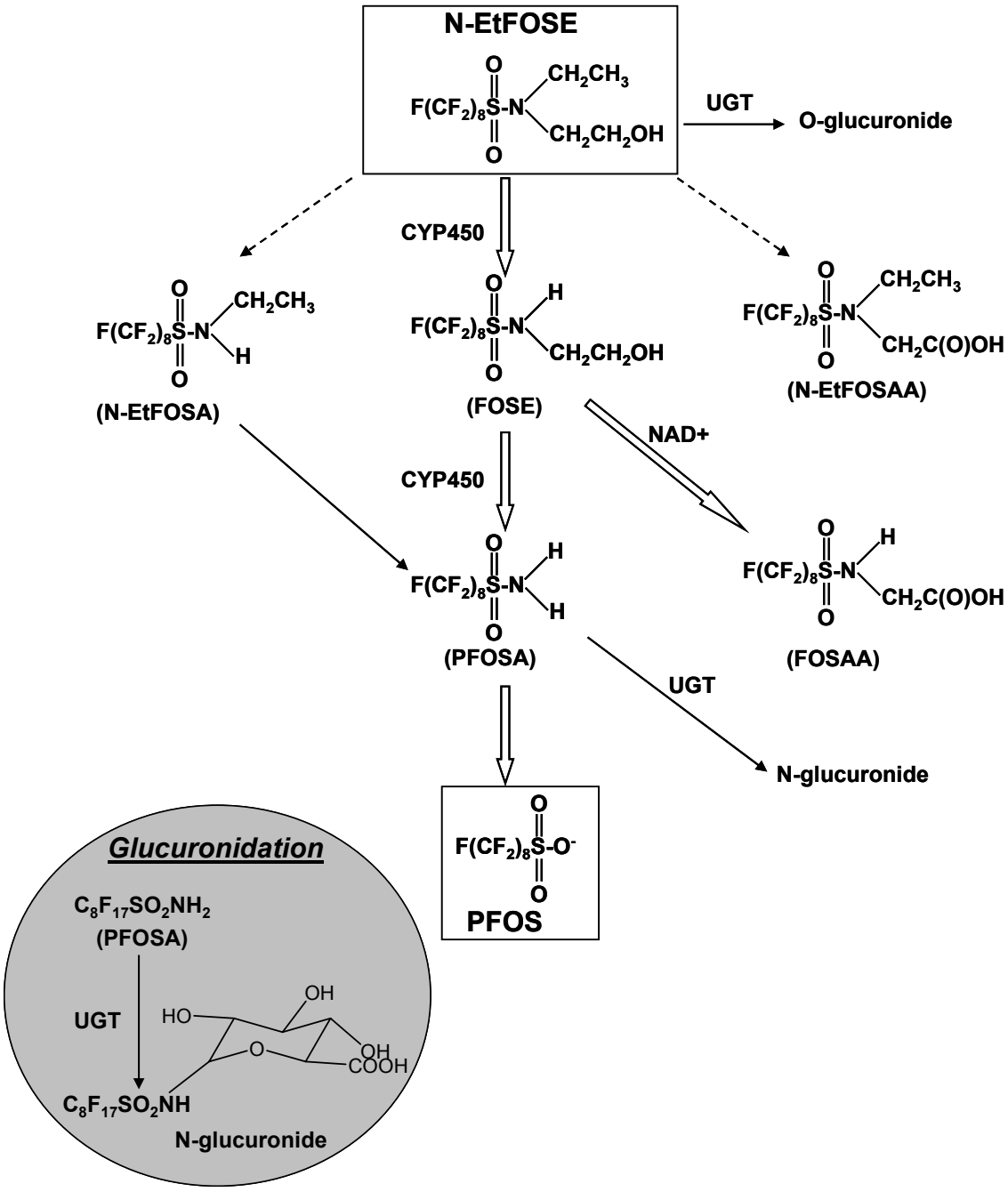

Figure 1.5. Metabolism of N-EtFOSE by CYP450, liver microsomes, and liver slices (adapted from [131]). Block arrows indicate major pathways. Example of glucuronidation reaction is presented shaded area. N-EtFOSE and FOSE may be transformed to O-glucuronide.

1.2.3 Atmospheric chemistry of fluorotelomer alcohols

Atmospheric oxidation of FTOHs was demonstrated in smog chamber under low NO_x conditions designed to stimulate remote or rural areas with little air pollution [106,133,134]. Under these constraints, a homologous series of PFCAs, varying in perfluoroalkyl length, were formed[133,134].

The mechanism was believed to be initiated by abstraction of a hydrogen on the carbon bearing the alcohol moiety by ·OH (Fig 1.6.). Further reactions yield fluorotelomer aldehyde ($F(CF_2)_xCH_2C(O)H$, *x*:2 FTAL) from which two pathways emerge, one leading to *x*:2 FTCA and the other to perfluoroaldehyde ($F(CF_2)_xC(O)H$, PFAL). PFAL may undergo photolysis to form perfluoroalkyl radical ($F(CF_2)_x\cdot$) and/or hydrogen abstraction by ·OH to produce perfluoroalkyl acyl radical ($F(CF_2)_xC(O)\cdot$). Further reactions of the acyl radical yield a PFCA containing *x*+1 carbons. Addition of oxygen to the perfluoroalkyl radical results in a peroxy radical ($F(CF_2)_{x-1}CF_2OO\cdot$), that can react with alkyl peroxy radicals containing an alpha hydrogen, the most common of which is $CH_3OO\cdot$. Reaction of the perfluoroalkyl peroxy radical with $CH_3OO\cdot$ forms perfluoroalkyl alcohol from which an acyl fluoride ($F(CF_2)_{x-1}C(O)F$) is obtained by heterogeneous decomposition. Hydrolysis of the acyl fluoride yields the PFCA containing *x* total carbons ($F(CF_2)_{x-1}C(O)O$-). An alternative fate of the peroxy radical is formation of an alkoxy radical ($F(CF_2)_{x-1}CF_2O\cdot$) followed by sequential loss of COF_2 units leading to shorter chain length PFCAs.

Formation of PFCAs from 8:2 FTOH (and other FTOHs) will decrease under increasing NO_x concentrations. Competition between NO_x (shaded box in Fig 1.6) and $CH_3OO\cdot$ to react with the perfluoroalkyl peroxy radical determines the yield of PFCA. The NO_x pathway results in primarily COF_2 production. Emmons et al. investigated NO_x concentrations in a variety of locations in the North American hemisphere[135]. Remote and rural areas such as Barrow, Alaska and Egbert, ON, had lowest concentrations of NO_x, 25 parts per trillion (ppt) and 2 parts per billion (ppb), respectively[135]. Urban areas including Los Angeles, CA, and Toronto, ON had high NO_x levels ranging from 40-200 ppb[135].

A summary of PFCAs generated by atmospheric oxidation of FTOHs is presented in Table 1.5. Included are expected products from analogous reactions of a hypothetical FTOH

containing an isopropyl odd-chain length perfluoroalkyl group ($(CF_3)_2CF(CF_2)_2CH_2CH_2OH$, 5:2 *iso*-FTOH). If the mechanism for *n*-perfluoroalkyl FTOHs apply then isopropyl PFCAs ($(CF_3)_2CF(CF_2)_yCOO$-) would be produced including *iso*-PFHxA, *iso*-PFPA, and *iso*-PFBA (y = 2). Via the unzipping cycle, presumably the perfluoroisopropyl radical ($(CF_3)_2C(\cdot)F$) would be generated and consequently the perfluoroisopropanol ($(CF_3)_2CFOH$). Loss of HF from perfluoroisopropanol produces perfluoroalkylketone. The ketone may undergo photolysis resulting in carbon monoxide and two perfluoroalkyl radicals or a perfluoroalkyl radical and a perfluoroalkyl acyl radical[115,136]. Atmospheric chemistry of branched perfluoroalkyl precursors is further discussed in Chapter 7.

Smog chamber studies with FTOs resulted in production of perfluoroalkyl aldehyde which subsequently generated perfluoroalkyl radicals[115,117]. These products were also noted in FTOH oxidation (Fig 1.6.) and it is thus likely that FTOs also yield PFCAs by analogous atmospheric reactions. Sulbaek Andersen et al. noted atmospheric reaction rates of FTOs to be independent of chain length, congruent with FTOH observations[116].

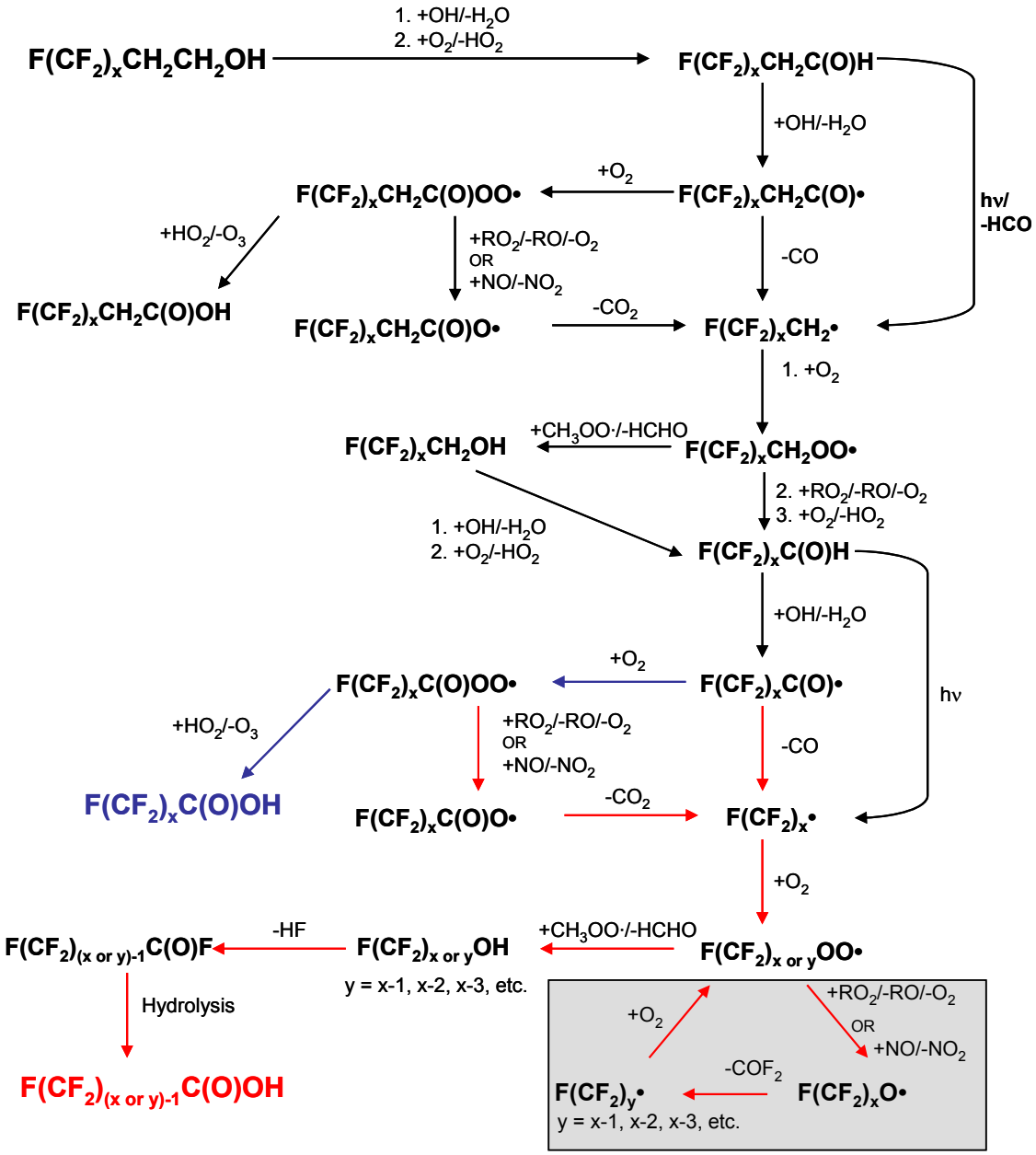

Figure 1.6. Atmospheric oxidation scheme of FTOH (adapted from [133]). The shaded box indicates perfluoroalkyl "unzipping" cycle. Blue arrows represent "pathway 1" and red arrows are "pathway 2", corresponding to Table 1.5.

Table 1.5. PFCA products from atmospheric oxidation of precursors (according to mechanism in [107,108,133]), with pathways 1 & 2 in Fig. 1.6.

Precursor (Parent)	**PFCA Products**		
	via pathway 1	**via pathway 2 (not unzipped)**	**via unzipping in pathway 2**
x:2 FTOH $F(CF_2)_xCH_2CH_2OH$	$F(CF_2)_xC(O)O$-	$F(CF_2)_{x-1}C(O)O$-	$F(CF_2)_yC(O)O$- y = x-2, x-3, …, trifluoroacetate (TFA)
4:2 FTOH $F(CF_2)_4CH_2CH_2OH$	*n*-PFPA $F(CF_2)_4C(O)O$-	*n*-PFBA $F(CF_2)_3C(O)O$-	PFPrA $F(CF_2)_2C(O)O$- TFA $CF_3C(O)O$-
5:2 *iso*-FTOH* $(CF_3)_2CF(CF_2)_2CH_2CH_2OH$	*iso*-PFHxA $(CF_3)_2CF\ CF_2CF_2C(O)O$-	*iso*-PFPeA $(CF_3)_2CFCF_2C(O)O$-	*iso*-PFBA $(CF_3)_2CFC(O)O$- perfluoroacetone $(CF_3)_2C(O)$
n-perfluoropentyl sulfonamide $F(CF_2)_5SO_2N(R)(R')$	*Not applicable*	*n-PFPeA*	*n*-PFBA *n*-PFPrA TFA
iso-perfluoropentyl sulfonamide** $(CF_3)_2CF(CF_2)_2SO_2N(R)(R')$	*Not applicable*	*iso-PFPeA*	*iso*-PFBA perfluoroacetone $(CF_3)_2C(O)$

* hypothesized products from 5:2 iso-FTOH based on mechanism by Ellis et al.[133]

** hypothesized products from iso-perfluoropentylsulfonamide based on mechanism by Martin et al.[107] and D'eon et al.[108]

1.2.4 Atmospheric chemistry of PFSAm

Two PFSAm have been investigated in smog chambers, N-methyl perfluorobutylsulfonamidoethanol ($F(CF_2)_4SO_2N(CH_3)(CH_2CH_2OH)$, N-MeFBSE)[108] and N-ethyl perfluorobutylsulfonamide ($F(CF_2)_4SO_2NH(CH_2CH_3)$, N-EtFBSA)[107]. In both studies production of PFCAs was observed. D'eon et al. proposed initial addition of ·OH to the S=O bond forms an unstable sulfonyl radical[108] (Fig 1.7.). Two pathways emerge, cleavage of the C-S bond or the S-N bond[108]. In the case of the C-S bond breaking, a perfluoroalkyl radical results and a mechanism analogous to those described in the previous section ensue. Perfluoroalkylsulfonate forms by breaking of the S-N bond. As depicted in Fig 1.7., the longest PFCA obtained via atmospheric oxidation of $F(CF_2)_xSO_2N(R)(R')$ is one with *x* carbons. In other words, perfluorooctyl-based PFSAm will form PFOA, PFHpA, PFHxA,…, and TFA but not PFNA (Table 1.5.). PFSAm originated from ECF production and are hence a mixture of isomers. Although not investigated, the mechanism presented in Figure 1.5. is likely applicable to branched PFSAm. Postulated PFCA products generated from a hypothetical example of a branched PFSAm is shown in Table 1.5. Presumably isopropyl version of the perfluoroalkylsulfonate would also ensue from an isopropyl PFSAm.

Atmospheric products are expected to conserve the perfluorocarbon arrangement of the parent product and thus, *n*-PFCA and *n*-PFOS would result from *n*-PFSAm. Similarly, the ECF isomer signature of PFSAm is expected to be maintained in the PFCA and PFOS product (and intermediates). The isomer-specific physical properties PFSAm may have an effect on transport. As presented in Chapter 2, branched PFSAm were more water soluble than linear PFSAm, however differences in log K_{OW} were not significant. Vapour pressures and air partition coefficients are not known for ECF isomers and it remains uncertain whether these properties would be significantly different among isomers. In earlier research, boiling points for branched perfluoroalkanes were marginally higher than the corresponding *n*-perfluoroalkane isomer[137]. For example, the boiling point of n-perfluoropentane was 29.3 °C compared to 1-methyl perfluorobutane (30.1 °C) and perfluoroneopentane (29.5 °C)[137].

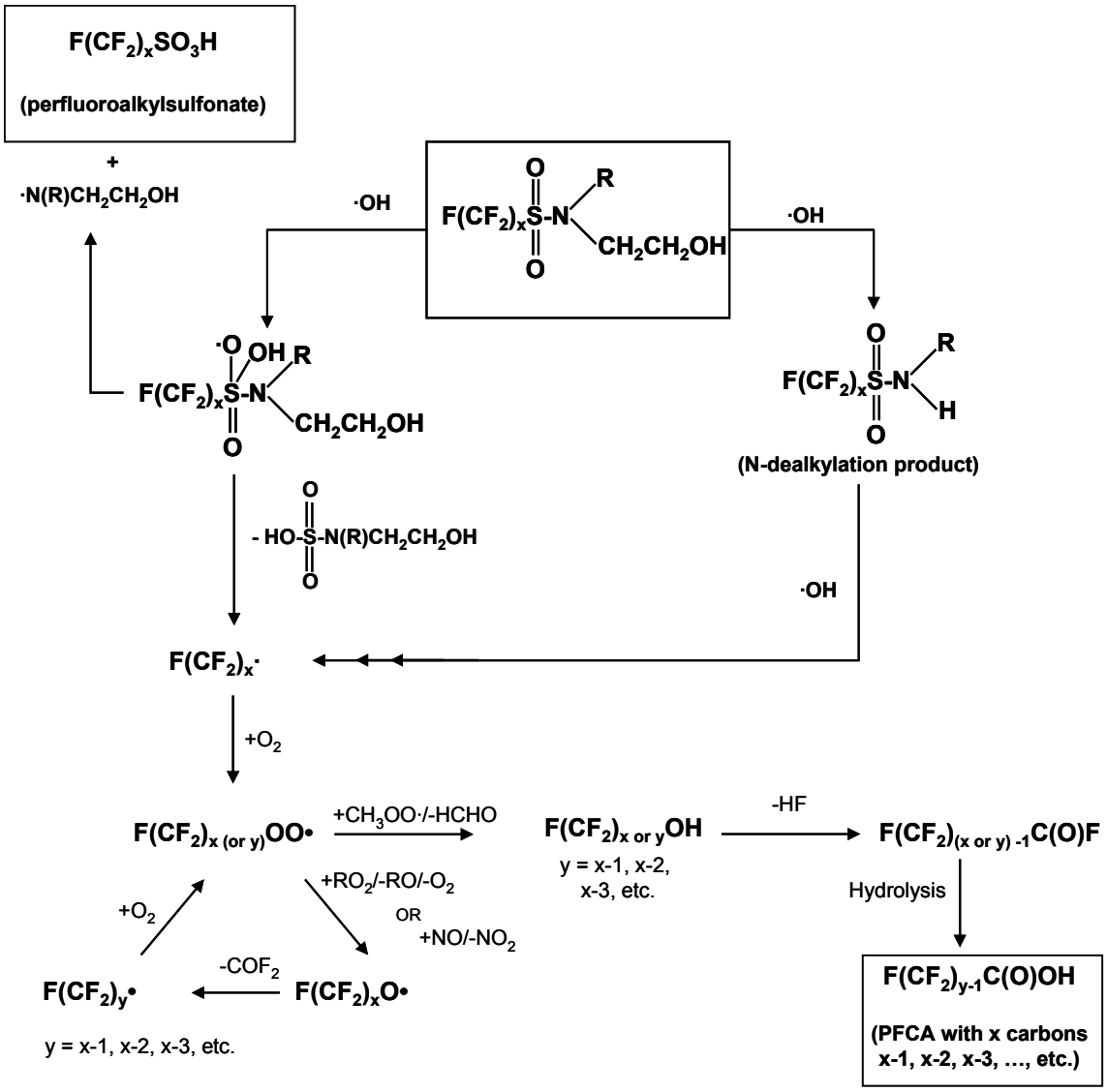

Figure 1.7. Atmospheric oxidation scheme of PFSAm (adapted from [133]and[107]).

1.3 Biological handling PFAs

PFAs do not undergo metabolism and as such, biological processes that are applicable to PFAs are absorption, dispersion and elimination. Biological handling, including toxicological effects, of PFOA and PFOS have been reviewed in a number of publications[138-142] and thus, only a brief summary of major findings are presented in this section. Both PFOS and PFOA have the ability to activate receptors inducing peroxisome proliferation and PFOA caused rodent tumors consistent with deleterious effects associated with peroxisomal proliferators[143]. In rodents, 2nd generation effects were apparent for both PFOS and PFOA maternal exposure(reviewed in [144]). PFOS caused mortality in neonates even though there was an absence of apparent maternal toxicity[145]. Similarly, PFOA resulted in growth and developmental delays in offspring with deaths attributed to low birth weight or immaturity at weaning[146]. The focus of the remainder of this section is on extrapolation of laboratory-based studies using rodents to humans and factors leading to prolonged half-lives.

1.3.1 Blood protein binding

Environmental biological monitoring of PFAs revealed the greatest accumulation occurs in blood and liver[35,38,62,147,148], results corroborated by laboratory-based exposure studies involving rodents[149-151], fish[152,153], and monkeys[13,154]. Unlike traditional persistent organic pollutants, PFAs do not significantly accumulate in fatty tissue, presumably due to the presence of a lipophobic perfluorinated tail and hydrophilic anion ($C(O)O^-$ and SO_3^-) group. PFAs may occur in fatty tissue as evidenced by PFOS detected in the blubber of harbor porpoises[155] but it is currently unclear whether the PFA resides in the blood capillaries or fat. Based on *in vitro* binding assays, PFOS binds strongly to albumin, the major protein in blood serum, in a 1:1 stoichiometric ratio[156]. In that study, to saturate albumin binding sites, PFOS concentrations would have to be greater than 50 – 100 mg/L in serum[156]. Akin to PFOS, PFOA also primarily binds to serum albumin with equivalent binding affinity in rat and human sera[157].

1.3.2 Enterohepatic recirculation

Clearance of xenobiotics from the body typically involves the liver via biotransformation or biliary excretion. Enterohepatic circulation is the movement of bile between the liver and GI tract via hepatic portal transport[158]. These bile salts are necessary in the digestion of some materials including fat. Both PFOS and PFOA also undergo enterohepatic circulation[159]. Together blood serum protein binding and enterohepatic recirculation are likely factors in the preferential accumulation of PFAs in liver as well as prolonged half-lives in organisms.

1.3.3 Pharmacokinetics of PFAs

Numerous studies detail pharmacokinetics of PFAs in a variety of organisms (Table 1.6). First order elimination kinetics have been established by monitoring concentrations in blood or by whole body sacrifice post-exposure in treated subjects. This data is expressed in the form half-life ($T_{½}$) as calculated using the first order elimination rate constant (k_e). Human data presented in Table 1.6 was acquired by measuring decreasing PFA blood concentrations from the blood of retired employees who were formerly employed at a fluorochemical production plant. More pharmacokinetic information is available for PFOA compared to PFOS, mainly because the biological behaviour of PFOA is less-straightforward. In particular, there are clear sex-related differences in accumulation of PFOA but the trend is inconsistent between species (Table 1.6). Although half-lives were not presented, Kennedy et al. highlighted some sex-related PFOA pharmacokinetic differences in hamsters whereby in five days following exposure, males excreted 99% of PFOA and female only 58%[138]. PFOS tends to have a longer half-life than PFNA, although both possess 8 perfluorinated carbons. Surprisingly, humans do not have a demonstrably different elimination rate constant for PFOS compared to PFOA. In addition, increasing chain length of PFAs results in a longer half-life but some exceptions were noted in Table 1.6.

Table 1.6. Elimination half-life ($T_{½}$) of PFAs. Where available, uncertainty on $T_{½}$ is in brackets.

Species	PFA	$T_{½}$ in days	Gender	Mode of Exposure	Tissue for $T_{½}$	Reference
Rat-PFOS						
Rat	PFOS	100	mixed	single dose i.v.	Blood	[160]
Sprague-Dawley	PFOS	82	male	84 day dietary	Blood	Chapter 5
Sprague-Dawley	PFOS	80	female	84 day dietary	Blood	Chapter 5
Birds – PFOS						
Mallard	PFOS	6.86	mixed	5-day dietary	Serum	
Mallard	PFOS	17.5	mixed	5-day dietary	Liver	[161]
Quail	PFOS	12.8	mixed	5-day dietary	Liver	
Chicken	PFOS	125	male	s.c. implant 4-wk continuous	Blood	[162]
Fish-PFOS						
Rainbow trout	PFOS	20 (±6)	mixed	34-day dietary	Liver	[153]
Carp	PFOS	50-152	mixed	58-day Water-borne	Whole body	Reviewed
Bluegill	PFOS	112	mixed	62-day water-borne	Whole body	in[142]
Invertebrate – PFOS						
Blackworm	PFOS	18	N/A	28-day sediment	Whole body	[132]
Primates – PFOS						
Cynomolgus Monkey	PFOS	200	mixed	26-week repeat oral	Blood	[13]
Humans	PFOS	5 years	male	occupational	Blood	[163]
Rats (male) – PFOA						
Sprague-Dawley	PFOA	15	male	single dose i.p.*	Whole body	[151]
Sprague-Dawley	PFOA	9	male	single dose i.p.*	Blood	[151]
Wistar	PFOA	4.4	male	single dose i.p.*	Blood	[164]
Wistar	PFOA	5.6 (±1.2)	male	single dose i.v.*	Blood	[165]
Sprague-Dawley	PFOA	9.0	male	84 day dietary	Blood	Chapter 5
Rats (female) – PFOA						
Sprague-Dawley	PFOA	<1	female	single dose i.p.*	Whole body	[151]
Sprague-Dawley	PFOA	0.17	female	single dose i.p.*	blood	[151]
Wistar	PFOA	1	female	single dose i.p.*	Blood	[164]
Wistar	PFOA	0.08 (±0.03)	female	single dose i.v.*	Blood	[165]
Birds – PFOA						
Chicken	PFOA	4.6	male	s.c. implant 4-wk continuous	Blood	[162]
Mice – PFOA						
ddY Mice	PFOA	12	male	single dose i.p.	Blood	[165]
ddY mice	PFOA	20	female	single dose i.p.	Blood	[165]
Rabbit – PFOA						
Rabbit	PFOA	0.23	male	single dose i.p.	Blood	[165]
Rabbit	PFOA	0.29	female	single dose i.p.	Blood	[165]
Dog – PFOA						
Beagle	PFOA	21.5 (±1.5)	male	single dose i.v.	Blood	[166]
Beagle	PFOA	10.6 (±2.2)	female	single dose i.v.	Blood	reviewed in [141]
Fish-PFOA						
rainbow trout	PFOA	5 (±0.5)	mixed	34-day dietary	Liver	[153]
Invertebrate – PFOA						
Blackworm	PFOA	17	N/A	28-day sediment exposure	Whole body	[132]
Primates – PFOA						
Cynomolgus Monkey	PFOA	30	male	single dose i.v.	Blood	[154]

Cynomolgus Monkey	PFOA	21	female	single dose i.v.	Blood	[154]
Humans	PFOA	4 years	male	occupational	Blood	[163]
Other PFAs						
Wistar rat	PFNA	29.5 (±2.3)	male	single dose i.v.*	Blood	[165]
	PFNA	2.4 (±0.4)	female			
	PFDA	39.5 (±8.6)	male			
	PFDA	58.6 (±5.8)	female			
Sprague Dawley	PFNA	47.5	male	84-day dietary	Blood	Chapter 5
Sprague Dawley	PFNA	2.3	female	84-day dietary	Blood	Chapter 5
Humans	PFHxS	7-9 years	male	occupational	Blood	[163]
rainbow trout	PFDA	14 (±2)	mixed	34-day dietary	Liver	[153]
	PFUnA	10 (±1)				
	PFDoA	15 (±2)				
Blackworm (aquatic oligochaete)	PFNA	42	N/A	28-day sediment exposure	Whole body	[132]
	PFDA	26				
	PFUnA	15				
	PFDoA	15				

* Abbreviations: i.p.= intraperitonal, i.v. = intravenous, s.c.= subcutaneous, Blackworm is an aquatic oligochaete, N/A = not available.

1.3.4 Extrapolation from laboratory-derived pharmacokinetics to humans

As demonstrated in Table 1.6., the elimination $T_{½}$ of PFAs varied widely between species. To date, humans have the longest $T_{½}$ reported for these compounds. Persistence in humans has lead to scrutiny of the applicability of laboratory animal based modeling of PFA pharmacokinetics[163,167,168].

Extrapolation from monkey data compared to that of rat and dog appears to best predict human clearance of pharmaceuticals[169,170]. Comparing pharmacokinetic properties such as $T_{½}$ between species, variables must be scaled appropriately. One technique[171] is to account for the different sizes of species by using blood flow volumes whereby

$$T_{½,species\#2} = T_{½,species\ \#1} * (\text{hepatic blood flow}_{species\ \#1} \div \text{hepatic blood flow}_{species\#2})$$

Typical blood flow (expressed per kg body weight) values are 85, 45, and 21 ml/min/kg for rat, monkey, and humans, respectively[170]. Based on these blood flow rates and a reported $T_{½}$ of 9 days in male Sprague-Dawley rats for PFOA, the estimated $T_{½}$ of PFOA in humans and monkeys are predicted as 17 and 61 days, respectively, dissimilar from the measured values of 30 days and

4 years[163]. Based on the lack of agreement in pharmacokinetics between different species and the observation of sex-dependent pharmacokinetics, the role of active transport (described in Section 1.3.5) mediated by proteins specific to species and gender has been postulated[167].

1.3.5 Biological Excretion of PFAs

When comparing renal (urinary) and biliary (fecal) excretion, clearance of PFOS and PFOA occurs primarily through urine[151,172,173]. Furthermore, differences in plasma elimination $T_{½}$ values between sexes of a species demonstrate correlation to PFA renal excretion rates[151,173,174]. Hundley et al. determined that female rat and male hamster administered a single dose of PFOA excreted 74% and 90%, respectively, of the dose in urine within 48 h[172]. During this same time period, urine collected from treated male rat and female hamster accounted for only 26 and 45% of the dose, respectively. In the feces of the male rat, female hamster, female rat and male hamster, 9%, 9%, 28%, and 8% of the dose was recovered, representing biliary excreted or unabsorbed PFOA[172]. Although the dominant excretion route of PFOS is also via urine, its clearance is less efficient than PFOA, which may be a leading factor in the prolonged residence time of PFOS in biota. The cause of this could be reabsorption from the kidney filtrate and/or enterohepatic circulation. In PFOS-treated rats, 30% of the dose was excreted into urine and 13% into feces[150]. This pattern is independent of sex and consistent with observation in monkeys[167].

Renal excretion

Renal handling of organic acids occurs in the nephron, the basic functioning unit of the kidney. Sub-structures within the kidney filter chemicals from plasma at the glomerulus[175]. Within the proximal tubules the compounds can be secreted into urine (passive clearance) or be reabsorbed into blood from distal tubule segments (active resorption). A scheme of active resorption and passive urinary elimination is presented in Fig. 1.8.

Filtering of plasma in the glomerulus of the nephron typically frees only unbound compounds (compounds not bound to plasma proteins). This is referred to as passive clearance or glomerular filtration. Many organic acids such as 2,4-dichlorophenoxyacetic acid (2,4-D) and

2,4,5-trichlorophenoxyacetic acid (2,4,5-T) [175], are bound to proteins and thus passive clearance is considered only a minor component in their overall clearance.

Active transport occurs when a compound is bound to a transporter protein. Once the compound is secreted from the proximal tubule, the compound may bind to a carrier protein which has broad substrate affinities[175]. This is a saturable process as there are a finite number of transporter proteins and associated binding sites. In laboratory-based animal studies, the administered dose is usually much higher than a typical environmental concentration and as such glomerular filtration (passive transport) may become increasingly significant if binding sites on proteins involved in active transport are saturated. Evidence of active renal tubular secretion occurs when a dose-dependence is observed in plasma pharmacokinetic properties. At a certain dose, the transport mechanism is saturated as plasma concentrations increase.

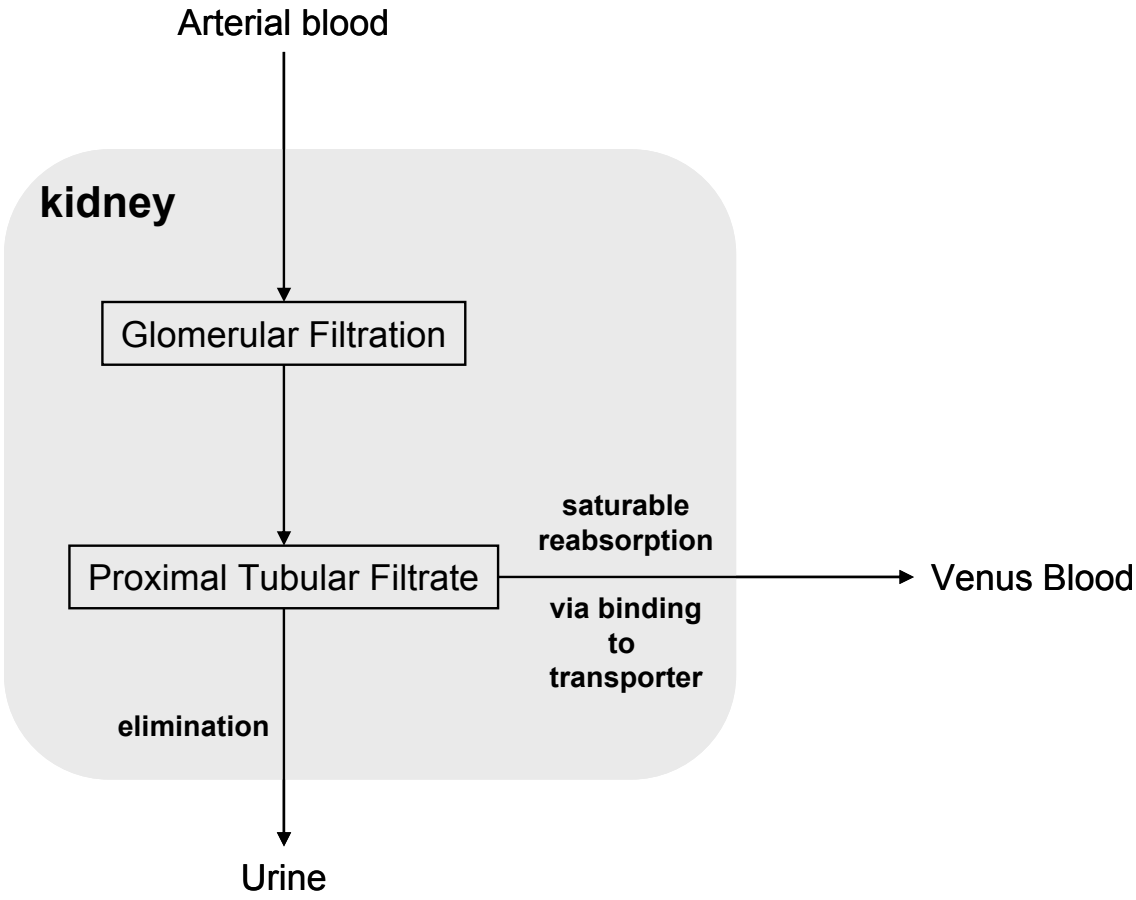

Figure 1.8. Passive renal clearance of a xenobiotic versus active reabsorption mediated by transporter proteins. Figure adapted from [176].

1.3.6 Saturable resorption of PFAs

Binding to blood proteins and enterohepatic circulation likely contribute to the long $T_{½}$ in organisms; however, these mechanisms alone do not account for gender-related pharmacokinetic differences because serum albumin profile and enterohepatic circulation are not sex-dependent. There is considerable evidence that PFOA and PFOS are subject to saturable resorption process whereby a carrier protein repeatedly transports PFOA and PFOS from the renal filtrate back into blood serum[167,177-179]. Organic anion transporter proteins (OATPs) are candidates for these proteins, especially because they are hormonally regulated and are thus characterized by a different pattern within a gender of certain species[180]. For example, in mice, OATP2 is expressed equivalently in kidneys of both females and males but OATP1 is more dominant in male kidney than in the female. In rats, OATP2 is predominant in female kidney but not in males[180]. Research of OATPs remains an emerging field and currently there is only limited information available on rodent OATPs and even less regarding OATPs in humans and other organisms[180].

Urinary elimination of PFOA is similar in genders in monkeys[167] and humans (as deciphered from samples collected from volunteers in Japan) [168]. Andersen et al. developed a physiological pharmacokinetic model that accounted for PFOS and PFOA pharmacokinetics in monkeys by renal resorption[167]. Resorption parameters were transporter protein maximum and transporter protein affinity. By scaling these parameters from a 4-7 kg monkey to the standard 70 kg human, the model was able to accurately predict the human 4-5 year $T_{1/2}$ of PFOS and PFOA[167]. Andersen et al. also noted that PFOS appeared to have a greater capacity for renal resorption than PFOA[167]. Renal resorption of other PFCAs has not been determined but in pharmacokinetic experiments with rodents, increasing chain length of PFCAs resulted in longer $T_{1/2}$ as well as diminished urinary excretion and greater biliary excretion[165,173].

1.4 Human exposure to PFAs

Perfluoroalkyl-containing compounds are of anthropogenic origin, intended for human use. As such, the routes of exposure for humans are numerous. PFAs are ubiquitously detected in the environment and as such may be ingested and inhaled. In addition, humans are likely subject to indirect exposure sources including inhalation and ingestion of precursors that are metabolized to the longer-lived PFAs.

Drinking water samples from Japan demonstrated regional differences in PFOS contamination where Morioka and Miyako samples contained 0.1-0.5 ng/L compared to tap water from urban Kyoto which was in the range of 3 – 3.5 ng/L[181]. FTOHs and PFSAm are more prevalent in indoor air than outdoor air (Section 1.1.2) but the opposite trend was noted for PFAs. Indoor air in residences of Tromsø, Norway had mean concentrations of 17.1 pg/m^3 PFHxA, 0.8 pg/m^3 PFHpA, 4.4 pg/m^3 PFOA, 2.7 pg/m^3 PFNA, 3.4 pg/m^3 PFDA, and 1.2 pg/m^3 PFDoA [98]. The units correspond to pg of PFA per volume of air sample although the acids were in the particulate phase[98]. PFOS concentrations were less than the method LOD[98]. Indoor dust in homes in Ottawa, ON contained 38 ng PFOS and 20 ng PFOA per g of dust[182].

In Section 1.2.1, the fate of ingested PFA precursors was discussed. Ingestion of PFAs in food also appears to occur. Human milk[183,184] and umbilical cord blood samples[185] also contain PFAs (PFOS, PFOA, and PFNA) in 10^1 - 10^2 ng/L levels, representing pre- and post-natal exposure to infants. Analysis of items from a Canadian diet comprised of meat, fish, and packaged food items (pizza, french fries, egg breakfast sandwich, chicken nuggets, etc.), by Tittlemier et al. revealed low ng/g levels of PFAs, 3-4 ng/g PFOA, <0.4-5 ng/g PFNA, and <1-3 ng/g PFOS[186]. Longer chain PFCAs were monitored but found to be <LOD. Falandysz et al. observed a correlation between PFA concentrations in humans in the Baltic coast and that in fish comprising a major portion of their diet[77]. Direct exposure to PFA via air, water and food does not necessarily represent industrially produced PFAs and could in fact arise from environmental degradation of precursors.

Although FTOHs, PFSAm, and FTOs have been determined in indoor and outdoor air, exposure to humans has not been estimated from other precursor-related sources. FTOH- and N-EtFOSE-based phosphate surfactants were used in a variety of food-contact paper coatings and D'eon et al. observed rodent-metabolism of fluorotelomer phosphates to PFCAs[119]. Begley et al. demonstrated migration of fluorotelomer phosphates from food packaging into Miglyol, a food oil stimulant, suggesting that ingestion of perfluoroalkyl phosphate esters is possible via food-contact packaging[187]. Research by Schultz et al. noted an increase in PFOA and some other PFAs in effluent from a municipal wastewater treatment plant compared to influent[188], suggesting that treatment processes do not remove PFAs and also that the treatment may be a site of PFA production via precursor biodegradation. According to 3M, the major source of PFOS in humans is expected to be PFSAm because PFOS itself was not a major commercial

product[189]. Evidence of PFSAm exposure is in the acetate metabolites of N-EtFOSE and N-MeFOSE observed in human blood from the general population[44,189]. N-MeFOSE is a residual component in certain fluoropolymers used in textile treatment, including carpet & upholstery. If this is indeed the primary source of PFOS in humans, then by analogy, FTOHs are a likely source of PFCAs in humans because FTOHs are used in similar applications.

Using available data on food, air and dust concentrations of PFAs and their precursors, relative exposure routes in humans can be estimated. Precursors exposure may be air and dust-borne. Humans typically spend 16 h per day engaged in light activity during which time air inhalation rates are estimated to be 20 L/min (19.2 m^3 air inhaled per d)[102,190]. Furthermore, adult humans have been suggested as ingesting 75 mg of dust per day[102,190]. Median indoor air concentrations in residential homes in Ottawa, ON, were measured by Shoeib et al. as 744 pg/m^3 N-EtFOSE, 1490 pg/m^3 N-MeFOSE, 2070 pg/m^3 8:2 FTOH and 890 pg/m^3 10:2 FTOH [102,191]. Indoor dust concentrations were also monitored and median values were 113 ng/g N-EtFOSE and 148 ng/g N-MeFOSE [102]. Inhalation exposure for FTOHs and PFSAm can be estimated as follows:

$$\text{Precursor exposure (ng/d)} = \text{indoor air concentration}(\text{pg/m}^3)*\text{volume of air inhaled per day (m}^3\text{/d)} + \text{indoor dust concentration (ng/g)}*\text{mass of dust ingested per day (g/d)}$$

Using N-EtFOSE as an example, exposure is estimated by (744 pg/m^3)*(19.2 m^3/d)+(113 ng/g)(0.075 g/d) = 22 ng/d. The same calculation applied to other precursors equate to 40 ng/d 8:2 FTOH, 17 ng/d 10:2 FTOH, and 35 ng/d N-MeFOSE, bearing in mind that these FTOH exposures did not include contributions from dust for which data was not available. Presumably further exposure occurs via ingestion of residuals migrating into food from packaging but data corresponding to that type of exposure is not available for precursor-related materials.

Fromme et al. created duplicate diet portions where one portion was used as subsistence for human subjects and the other portion was analyzed for PFOA and PFOS in Munich, Germany[192]. Females and males in that study consumed 2464 and 3324 g of food per day[192]. Median PFOS and PFOA intake corresponded to 80 and 132 ng/d in females and 93 and 242 ng/d in males[192]. Thus food exposure is much more dominant than inhalation of PFAs sorbed to particles. Using a mean value of 4.4 pg/m^3 PFOA in air[98] and 20 ng/g PFOA

in dust[182], together air inhalation and dust ingestion account for 1.58 ng PFOA per day. Indoor air concentrations are not available for PFOS but the mean dust concentration was 38 ng PFOS/g in homes as reported by Kubwabo et al.[182].

Yields from human metabolism are also necessary in order to predict the contribution of precursors to the PFA body burden. These yields have not been reported in the literature. However, Martin et al. observed 1.4% transformation of 8:2 FTOH in rats to PFOA[124]. Assuming this transformation yield is consistent in humans, the estimated 40 ng of inhaled 8:2 FTOH per day by the average human is transformed to 0.56 ng PFOA. If one makes the further assumption that PFSAm metabolism to PFOS yield is also 1.4%, then the total ingested and inhaled 60 ng of combined N-EtFOSE and N-MeFOSE would be transformed to 0.84 ng PFOS.

All biotransformation products are expected to conserve the perfluorocarbon arrangement of the parent product and thus, *n*-PFCAs would result from metabolism of *n*-FTOHs. Similarly, the ECF isomer signature of PFSAms is expected to be maintained in the PFOS product (and intermediates). This is corroborated by the PFOS isomer profile in human blood which is consistent with an ECF signature[9,83]. In addition, metabolism of N-EtFOSA isomers by human microsomes were found to yield the corresponding isomer of PFOSA[9]. The potential for biological discrimination to alter the isomer profile is investigated in Chapters 5 and 6, in which fish and rats were administered a dietary dose of ECF isomers and isomer profiles in tissues were determined in a time course. Previously, Loveless et al. observed a dose-dependency in isomer discrimination in rodents. With increasing dose, rats and mice preferentially excreted branched isomers of PFOA and the degree of isomer discrimination was more pronounced in rats compared to mice[193]. The work of Loveless et al. is elaborated upon in Chapter 5.

1.5 Trends in isomer and congener patterns of other environmental pollutants

Like PFAs, other anthropogenic contaminants are also isomer mixtures in the technical grade product. In other cases, some pollutants are considered congener mixtures. Congeners are chemicals that belong to the same class due to structural similarities but do not necessarily have to be isomers (identical molecular formula). For example in the following series of polychlorinated biphenyls (PCBs), 2-chlorobiphenyl, 3-chlorobiphenyl and 2,3-dichlorobiphenyl,

all three are considered congeners because they share the biphenyl substructure and possess some degree of chlorination, however, only the first two compounds are isomers. The fate and disposition of congeners and isomers of other contaminants may provide insight regarding that of PFA and precursor isomers and vice versa. A brief description follows, highlighting current findings in emerging pollutants, polybrominated diphenyl ether congeners, Dechlorane Plus and alkylphenol ether isomers as well as the legacy pollutant polychlorinated bornanes (toxaphene).

1.5.1 Polybrominated Diphenyl Ethers (PBDEs)

PBDEs are additives in flame retardants and have been used in the treatment of textiles, furniture, computers and electronics. Three formulations of PBDEs were sold, penta-, octa-, or deca-BDE, with the prefix referring to the average number of bromine atoms per PBDE (Fig. 1.9)[194]. Analysis of PBDE congener patterns in the environment has garnered information regarding the fate and transport of this group of compounds. Soils collected from UK and Norway revealed a PBDE congener pattern resembling that of the technical penta-BDE formulation, suggesting resistance to environmental degradation[195]. Based on analysis of soils along a latitudinal gradient it was determined that less-brominated congeners were enriched in areas further from source regions, implying diminished transport efficiency of the more brominated congeners[195]. Similarly, in PBDE congener profiles of Arctic organisms such as polar bears and glaucous gulls, BDE-47 (2,2',4,4'-tetra PBDE) dominated followed by BDE-99 (2,2',4,4',5-penta BDE) with very little (<0.1-1%) BDE-209 (2,2',3,3',4,4',5,5',6,6'-deca BDE)[196]. Schecter et al. determined that BDE-209 was the most prevalent congener in computer wipe and vacuum dust samples[197], however human milk and blood contain predominantly BDE-47, BDE-99 and BDE-153 (2,2',4,4',5,5'-hexa BDE). This suggested food containing tetra, penta, and hexa-brominated BDEs was potentially a more dominant source of exposure to humans. This assumes PBDEs are environmentally stable and that congeners have similar accumulation potential. However, there is evidence of biologically and photochemically mediated debromination of PBDEs forming PBDE metabolites with fewer bromine atoms than the parent compound[198-203]. In two separate studies, both photodegradation[203] and metabolism of BDE-209[198] yielded a series of octa-brominated BDEs. This suggested BDE-209 exposure could be detected based on the presence of these octa-brominated BDEs. In addition, some PBDE congeners can undergo metabolic transformation to hydroxylated and methoxylated analogs[196,204]. The fate of hydroxylated PBDE metabolites requires some

attention as conjugation or further reactions could promote preferential excretion. Transformation of PBDEs by biotic and abiotic reactions occurring in the natural environment may be an obstacle in interpreting congener patterns for source elucidation.

1.5.2 Polychlorinated bornanes (Toxaphene)

Toxaphene was one of the most widely applied chlorinated insecticides used globally during the 1970s. Technical toxaphene is mainly a mixture of hexa- to deca- chlorinated bornanes (Fig. 1.9). Vapour pressures measured at 25 °C of 21 toxaphene congeners ranged from 0.22 to 7.1 mPa, with the least volatile corresponding to decachlorobornane[205]. More than 1000 polychlorinated bornanes have been resolved by GC in technical toxaphene[206]. Global dispersion of toxaphene has occurred largely by atmospheric transport[207,208]. In addition, toxaphene is recalcitrant with high bioaccumulation potential[209]. The toxaphene congener profile in environmental samples reflects changes in composition due to environmental reactions and properties that affect transport. Compared to technical toxaphene, air samples in both remote and temperate regions are enriched in the less chlorinated bornanes whereas agricultural soils are dominated by octa-chlorinated bornanes[208,210]. Vapour pressure was inversely related to the number of chlorinated atoms per congener, as expected but also to the substitution pattern within a homolog. For example, within the heptachlorinated bornanes, those with a Cl substituents on 9,9 or just 9 were more volatile than those with a 8,9 Cl substitution pattern (numbering indicated in Fig. 1.9)[205]. Amongst octa-chlorinated bornanes, 8,8 and 8,8,9 and alternating endo-exo Cl substitution all favour enhanced vapour pressure compared to 8,9 or 8,9,9 substituents[205]. Hexa-chlorinated toxaphene congeners are more prevalent in environmental samples compared to hepta-chlorinated analogs[211,212], which is likely evidence of dechlorination [213]. Research has demonstrated that the more chlorinated toxaphene congeners undergo dechlorination rapidly and that a *gem*-dichloro group on C2 (Fig 1.9) was most susceptible to dechlorination[213].

1.5.3 Dechlorane Plus

Dechlorane Plus ($C_{18}H_{12}Cl_{12}$, DP) was an additive in flame retardants used in electrical wires and cables[214]. The technical product is comprised of two stereoisomers, *syn* and *anti* configurations (*syn*-DP and *anti*-DP) in which the *anti*-DP comprises 75-80% of the technical

product[214]. The *syn*-DP isomer has cyclooctane in a U-shape with both dichlorocarbon bridges on the same side of the octane. The *anti*-DP isomer has a chair configuration for the cyclooctane and dichlorocarbon bridges on opposite sides of the cyclooctane. Both isomers are expected to be very hydrophobic as noted by the USEPA estimate of log K_{OW} = 9.3. Hoh et al. first reported the environmental presence of DP, in fish, sediment and air particulate from the Great Lakes area[214]. The stereoisomer profile of DP in air particulate and sediment cores was similar to technical DP but fish had a lower proportion of *anti*-DP compared to the abiotic samples which may be linked to bioavailability[214]. A sediment core from the centre of Lake Ontario revealed DP increasing in concentration (total *syn* + *anti*) from the mid-70s to the present with peak concentration in the late 90s[215], however, the isomer pattern was not consistent throughout the core. The isomer ratio in surface sediments was close to the technical product but with increasing depth, the proportion of *anti*-DP rose, suggesting that *anti*-DP is more environmentally stable than *syn*-DP[215]. Tomy et al. examined DP isomers in the Lake Ontario food web, including sediment[216]. All samples were enriched with *anti*-DP but to varying degrees. In fish samples, trout, smelt, alewife, and sculpin, *anti*-DP comprised ~50% of the profile whereas in benthic and pelagic invertebrates, *anti*-DP was 65 to 75% of the total DP and in sediment 85%[216]. Of the two isomers, *anti*-DP would be expected to be more susceptible to biotransformation reactions because the four interior carbons in the central cyclooctane portion of the molecule are less shielded by Cl atoms compared to the *syn*-DP configuration[214]; however, in environmental samples, *anti*-DP appears more prevalent. Bioaccumulation experiments, which could yield valuable information regarding bioavailability and also the potential for metabolism, have not yet been conducted for DP isomers. Diporeia are considered to have little metabolic capability and use mainly sediment as a food source. The difference in DP isomer profiles in diporeia and sediment suggest isomer-selective accumulation may occur with preferential retention of the *syn*-isomer. Human exposure to DP is likely given the anthropogenic usage of these compounds and in fact, DP isomers were observed in indoor dust in a recent investigation with concentrations ranging from 2 – 200 ng/g (total DP)[217]. The average % of *anti*-DP in the DP isomer pattern in house dust was 67% but with a wide range consisting of 0.1 to 95%[217]. Further research is necessary to determine factors altering the environmental DP isomer signatures.

1.5.4 Nonylphenol ethoxylates (NPEs) and nonylphenol (NP)

NPEs are a class of nonionic surfactants that were used as detergents, dispersants, emulsifiers and solubilizers in a number of industrial applications such as ink removal from paper at recycling plants[218]. NPE is a mixture of 22 isomers, comprised of linear and branched versions of the nonyl group[219]. The position of nonylphenol relative to the ethoxylate in technical NPE is mainly *para*-substituted with minor amounts of *ortho*-substituted[220]. Both aerobic and anaerobic biodegradation of NPE (Fig 1.9) proceeds via progressive shortening of the ethoxylate chain to eventually yield nonylphenol monoethoxylate (n=1) and subsequently the entirely de-ethoxylated product nonylphenol (NP)[221]. Some NP isomers may undergo further degradation by ring cleavage and oxidation of the nonyl group, however, technical NP is considered recalcitrant to biodegradation[221] because >85% of the isomers have a quaternary alpha carbon on the branched alkyl chain, rendering the nonyl chain resistant to oxidation[219]. Environmental monitoring reveals NP and monoethoxylated NPE in water and sediment from rivers near Tokyo Bay, Japan [222] and Ontario recycling plant sludge[223]. These revealed a similar NP isomer pattern to the technical mixture throughout sediment core slices representing a 40 year period[222]. This is surprising given recent research by Das and Xia in which isomer specific biotransformation of NP was observed[224]. In that paper, NP isomers in which the alpha carbon of the nonyl chain was tertiary were slowest to transform. Bulkiness of the substituents on the alpha carbon also mattered suggesting steric hindrance to be an obstacle in microbial degradation. For example when the alpha carbon substituents were –(C_3H_7), -(CH_3), and -(C_4H_9), the half life was 30 days but when the substituents were –(CH_3), -(CH_3), and -(C_6H_{13}), the half life was reduced to 15 days[224]. Structure elucidation of technical NP is necessary for future experiments because there are isomers even within these classes of NP substituents. Due to the absence of purified standards, peaks noted on NP chromatograms are typically classified by similarity in substituents based on MS fragmentation patterns but have not been specifically identified.

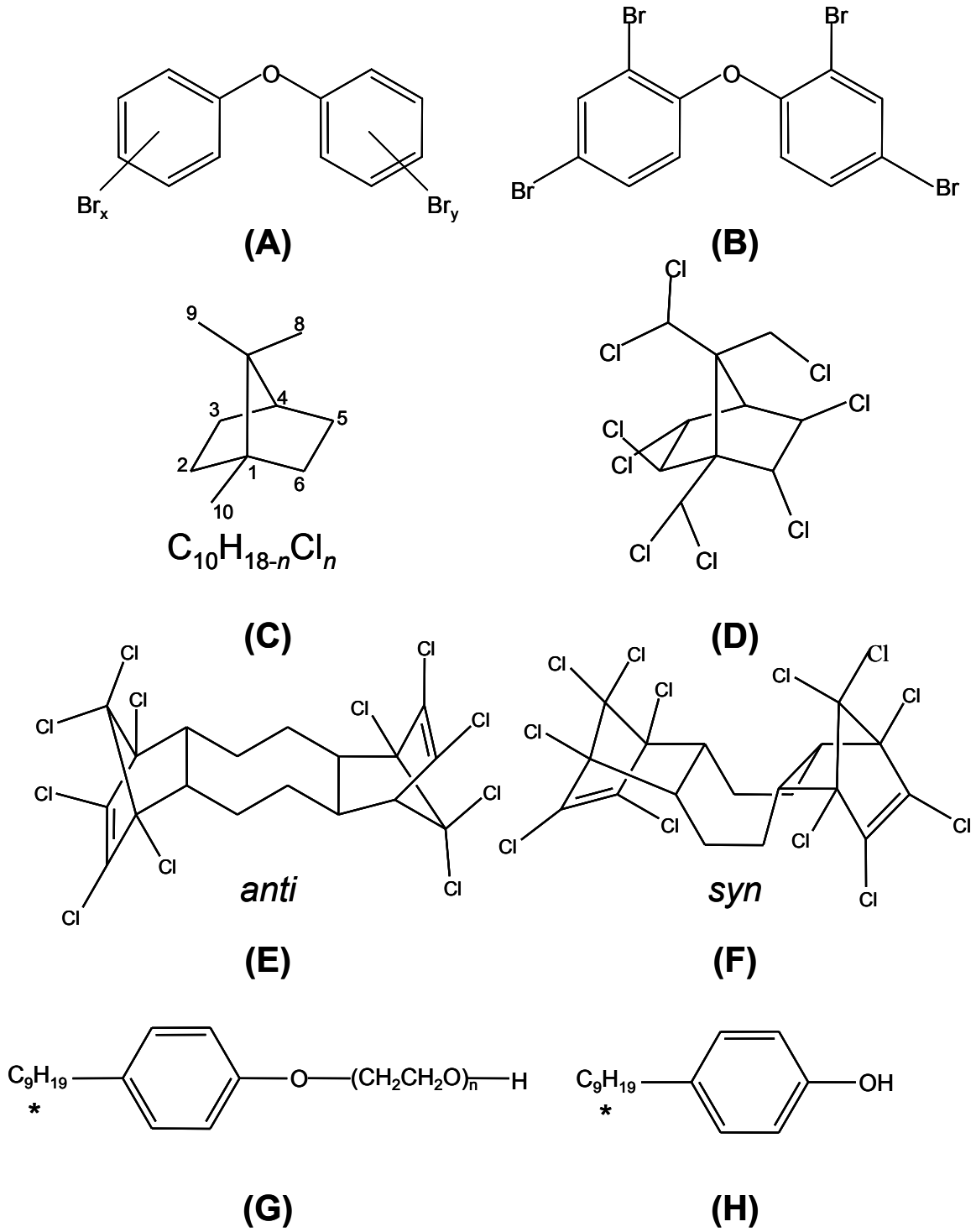

Figure 1.9. Some classes of environmental pollutants which are characteristically an isomer or congener mixture in the technical product: A) general structure of PBDE congeners, B) 2,2',4,4'-tetra BDE congener (PBDE-47), C) general structure for chlorinated bornane (toxaphene) congeners; numbering of carbon atoms is referred to in text, D) toxaphene congener B9-1679, E) *anti*-DP isomer, F) *syn*-DP isomer, G) general structure of NPE, H) general structure of NP. *In G) and H) nonyl group may be branched or linear and *para* or *ortho* to the ethoxylate.

1.6 Goals and Hypotheses

PFCAs are ubiquitously dispersed in the environment with plausible direct and indirect sources. Further complicating source elucidation are potential inputs from two industrial pathways, ECF and telomerization. Understanding the dominant source of PFCAs is pertinent for a number of reasons. Although ECF perfluorooctyl –based production has been phased out since 2002, fluorotelomer chemicals including FTOHs, FTOs, PAPs, monomers and polymers are still in production. Whether the major source of PFCAs is via exposure to fluorotelomer precursors is toxicologically relevant given the existing evidence of enhanced toxicity of fluorotelomer biological intermediates compared to PFCA end products. Some of these intermediates are also produced by abiotic reactions and regardless of abiotic or biotic origin, FTCAs and FTUCAs have apparent environmental presence. Another reason why source determination is important is because fluorotelomer products are a characteristic mixture of perfluorocarbon chain lengths. This is of concern because toxicity of FTCA and FTUCA increases with chain length and secondly, there is an enhancement of bioaccumulation potential of PFCAs as the perfluorocarbon chain lengthens. Based on environmental monitoring, the PFCA contamination is not constrained to source regions. Identification of the sources responsible for PFCA dissemination may lead to better engineering in the fluorochemical industry whereby residual emissions are reduced, stability of polymer linkages are improved, and production volumes are restricted.

Although ECF and telomerization were/are used in industrial synthesis of PFCAs and precursors, only ECF products are mixtures of structural isomers whereas telomer products are generally considered to be of a single geometry. The overall goal of this thesis was to determine whether environmental PFCA isomer analysis could be used as a tool to decipher industrial contributions.

Clearly environmental monitoring of PFCA isomers would not be sufficient to determine the relative inputs from ECF and telomer sources. A hypothesis of this thesis is that abiotic and biotic factors would alter the pattern of isomers in the environment compared to emission sources. Thus, in Chapter 2, the abiotic influence of this hypothesis was tested by investigating the physical properties of a group of isomers in technical ECF N-EtFOSE.

In order to gain an understanding of PFA isomer distribution in the environment, an analytical method was necessary. The method, a derivatization technique, was applied to PFCAs from PFOA to PFTA, with isomer resolution accomplished by GC-MS. This is presented in Chapter 3. The utility of the method was demonstrated with PFCA isomer analysis in two polar bear populations in Greenland and Canada. In addition, the hypothesis that some branched PFOA isomers would be present in these samples given the legacy of ECF PFSAm production, was tested.

In Chapter 4, PFCA isomers in human blood samples were determined. The differences in isomer profiles in humans compared to polar bears addressed contrasting sources between an apex predator residing in the Arctic versus an organism with different usage patterns from a temperate region.

Testing the hypothesis that biological handling alters the PFCA isomer profile was conducted in Chapters 5 and 6. Uptake, accumulation and elimination of PFCA isomers in rodents and fish, respectively, was explored.

Chapter 7 builds upon the environmental monitoring of PFCA isomers that was presented in Chapters 3 and 4. A diverse set of samples, both abiotic and biotic, from temperate and remote areas in North America, were analyzed for PFCA isomers. Incorporating information acquired from biotic and abiotic isomer discrimination, the results demonstrate the potential for source illumination from isomer analysis when assessing a variety of sample types.

The final chapter summarizes the findings of environmental PFCA isomer analysis and conclusions regarding the hypothesis of using isomer patterns for deciphering source. The future of isomer analysis for further source refinement is discussed. Other research directions related to this goal are also presented.

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CHAPTER TWO

Water Solubility and Octanol-Water Partition Coefficient of Perfluorooctylsulfonamides and Fluorotelomer Alcohols

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Contributions – Measurement of octanol-water partition coefficients (K_{OW}) of 4:2 FTOH, 6:2 FTOH, 8:2 FTOH, 10:2 FTOH, N-EtFOSA, and PFOSA was conducted and originally written as a report by undergraduate student Julia Bonin under the guidance of graduate student Naomi Stock . Analytical chemistry for isomer separation and determination of water solubility of N-EtFOSE isomers was by undergraduate student Grace Wong with assistance of Amila De Silva and was originally written as a report by G. Wong. K_{OW} measurements of N-EtFOSE isomers and 10:2 FTOH was by A. De Silva. Preparation of the manuscript was by A. De Silva and involved adaptation of reports by G. Wong and J. Bonin as well as inclusion of editorial comments by N. Stock and C. Young. The manuscript and research herein was conducted under the supervision and direction of Scott Mabury.

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2.1 Introduction

Polyfluorooctylsulfonamides ($C_xF_{2x+1}SO_2NRR'$, PFSAm) and fluorotelomer alcohols ($F(CF_2)_yCH_2CH_2OH$, y = 2,4,6,…18, y:2 FTOH) are two classes of compounds known to yield perfluorinated acids, including perfluorocarboxylates ($C_zF_{2z+1}C(O)O^-$, PFCAs) via environmental reactions. PFSAm and FTOHs (Table 2.1) are believed to have contributed to the environmental burden of PFCAs based on their tropospheric detection in remote[1-3] and temperate[4,5] regions and their historic industrial production. This is of interest because PFCAs are recalcitrant compounds that have been globally detected in sediment, precipitation, water, biota, ice and air[6-10].

From the 1960s until 2002, octyl-PFSAm, such as N-ethyl perfluorooctanesulfonamidoethanol (R =CH_2CH_3, R' = CH_2CH_2OH, N-EtFOSE) and N-methyl perfluorooctanesulfonamidoethanol (R = CH_3, R' = CH_2CH_2OH, N-MeFOSE), were industrially synthesized in large quantities for use in surface protectants[11]. N-EtFOSE was targeted to be chemically bound as phosphate esters in a surfactant formulation intended for use in paper and packaging applications[11], however, the commercial product has been known to contain residual quantities of the free PFSAm[12]. N-MeFOSE was used to treat carpets, upholstery and textiles for water and oil repellency[11].

Use of these products may contribute to the presence of PFSAm in indoor air and dust[13]. Furthermore, N-EtFOSE phosphate ester surfactants were used to treat food-contact paper, which could lead to ingestion of PFSAm[11]. Human exposure to PFSAm is supported by the detection of PFSAm metabolites, including N-methyl perfluorooctanesulfonamidoacetate ($C_8F_{17}SO_2N(CH_3)CH_2COO$-), N-ethyl perfluorooctanesulfonamidoacetate ($C_8F_{17}SO_2N(CH_2CH_3)CH_2COO$-), perfluorooctanesulfonamidoacetate ($C_8F_{17}SO_2N(H)CH_2COO$-), and perfluorooctane sulfonate ($C_8F_{17}SO_3$, PFOS) in blood monitoring studies from the general human population[14]. PFSAms are considered semi-volatile and their detection in remote environments[1-3] far from production sources and commercial use is evidence of their long range transport potential.

Through atmospheric oxidation, perfluorooctyl PFSAm form PFCAs, ranging from perfluorooctanoate ($C_7F_{15}COO$, PFOA) and shorter chain lengths down to trifluorooacetic

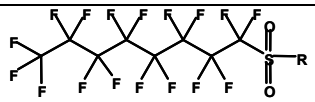
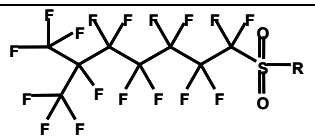
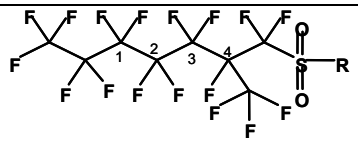
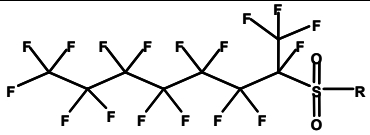
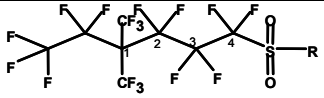
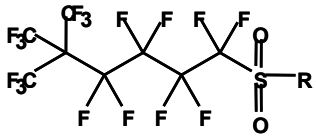
acid[15,16]. PFSAm produce perfluoroalkylsulfonates ($C_xF_{2x+1}SO_3$-) both abiotically[16] and biotically[17,18] (i.e. PFOS is the reaction product of perfluorooctyl-based PFSAm). Because neither perfluoroalkylsulfonates nor PFCAs undergo environmental reactions, they are regarded as highly persistent.

Historical PFSAm production was predominated by electrochemical fluorination (ECF). As with other ECF products, PFSAm are a characteristic mixture of linear and branched isomers, varying in the carbon arrangement of the perfluoroalkyl tail (Table 2.1). Presumably PFOS and PFSAm are comprised of the same isomer profiles because both are derivatives of ECF perfluorooctanesulfonyl fluoride.

Akin to PFSAm, FTOHs were historically, and are currently, manufactured for their use as intermediates in formulations for surface protection, inks, paint, polymers, adhesives, polish, wax and caulk. PFCAs are the terminal products of FTOH microbial degradation, rodent metabolism, indirect photolysis and atmospheric oxidation [19-23]. Research has indicated FTOHs ranging from 6:2 to 12:2 were residual unreacted components in some fluorotelomer products such as polymers and phosphate surfactants[12]. FTOHs are synthesized by telomerization, which unlike ECF products, are generally understood to have their perfluorinated carbons in a linear/normal arrangement[24]. However, in the telomerization reaction, products maintain the geometry of the starting material and as such, branched versions of FTOHs are possible if a branched perfluoroalkyl iodide is employed as a reagent telogen.

The transport and fate of FTOHs, PFSAm and PFCAs are dependent in part, upon their physical properties. For these PFCA precursors, the most widely measured physical property is vapour pressure and air partitioning coefficients but there is a lack of information regarding their octanol-water partition coefficients (K_{OW})[25-29]. K_{OW} and water solubility (C^{sat}_w) may permit inferences of relative residence times in biota, sorption to organic matter such as soil or sediment, and likelihood of aquatic transport. The objective of this study was to determine the K_{OW} of a variety of FTOHs and PFSAm. By studying a homologous series of FTOHs with increasing number of perfluorocarbons, the influence of perfluorinated chain length can be derived. Furthermore, the influence of perfluoroalkyl geometry was investigated by focusing upon the ECF isomers of N-EtFOSE.

Table 2.1 Perfluoroctylsulfonamide structures and Hyperchem molar volume estimates

Name (Abbreviation)	Structure	% in ECF standard by weight[30]	Estimated molar volume* cm^3/mol
Perfluorooctanesulfonamide (PFOSA)	$C_8F_{17}SO_2NH_2$	N/A**	483
N-ethyl perfluorooctanesulfonamide (N-EtFOSA)	$C_8F_{17}SO_2N(CH_2CH_3)H$	N/A**	548
N-EtFOSE R = $N(CH_2CH_3)(CH_2CH_2OH)$	 *n*-isomer	70	605
N-EtFOSE R = $N(CH_2CH_3)(CH_2CH_2OH)$	 isopropyl isomer (*iso*)	11	603
N-EtFOSE R = $N(CH_2CH_3)(CH_2CH_2OH)$	 Internal monomethyl isomer, methyl branch can be on carbons 1 through 4	17	599 (branch on C1) 599 (branch on C2) 598 (branch on C3) 588 (branch on C4)
N-EtFOSE R = $N(CH_2CH_3)(CH_2CH_2OH)$	 alpha methyl branch isomer	1.6	595
N-EtFOSE R = $N(CH_2CH_3)(CH_2CH_2OH)$	 gem-dimethyl branch isomer, dimethyl branch can be on carbons 1 through 4	0.13	582 (when dimethyl branch is on carbon labeled 1)
N-EtFOSE R = $N(CH_2CH_3)(CH_2CH_2OH)$	 *t*-butyl isomer	0.23	589

** data not available

2.2 Materials and Methods

2.2.1 Chemicals and Sampling Media.

The following were purchased from Oakwood Research Chemicals (West Columbia, SC) 4:2, 6:2, 8:2 and 10:2 FTOHs (all 97%). N-EtFOSA (95%) was from Lancaster (Pelham, NH) and PFOSA (97%) from Synquest (Alachua, FL). Technical ECF N-EtFOSE was obtained from 3M (St. Paul, MN). Ethyl acetate and acetone of highest grade (>99.8 %) was acquired from VWR International. HPLC grade *n*-octanol (99+%) and borosilicate solid glass beads (1 mm diameter) were from Sigma-Aldrich (Oakville, ON). HPLC grade methyl *tert*-butyl ether (MTBE) and reagent grade methanol and dichloromethane were obtained from Fisher Scientific (Ottawa, ON).

2.2.2 Slow-stirring K_{OW} Determination of 4:2, 6:2, 8:2 FTOH, 10:2 FTOH, PFOSA and N-EtFOSA.

The K_{OW} slow-stirring method was originally developed by Brooke et al.[31]. Reaction vessels consisted of a Pyrex glass flask, approximately 1 L capacity, with a tapered top and a tap located 2 cm from the bottom. Both the top and bottom tap were sealed with Mininert caps (Mandel, Guelph, ON) allowing for octanol sampling in an airtight environment via syringe. Water samples were taken from a tap 2 cm from the bottom of the vessel. Initially 970 ml of Millipore 18 MΩ water was added into the reaction vessel along with a Teflon coated stir bar. This was followed by the careful addition of 20 to 50 ml of water-saturated *n*-octanol solutions of the test compounds (12-50 mg/ml). The mixture was then stirred at 200 rpm for 4 days, which was verified as necessary to establish equilibrium. Both octanol and aqueous phases were sampled in triplicate. Aliquots of octanol (2 µl) were diluted in 10 ml of MTBE. Water aliquots (5 ml) extracted as follows: sodium chloride (approximately 0.2 g) and MTBE (5 ml) were added to each sample and the mixture was shaken. After centrifugation, the MTBE layer was collected. This was repeated using an additional 5 ml of MTBE to consolidate both MTBE layers. The extract was concentrated under a gentle stream of nitrogen to 1 ml. The extracted aqueous phase and diluted octanol phase were then analyzed using gas chromatography (GC). Performance of the methodology was assessed by measuring the K_{OW} of 1,2-dichlorobenzene (1,2-DCBz) and comparing to literature values.

The generator column method[32], was also used to determine K_{OW} of 8:2 FTOH for comparison to slow-stirring results. The glass generator column was 45 cm in length with a 1 cm internal diameter and packed with borosilicate glass beads. The column was temperature regulated at 25.0 ± 0.1 °C by pumping water from a constant-temperate bath through a jacket enclosing the column. Approximately 2 ml of a solution of 8:2 FTOH (50 mg/ml in *n*-octanol) was slowly poured into the clean, dry generator column until saturation. A separatory funnel was used to introduce water drop-wise into the generator column. Eluting aqueous samples were collected at a rate of 1 ml/min at the exit end of the column and extracted as above for further GC analysis.

2.2.3 Slow stirring-K_{OW} Determination of N-EtFOSE Isomers and 10:2 FTOH.

Closed vessels consisted of polypropylene centrifuge tubes with the tapered end removed to form a 12 ml hollow tube, 1.3 cm in diameter. Each end was capped with rubber septa to permit sampling via syringe. Approximately 2.38 mg of N-EtFOSE isomer mixture (or 5-7 mg 10:2 FTOH) was combined with equal amounts (6 ml each) of water-saturated *n*-octanol and 18 MΩ water in the closed vessel. Headspace was eliminated from the vessel by ensuring complete occupation of space by *n*-octanol and water. The system was stirred on a magnetic stir plate using a Teflon coated stir bar for 7 days to achieve equilibrium at room temperature (23 ± 1 °C), as judged by determining K_{OW} at 7, 21, and 28 days. Stirring speed was adjusted to create a vortex height of no more than 1 cm at the interface of the two phases. Accurately measured 0.10 to 0.15 ml of the upper octanol phase was sub-sampled and diluted to 50 ml with ethyl acetate. Of the lower water phase, 5 to 5.5 ml was collected. Within 1 cm of the interface between both layers was avoided when sampling either phase. The aqueous subsample was extracted with ethyl acetate (0.5 ml x 2) after the addition of NaCl (1 g). Anhydrous sodium sulfate was introduced to the ethyl acetate extract to remove any residual water. Our ability to accurately determine K_{OW} was supported by measuring K_{OW} of two compounds with literature reported values, atrazine (ATR) and hexachlorobenzene (HCBz). Gas chromatography – mass spectrometry (GC-MS) was employed for N-EtFOSE isomer analysis and the two test compounds as described below. This volume of this system was 500 times smaller than the system described in part I. Use of two systems did not have any apparent effect on the outcome of K_{OW} determination based on the overlapping values for 10:2 FTOH in both systems.

2.2.4 Saturated Water Solubility (C^{sat}_w)of N-EtFOSE Isomers Using the Generator Column Method.

The generator column method for determining C^{sat}_w was originally developed by May et al.[33] using a liquid chromatography column. The apparatus used in this experiment was based on the same principle as the set up by May et al. and was adapted from a similar investigation by Gauthier[34]. Approximately 125 g of glass beads were cleaned and sonicated with hexanes, dichloromethane, and then methanol and dried for twelve hours at 120 °C. The beads were then immersed in technical N-EtFOSE isomer mixture dissolved in acetone (28.50 mg in 50 ml acetone). After permitting complete evaporation of acetone (3 hours at room temperature), the N-EtFOSE coated beads were packed into a glass buret (108 cm length, 1.5 cm diameter). Temperature was controlled using the water circulator connected to the glass jacket surrounding the column. A separatory funnel connected to the top of the buret was used to introduce a slow flow of water, controlled by opening the stopcock at the base of the buret. After discarding the initial 100 ml, consecutive 10 ml fractions of water eluting from the column at a flow rate of approximately 1 ml/min were collected. Five collections were performed each at 15, 20, 25, and 30 °C, after allowing 1 hour equilibration. N-EtFOSE was extracted from the collected water fractions by performing a liquid-liquid extraction with ethyl acetate as follows. Sodium chloride was added to each fraction (approximately 0.5 g) and then two consecutive 3 ml volumes of ethyl acetate were shaken with the aqueous phase. After centrifuging and isolating the ethyl acetate layer, anhydrous sodium sulfate was added to trap any residual water. GC-MS was employed for instrumental analysis.

2.2.5 Analysis of 6:2, 8:2 and 10:2 FTOH, PFOSA, N-EtFOSA, and 1,2-DCBz.

Analytes were separated on a DB-35 column (30 m x 0.25 mm x 0.5 μm film thickness) (Phenomenex, CA) using a Hewlett Packard 5890 series 2 GC equipped with an electron capture detector (ECD). The oven program for the FTOHs began with an initial temperature of 60 °C and held for 2 min, followed by ramps of 10 °C/min to 95 °C, 30 °C/min to 150 °C and 40°C/min to 230 °C. The oven program for N-EtFOSA began at a 60 °C hold for 2 min, followed by ramps of 10 °C/min to 160 °C, held for 1 min and finally 40 °C/min to 240 °C. The PFOSA oven program began at 60 °C followed by a 10 °C/min ramp to 95 °C and then 30 °C/min to 230 °C. The oven program for 1,2-dichlorobenzene started with a 2 min hold at 50 °C and ramped to

150 °C at a rate of of 15 °C/min and 40 °C /min to 230 °C. Pressure for all oven programs was held constant at 5 psi using H_2 gas and injection volumes were 1 µl. Analytes were identified based on comparison to retention times of authentic standards.

2.2.6 Analysis of 4:2 FTOH.

Analysis of 4:2 FTOH was carried out on an Agilent Technologies 6890N GC and a 5973 inert MS using a DB-WAX column (30 m x 0.25 mm x 0.25 µm film thickness). The oven program employed was that used for 8:2 FTOH. The system was operated in positive chemical ionization mode using methane reagent gas. Ions 227 and 265 m/z were monitored with quantification using 265 m/z.

2.2.7 Analysis of N-EtFOSE Isomers.

N-EtFOSE isomers were separated on a 60 m DB-WAX (0.25 mm ID x 0.25 µm FT, Phenomenex, Torrance, CA) using GC-(EI)MS (system described above in V.) . The oven program began at 70 °C and held for 1 min, was ramped to 173 °C at 30 °C/min and then to 177 °C by 0.5 °C/min, and finally 20 °C/min to 225 °C at which point the temperature was held for 5 min. Samples and standards were injected into the 200 °C inlet using 1 µl volume and the He flow rate was constant at 1.3 ml/min. Ions 448, 462, 526, 540 m/z were monitored and quantification was done using 540 m/z.

2.2.8 Analysis of Atrazine (ATR) and Hexachlorobenzene (HCBz).

HCBz and ATR were analysed by GC-(EI)MS (system described above in V.) using a 15 m DB-5 column (0.25 mm ID, 0.25 µm film thickness, Restek, Bellefonte, PA) with an oven program as follows: the initial temperature was set to 70 °C and held for 1 min, followed by 30 °C/min ramp to 175 °C, 10 °C/min ramp to 240 °C with a hold of 5 min. He carrier gas flow rate was set to 1 ml/min, injector temperature at 240 °C and transfer line at 250 °C. Ions monitored for HCBz were 142, 249, 282, and 286 m/z (quantified with 286 m/z) and for ATR 173, 200 and 215 m/z (quantified with 200 m/z).

2.2.9 Computer Modeling

HyperChem (HyperChem 7.52, Hypercube Inc., Gainesville, FL, USA) modeling software was used to estimate the molar volume of different N-EtFOSE isomers in the ECF N-EtFOSE standard, N-EtFOSA, PFOSA and the FTOHs.

2.3 Results and Discussion

Chromatographic separation of N-EtFOSE isomers revealed differential interaction with the stationary phase (Fig. 2.1 A), suggesting differences in physical properties of the isomers. However, a polar stationary phase was used and therefore retention time differences may not be solely based on vapour pressure. Nine prominent peaks were observed on the ECF N-EtFOSE chromatogram. Each was believed to correspond to N-EtFOSE based on the characteristic mass fragments obtained with EI. The dominant peak presumably corresponded to the *n*-isomer constituting 79% of the isomer mixture. The peak labeled '6' was tentatively designated the isopropyl isomer based on relative retention and proportion (9% of total peak area). This inference was re-inforced by comparison to an ECF PFOA (derivatized) chromatogram and an *iso*-PFOA standard on the same stationary phase.

2.3.1 Water Solubility (C^{sat}_{w}) of N-EtFOSE

At temperatures ranging from 15 °C to 30 °C, C^{sat}_{w} of the *n*-isomer of N-EtFOSE isomer was investigated (Table 2.2). At least 5 replicates were collected in order to assess precision as denoted by standard error of mean (S.E.M., standard deviation/(number of samples)$^{1/2}$). A linear relationship (r^2 0.9700) between log (C^{sat}_{w}) and inverse temperature (expressed in K^{-1}) was observed and from the slope of the line, the enthalpy of solution was calculated to be 23.35 kJ/mol (standard error ± 2.9 kJ/mol). Over the 15 °C range, the C^{sat}_{w} increased by 46 µg/l. The C^{sat}_{w} observed here for the *n*-isomer of N-EtFOSE is similar to that of some tri- and tetra-chlorinated biphenyls (PCBs)[35] as well as that of the 8:2 FTOH based on two literature values ranging from 137[28] to 194[36] µg/l at 25 and 22 ºC, respectively.

Table 2.2 C^{sat}_w of N-EtFOSE *n*-isomer (from ECF mixture) at different temperatures, mean ± standard error of the mean (S.E.M.), N=5.

Temperature (°C)	Mean (μg/l)	S.E.M. (μg/l)
15.0	77.3	2.2
20.0	83.9	2.9
25.0	104.5	1.3
30.0	123.0	3.2

The C^{sat}_w of the N-EtFOSE *n*-isomer was determined using the ECF isomer mixture. Based on chromatographic peak area, the linear isomer constitutes approximately 79%, assuming detector response of isomers is equivalent (Fig. 2.1 A). The isomer profile in the saturated water eluant from the generator column was compared to original standard, for a sense of relative C^{sat}_w of the branched isomers. Aqueous collections from the generator column were enriched in branched isomers compared to the composition of the original standard (Fig. 2.1 B). In fact, many of the branched N-EtFOSE isomers that were minor components in the standard were noticeably higher in the water fractions (Fig. 2.1 B).

Previously, van Haelst et al. found C^{sat}_w to be inaccurate for some tetrachlorobenzyltoluene isomers and accurate for others when determined using a technical isomer mixture compared to using the individual isomer components[37]. One theory is that the mixture can result in changes to the water network[38]. The apparent solubility of a component in a mixture compared to the measurement obtained using the individual compound can be enhanced, depressed or have no effect[38]. Thus caution in interpreting apparent solubility is advised.

One interpretation of Fig. 2.1 B is based on comparing the isomer profile of the technical mixture to that of the saturated aqueous extract, similar to the approach employed by Sokol et al. for PCBs[39]. Enrichment of the branched isomers in the aqueous collection compared to technical N-EtFOSE could suggest that branched isomers are apparently more water soluble than the *n*-isomer. This trend was previously observed for isomers of PFOS[40]. PFOS and perfluorooctyl PFSAm originate from perfluorooctylsulfonyl fluoride ($C_8F_{17}SO_2F$) which is generated by subjecting *n*-octylsulfonyl fluoride ($C_8H_{17}SO_2F$) to ECF. It is this initial step that

produces the mixture of isomers. The ECF perfluorooctylsulfonyl fluoride isomer mixture is then reacted to form PFOS or PFSAm which consequently contain the same isomer pattern as the ECF perfluorooctylsulfonyl fluoride. A comparison between isomer profiles of perfluorooctanesulfonyl fluoride and PFOS revealed a higher percentage of branched isomers in perfluorooctanesulfonyl fluoride[41]. ECF perfluorooctanesulfonyl fluoride was comprised of 26%- 30 % branched isomers and ECF PFOS, 18 – 24 % branched isomers, which the authors attributed to selective removal of the branched isomers during aqueous washing steps of the purification process[41].

The molecular volume of a compound can describe, in part, the ability of water to accommodate the compound as it relates to size. To this end, molar volume has served as a predictor for ranking the hydrophobicity of a series of structurally similar compounds such as polyaromatic hydrocarbons (PAHs), whereby the larger the molecular volume, the lower the C^{sat}_{w} [42,43]. HyperChem software was used to estimate molecular volume of the N-EtFOSE isomers (Table 2.1). The molar volumes obtained with Hyperchem were plotted against those calculated using a modified McGowan equation[29,44]. The McGowan method is a fragment-based method by which tabulated atomic volumes are added[44]. Using density measurements with FTOHs and other fluorinated compounds, Goss et al. noted that the McGowan method under-predicted molar volume of fluorinated organics and introduced an empirically-derived molar volume corresponding to the fluorine fragment (12.48 cm^3/mol) to improve accuracy when employing the McGowan equation[29]. Excellent linear correlation (r^2 0.9987) was observed but also a systematic bias between our Hyperchem modeled molar volumes and those obtained with the modified McGowan equation (Appendix A). Although the absolute values of molar volume for N-EtFOSE may not be accurate, the trends amongst the Hyperchem predicted values provide a basis to investigate the role of size on C^{sat}_{w}. Since the McGowan equation is based on summation of atomic molar volume, it does not distinguish between different molecules which share the same molecular formula. Hence, it was not used in this experiment beyond the purpose of verifying the Hyperchem modeled volumes.

As expected, for every branched N-EtFOSE isomer modeled, the estimated molar volume was less than that of the *n*-isomer. Many of the branched isomers that constituted a minor portion of the ECF mixture, such as the *gem*-dimethyl and alpha branched isomers, were largely enriched in C^{sat}_{w} measurements and were found to have the smallest molar volumes. This is

consistent with the increase in relative concentration of the branched isomers compared to that of the *n*-isomer in aqueous eluant (Fig. 2.1 B). Of the branched isomers, the isopropyl isomer was the least different from *n*-isomer in molar volume. Perhaps correlated, isopropyl isomer was also the least enriched in the aqueous fraction.

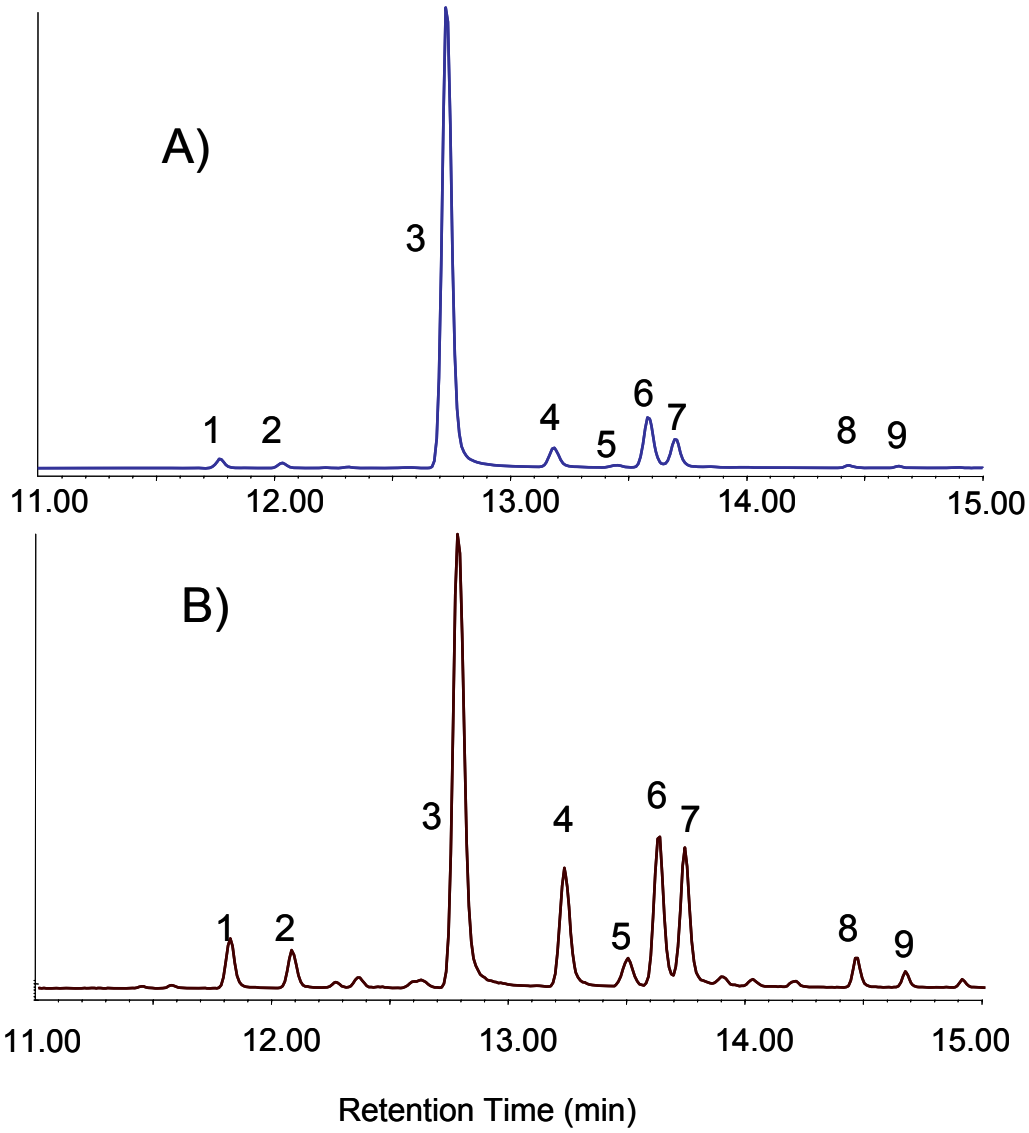


Figure 2.1. Comparison of N-EtFOSE isomer profile in **A)** ECF mixture and **B)** saturated water extract from generator column in C^{sat}_{w} experiment at 25 °C. Peak 3 is *n*-isomer and represents 79% peak area in A).

2.3.2 K_{OW} of FTOH and PFSAm.

The slow-stirring method[31] was used for determination of K_{OW} of the FTOHs and PFSAm. This technique is considered advantageous over the shake-flask method because of the avoidance of emulsion formation during shaking[31,45]. Contamination of the aqueous phase with octanol and under-estimation of K_{OW} are thereby minimized[45]. The generator column technique also circumvents this obstacle but involves a more cumbersome set up and does not permit sampling of the octanol phase at equilibrium[32]. Two slow-stir systems were used to measure K_{OW}: one for FTOHs, N-EtFOSA, and PFOSA and another for N-EtFOSE isomers. Our ability to use the methods with good performance were assessed by using the experimental setup to measure K_{OW} of HCBz, ATR and 1,2-DCBz was compared to literature values (Table 2.3). The values reported here are in good agreement with literature values. Additionally, K_{OW} for the 8:2 FTOH determined using the generator column technique was consistent with that obtained via the slow-stirring technique (Table 2.4).

Table 2.3. Experimental K_{OW} of test compounds, 1,2-dichlorobenzene, hexachlorobenzene, and atrazine.

Test Compound	log K_{OW}		
	This Research (mean ± standard deviation)	**Literature**	**Ref.**
1,2-DCBz	3.31 ± 0.09 N = 12	3.43 ± 0.02 (slow stir)	[46]
HCBz	5.64 ± 0.09 N = 3	5.24 to 5.93 (slow stir) 5.67 to 5.78 (slow stir) 5.73 (slow stir)	[45] [47] [46]
ATR	2.72 ± 0.02 N = 3	2.63 ± 0.07 (shake flask) 2.47 ± 0.15 (reversed phase HPLC)	[48]

Based on 8:2 FTOH measurements equilibrium was achieved at day 4 and hence sampling of the other FTOHs, N-EtFOSA and PFOSA was conducted after a 4-day equilibration period. For N-EtFOSE isomers, 7 days were allotted for equilibration because K_{OW} found on day 7 was not significantly different than on days 21 and 28. Initially the N-EtFOSE K_{OW} experiment was conducted with 160 μg of ECF N-EtFOSE but due to the relatively high K_{OW} of N-EtFOSE, only the *n*-isomer could be detected in the water phase. Therefore to determine K_{OW} of nine N-EtFOSE isomers, 2.4 mg of N-EtFOSE was used in the slow-stirring vessel. Both high and low treatments yielded K_{OW} values for the *n*-isomer with no distinguishable difference within the experimental error.

The log K_{OW} values for all of the fluorinated compounds tested in this study are presented in Table 2.4. With increasing perfluoroalkyl chain length of FTOH, there was an increase in log K_{OW}, reflecting enhanced hydrophobicity with size. Each additional CF_2 unit in the FTOHs, resulted in an increase in log K_{OW} by 0.65 log units. This was similar to the 0.60 log unit contribution per CF_2 moiety for water-humic acid partition coefficients of FTOHs reported by Goss et al.[29], 0.5 to 0.6 log units increase in sediment sorption coefficients per CF_2 unit reported by Higgins and Luthy for perfluorinated acids[49]. Liu and Lee observed a slightly larger contribution where aqueous solubility decreased by 0.78 log units per additional CF_2 unit in FTOHs[50]. Martin et al. observed an increase in the bioconcentration factor (BCF) by 0.78 – 1.0 log units with the addition of each CF_2 for a series of PFCAs[51]. Increasing K_{OW} was demonstrated in the series of PFOSA, N-EtFOSA, and N-EtFOSE which may be attributed to increasing size.

The results in Table 2.4 are similar to those generated by Arp et al. using COSMOtherm commercial software, which accounted for modeled 3D conformers for each compound[27]. In that study, predicted values of log K_{OW} were 2.31 (2.17 to 2.43) for 4:2 FTOH , 3.32 (3.22 to 3.49) for 6:2 FTOH, 4.31 (4.17 – 4.55) for 8:2 FTOH, 4.35 for PFOSA, 5.49 for N-EtFOSA, and 5.39 (5.32 – 5.51) for N-EtFOSE[27]. Arp et al. reported a higher K_{ow} via COSMOtherm for the stretched conformation of FTOHs than for a conformation containing an electrostatic intramolecular interaction between a hydroxyl proton and fluorine atom[27]. For example, log K_{ow} of the stretched 8:2 FTOH conformer was 4.55 and 4.37 for the 8:2 FTOH conformer possessing an intramolecular H-F interaction[27]. Arp et al. postulated that the hydroxyl proton within an H-F intramolecular interaction can still undergo intermolecular H-bonding based on

surface charge density modeling[27]. The results are unexpected given that intramolecular interactions are typically considered to enhance hydrophobicity by hindering the ability of a heteroatom from H-bonding to water molecules. For example, calculations of log K_{OW} using the Hansch and Leo fragment-based method, intramolecular H-bond with an oxygen atom are assigned a contribution of +1.0 to log K_{OW}[52].

Prediction of K_{OW} may be performed using available C^{sat}_{w} values and linear free energy relationships (LFER). One such common LFER introduced by Isnard and Lambert corresponded to log K_{OW} = 4.62-0.72* logC^{sat}_{w} (in mg/L) which using a data set of 300 compounds, produced excellent linear correlation (r^2 0.93)[53]. Liu and Lee recently reported C^{sat}_{w} for 4:2, 6:2, 8:2, and 10:2 FTOHs corresponding to 974, 18.8, 0.194, and 0.011 mg/L at 22.5 °C[50]. Application of these values and our C^{sat}_{w} measurement for N-EtFOSE in the LFER estimates log K_{OW} to be 5.31, 2.45, 3.68, 5.11, and 6.01 for N-EtFOSE, 4:2 FTOH, 6:2 FTOH, 8:2 FTOH, and 10:2 FTOH. With the exception of 10:2 FTOH, these predicted log K_{OW} demonstrated consistent trends (r^2 0.9979) with our measurements but the LFER estimated values were approximately 1.15 times greater than our measured values (residual was< 0.5 log units). It should be noted, however, that Isnard and Lambert did not use any fluorinated chemicals to develop their LFER[53]. The discrepancy between K_{OW} estimates for 10:2 FTOH with measured values is discussed in the following paragraphs.

Although we observed an increasing trend of K_{ow} with perfluoroalkyl chain length, it is likely that the trend would begin to deteriorate at a certain chain length. K_{OW} is the ratio of the activity coefficients of a chemical in water (γ_w) and *n*-octanol (γ_o). For most organic solutes, γ_w is highly variable, ranging from 0.1 to 10^{10}[54] but *n*-octanol, is a less discriminating solvent and thus, γ_o for the majority of organic solutes is in the range of 1 and 10^2[54]. As such, K_{OW} is typically dominated by γ_w and because γ_w describes nonideal behaviour of a chemical in an aqueous medium, K_{OW} is considered a measure of hydrophobicity. Fluorinated organics including PFSAm and FTOHs are unique in that they are both hydrophobic and oleophobic. It is thus reasonable to assume that γ_o would be much larger for FTOHs and PFSAm than for typical nonfluorinated organic compounds and also that both γ_w and γ_o would increase with lengthening perfluoroalkyl moiety. Therefore, unlike typical organic solutes, it is probable that the magnitude of both γ_w and γ_o are significant to the overall K_{OW}. At some chain length, we expect

that the water and octanol repellency of the fluorinated chemical could be great enough that instead of partitioning into either phase, the fluorinated molecules would arrange to form a third phase at the interface. This could lead to low estimates of K_{OW} due to diminished amounts of chemical in the bulk octanol and aqueous phases.

The measurement for 10:2 FTOH was consistent even though contrasting experimental setups described in Section 2.2.2 and 2.2.3 in which the K_{OW} vessel consisted of a 1 L Pyrex flask or a 12 mL polypropylene tube. Based on twelve measurements, log K_{OW} for 10:2 FTOH was 2.91±0.50. This value was surprisingly low and similar in magnitude to 6:2 FTOH. One explanation is that 10:2 FTOH readily forms micelles enhancing its ability to partition into the water phase. This theory was not supported by the mass balance calculations of the bulk octanol and aqueous phases. The mass of 10:2 FTOH recovered in the octanol phase was approximately 300-fold greater than that in the aqueous phase. Taken together, the 10:2 FTOH mass recovered from both octanol and aqueous phases only accounted for 31 to 43% of the 5 to 7 mg of 10:2 FTOH originally inserted into the vessels. These findings suggested that the unrecovered 10:2 FTOH may reside at the interface. This would be consistent with the hypothesis of hindered partitioning into octanol due to excessive γ_o. Calculations of γ_o using measured K_{OW} for the FTOH series corresponded to 160, 540, 1800, and 13000000 for 4:2, 6:2, 8:2 and 10:2, respectively (calculation in Appendix A). Delicate sampling or spectroscopic analysis of the interface would be useful to assess whether the interface is a site of 10:2 FTOH accumulation.

A linear relationship between the molar volume estimated using HyperChem and the experimental log K_{OW} was observed (Fig. 2.2). Both PFOSA and 8:2 FTOH have similar molar volumes but very different log K_{OW}, which may suggest that FTOHs should be considered separately from PFSAm when developing predictive models for K_{OW}. Further measurements for a larger test set are warranted to build a model with accurate predictive capability and also to determine the chain length at which the trend does not hold. Some candidates are N-MeFOSE, N-methyl perfluorooctanesulfonamide ($C_8F_{17}SO_2NH(CH_3)$), N-ethyl perfluorobutanesulfonamidoethanol ($C_4F_9SO_2N(CH_2CH_3)CH_2CH_2OH$), N-ethyl perfluorobutanesulfonamide ($C_4F_9SO_2NH(CH_2CH_3)$), 10:2 FTOH and 12:2 FTOH. The perfluorobutyl-based compounds are of current interest because they have replaced perfluorooctyl PFSAm[55].

Table 2.4. Log K_{OW} (average ± standard deviation) of 4:2, 6:2, and 8:2 FTOH, PFOSA, N-EtFOSA, and N-EtFOSE isomers (labels correspond to Fig. 2.1) as determined using slow-stirring method.

Compound	**Mean Log K_{OW}**	**Standard deviation (log units)**
4:2 FTOH	1.97	0.17, N = 12
6:2 FTOH	3.30	0.41, N= 12
8:2 FTOH	4.88*	0.11, N = 12
8:2 FTOH (generator column)	4.96*	0.20, N = 9
10:2 FTOH	2.91	0.51, N=12
PFOSA	4.08	0.48, N = 12
N-EtFOSA	4.51	0.22, N=12
N-EtFOSE isomers		
1	5.47	0.09, N = 5
2	5.56	0.15, N = 5
3 (*n*-isomer)	5.33**	0.09, N = 5
4	5.38	0.07, N = 5
5	5.58**	0.15, N = 5
6 (isopropyl)	5.34	0.07, N = 5
7	5.37	0.06, N = 5
8	5.40	0.07, N = 5
9	5.38	0.20, N = 5

* not statistically different using t-test (t = -1.175, 19 degrees of freedom, p = 0.225)

** statistically different based on ANOVA (p = 0.007) and post hoc Tukey test (P = 0.036, q = 4.851 and p = 9).

Based on correlation of K_{OW} to molar volume , it was expected that the branched N-EtFOSE isomers would have smaller K_{OW} compared to the *n*-isomer. The lowest K_{OW} of the nine isomers corresponded to the *n*-isomer. However, using a one-way ANOVA test with post hoc Tukey test (P = 0.036), the only statistically different mean K_{OW} occurred between the n-isomer and isomer 5. Amongst the remaining isomers, there were no statiscially significant differences in K_{OW} (Table 2.4). Given that C^{sat}_{w} has long been correlated to K_{OW} due the relation

of both properties to the activity coefficient in water, γ_w [56], we were surprised that differences in water solubility observed for branched N-EtFOSE isomers did not appear to translate into correspondingly lower K_{OW}. Generally the same isomer profile was observed in the octanol and water phases at equilibrium (Appendix A). This could be due to the relatively small differences in molar volume for the ECF isomers (at most 4% difference) compared to the precision associated with the K_{OW} data (40% difference for isomers).

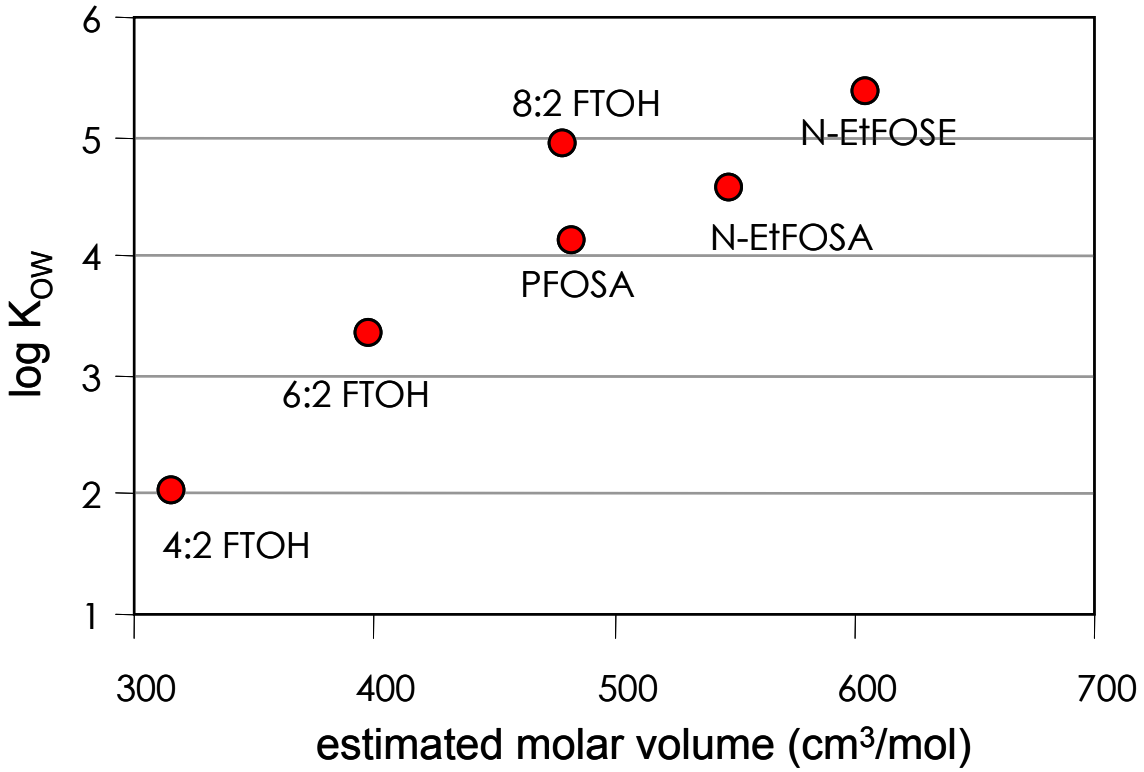


Figure 2.2 Correlation of measured log K_{OW} of FTOHs and PFSAm with estimated molar volume (Hyperchem).

Other research has noted discrepancies between C^{sat}_w and K_{OW}. De Maagd et al. observed that while C^{sat}_w was inversely linearly correlated to molar volume for a set of PAHs, the trend was not as consistent between molar volume and log K_{OW} [43]. For a series of halogenated dimethyl bipyrroles, calculated C^{sat}_w values using log K_{OW} did not correspond well with experimentally determined C^{sat}_w [57]. Mackay et al. proposed that with increasing molecular size, a compound can become less soluble in octanol as its γ_o increases[58] whereas the opposite trend was observed by Xiao and Wania in which γ_o was larger with greater chlorination[59].

Another contrast between the K_{OW} and C^{sat}_{w} is that K_{OW} represents partitioning between octanol and water to which γ_o and γ_w are both important. It is expected that the γ_o values for organofluorines are significant. Results of the C^{sat}_{w} experiment demonstrated a dependence of γ_w on perfluorocarbon arrangement because the isomers exhibited differences in solubility. Equivalent K_{OW} values for the isomers perhaps could mean that although γ_o and γ_w are simultaneously variable, the ratio between them is similar. The lack of variability in K_{OW} and yet varying C^{sat}_{w} for N-EtFOSE isomers implies that the structural arrangement of perfluorinated carbons affects both water solubility and octanol solubility but the combination of these parameters ultimately has a canceling out effect on K_{OW}.

Another possibility is that the C^{sat}_{w} trends observed for the N-EtFOSE isomers did not strictly reflect partitioning between the compound itself and water. Rather, sorption may have occurred with the glass beads packed in the column. The surface active nature of perfluorinated compounds has been exploited for industrial and commercial uses. Interaction between the N-EtFOSE isomers and the glass beads could be a source of discrepancy between K_{OW} and C^{sat}_{w} and may lead to error in C^{sat}_{w} measurements. Affinity of these compounds to glass has been both observed and discounted and remains a controversial issue [27,29] but differential affinity of linear versus branched isomers has never been assessed.

Given that all of the N-EtFOSE isomers show similar K_{OW}, it is anticipated that each isomer would have near equivalent kinetic rate constants for crossing lipid bilayers in organisms by passive diffusion, however, the mechanism of uptake of PFSAm and FTOHs in the body has not been reported in the literature. Both PFOA and PFOS are believed to bind to serum and organic anion transporting proteins as well as subjected to enterohepatic circulation leading to [60-62]. However, the influence of K_{OW} on uptake and elimination cannot be ruled out because incrementing chain length of PFCAs was correlated to bioconcentration factor[51], bioaccumulation factor in fish[63], sorption[49], and inversely to elimination rates from rats[64]. In addition, faster elimination of branched ECF PFOA isomers from rats[65,66] and fish[67] also occurs. Ultimately, the isomer profiles of perfluorinated compounds in biota is likely due to a combination of factors such as protein binding efficiency (which may be influenced by hydrophobicity), kinetics of metabolism[68] and if inhalation is the primary route of exposure to PFSAm and FTOHs, air partitioning coefficients will be an important parameter. To date, the

vapour pressures of branched PFSAm isomers have not been reported, nor has the isomer profile of PFSAm in air samples. A recent study reported differential kinetics of metabolism of PFOSA isomers[68], however, PFOS isomer profiles in human blood of the general population do not appear to be depleted in branched isomers relative to the ECF PFOS isomer mixture[68,69].

2.4 Conclusions

K_{OW} measurements of FTOHs and PFSAm were done by the slow stirring technique and ranged from 1.97 to 5.58. An increase in K_{OW} corresponded to increasing molar volume, as predicted using HyperChem software. Linear and branched N-EtFOSE isomers did not demonstrate significant differences in K_{OW}. Saturated aqueous fractions collected from the generator column revealed enrichment of branched N-EtFOSE isomers compared to the technical mixture which may be due to enhanced water solubility. These trends in hydrophobicity may contribute to the bioavailability of these compounds and their bioprocessing.

2.5 Acknowledgements

The authors thank 3M for the contribution of ECF N-EtFOSE. Gratitude is expressed to Suzanne Gauthier for her expertise in the generator column method, Derek Jackson for HyperChem modeling, and David Ellis for helpful discussions. Funding from NSERC is gratefully acknowledged.

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CHAPTER THREE

Isolating Isomers of Perfluorocarboxylates in Polar Bears *(Ursus maritimus)* From Two Geographical Locations

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Contributions – Amila De Silva conducted method development, data collection, interpretation, and manuscript preparation under the guidance of Scott Mabury.

3.1 Introduction

The United States Environmental Protection Agency (USEPA), in 2003, announced its plans to conduct a risk assessment of perfluorooctanoate (PFOA) based on preliminary animal toxicity studies and presence in human tissues[1-3]. PFOA has been found to be a peroxisome proliferator and an inhibitor of gap junctional intercellular communication, both of which may result in hepatocarcinogenic behaviour[4]. In addition to concerns regarding its toxicity and occurrence in human tissues, PFOA has been found in species of birds, fish and mammals globally[5-11], including those from remote regions[12,13]. Longer chain PFCAs (see Table 3.1 for full names of PFCAs and acronyms) in biota were first discovered by Moody *et al*. who reported the detection of PFHpA, PFOA, PFDA, PFUnA, PFDoA and PFTA in fish collected to evaluate background levels of perfluorinated compounds[7]. More recently, Martin *et al*., found ng/g levels of PFCAs ranging from 8 to 15 carbons in Canadian Arctic biota samples[12]. Given that PFCAs are relatively involatile and are only attributable to anthropogenic activity, the source of these compounds in such remote regions is of both public and research interest.

Table 3.1. Perfluorinated carboxylates: acronyms and molecular ion of 2,4-difluoroanilide derivative used for determination.

Name	Acronym	Molecular Ion of 2,4-difluoroanilide derivative
perfluoroheptanoate	PFHpA	475*
perfluorooctanoate	PFOA	525
perfluorononanoate	PFNA	575
perfluorodecanoate	PFDA	625
perfluoroundecanoate	PFUnA	675
perfluorododecanoate	PFDoA	725
perfluorotridecanoate	PFTrA	775
perfluorotetradecanoate	PFTA	825*

* not determined in this study

PFCAs are unlikely to move long distances in the atmosphere due to low volatility and efficient scavenging by wet and dry deposition[14]. Therefore, it has been hypothesized that

volatile precursor compounds, with the ability for long-range atmospheric transport, will eventually break down to the persistent PFCAs[15,16]. Telomer alcohols, which are volatile, have been found to degrade both atmospherically[17] and biologically[18,19] to PFCAs. Given the large-scale production of telomer alcohols worldwide, these compounds are candidates for contributors to global PFCA levels[20]. In fact, analysis of North American air samples show widespread tropospheric distribution of fluorotelomer alcohols at significant concentrations[15,21]. Ellis *et al.*, have indicated that telomer alcohols may be responsible in part for the presence of PFCAs in the Arctic and other non-urban areas where atmospheric levels of peroxy radicals far exceed that of NO_x (NO and NO_2)[17].

Although atmospheric transport of volatile precursor compounds is a plausible theory for explaining the presence of PFCAs in Arctic biota, marine transport should be considered. Marine transport of perfluorinated compounds including PFOA and PFNA was recently examined by So *et al.* [22]in southern China, Hong Kong and Korea[22]. It was speculated that emission of perfluorinated compounds to Pearl River and subsequent circulation by coastal currents resulted in a distribution of these compounds to locations on the western coast of Hong Kong remote from point sources[22]. However, the oceanic waters of eastern Hong Kong region also contained low levels of PFOA suggesting either PFCA input from mainland China and/or atmospheric input. It is not clear though that waters of the Atlantic or Pacific, not impacted by local sources, contain sufficient quantities of PFCAs to represent a plausible oceanic transport mechanism for the rather high concentrations observed for these materials in the Arctic. Further, oceanic transport would likely not explain the even/odd pattern, whereby [PFNA]>[PFOA], [PFUnA]>[PFDA], and [PFTrA]>[PFDoA], that has been observed in Arctic biota as determined by the Martin *et al.* [12]and Smithwick *et al*.[13].

Fluorinated alkyl compounds are primarily produced either by Simons electrochemical fluorination (ECF) or telomerization. In ECF, HF is used to replace hydrogen with fluorine atoms in hydrogen-carbon bonds of organic compounds[23]. This process yields constitutional isomers (also known as structural isomers) where the majority of the perfluoroalkyl chains in the product are in a linear arrangement and to a lesser extent, branched chain isomers are also formed[24]. Another feature of ECF products is that shorter and longer homologues are produced as impurities. Telomerization, on the other hand, is classified as a polymerization reaction that involves reaction of perfluoroalkyl iodides with perfluorinated alkenes, such as

tetrafluoroethene. This synthetic method merely increases chain length of the reacting perfluoroalkyl iodide and does not produce isomeric mixtures[24].

ECF is used to convert alkanesulfonyl fluorides and alkanecarbonyl fluorides into their perfluorinated counterparts. A number of polyfluorinated compounds can be made from perfluoroalkanecarbonyl fluoride and perfluoroalkanesulfonyl fluoride (PFOSF). Examples of compounds derived from perfluorooctanesulfonyl fluoride include perfluorooctane sulfonate (PFOS), *N*-ethyl perfluorooctanesulfonamidoethanol (*N*-EtFOSE), *N*-methyl perfluorooctanesulfonamidoethanol (*N*-MeFOSE), *N*-ethyl perfluorooctanesulfonamide (*N*-EtFOSA), and perfluorooctane sulfonamide (PFOSA). These latter four compounds are volatile precursor compounds with potential to degrade to persistent perfluorinated compounds. Metabolism of *N*-EtFOSA was first investigated 15 years ago in rats[25]. At that time, PFOSA was identified as the major metabolite as instrumentation was not available to determine production of PFCA or perfluoroalkyl sulfonate metabolites. Recently, PFOS was confirmed to be a biodegradation product of *N*-EtFOSA using fish liver microsomes[26]. Similarly, using municipal wastewater treatment sludge as a source of microbial inoculum, PFOS was identified as a biodegradation product of *N*-EtFOSE[27].

The ratio of linear to branched isomers in PFOSF-derived compounds can vary from 90:10 to 70:30[28,29]. The Certificate of Analysis for *N*-EtFOSE obtained from 3M (St. Paul, Minnesota, U.S.A.) states constitutional isomer composition based on ^{19}F NMR data. The linear:branched isomer ratio in this batch of *N*-EtFOSE was 70:30[30]. Table 3.1 depicts the structures of each of the types of isomers associated with ECF products. The most abundant branched isomers of *N*-EtFOSE were found to be the isopropyl branched isomer (~11%) and the internal mono-methyl branched isomer (~17%). Identified branched isomers comprising the remaining ~ 2% consist of the terminal *tert*-butyl branched isomer, alpha methyl branched isomer, and *gem*-dimethyl branched isomer[30]. A document submitted to the USEPA by 3M provides the Certificate of Analysis describing constitutional isomer composition for PFOA derived from perfluorooctanecarbonyl fluoride, a compound fluorinated by ECF[30]. These isomers are shown in Table 3.2. The same classes of branched isomers found in *N*-EtFOSE were identified in this batch of PFOA. Again, the most abundant branched isomers were the isopropyl branched isomer and the mono-methyl branched isomer. Contrary to *N*-EtFOSE, the ratio of linear to branched isomers in this sample of PFOA was found to be 78:22 [30]. This indicates

that there is some batch-to-batch variation in the isomer component of ECF products. Like *N*-EtFOSE, PFOS is synthesized from PFOSF. Isomer analysis of a batch of PFOS from 3M has been reported[31]. The branched isomer content of this sample of PFOS is the same (30%) as the *N*-EtFOSE described above. However, the individual branched isomer content varies by +/- 0.4%. Although it is believed that the branched isomer composition of ECF products can range from 10% to 30%, this may be an over-estimate. Thus, isomer determination of a wider range of compounds of ECF origins is necessary to gain appreciation for batch to batch variation.

Commercially, telomerization describes a polymerization reaction between a taxogen olefin, often tetrafluoroethylene ($CF_2=CF_2$), with a perfluoroalkyl iodide (telogen) in the presence of a catalyst to produce longer perfluorinated iodides[32]. The perfluoroalkyl iodide polymer (telomer) synthesized consists of a mixture of linear compounds varying in even-numbered carbon chain length[24]. These perfluoroalkyl iodides react with ethene to form 2-perfluoroalkyl-ethyl iodides ($CF_3(CF_2)_nCH_2CH_2I$). The perfluoroalkyl-ethyl iodides can undergo hydrolysis forming perfluoroalkyl-2-ethanols, also known as fluorotelomer alcohols (FTOH)[24]. Fluorotelomer alcohols have the form $CF_3(CF_2)_n(CH_2)_2OH$ and are named (n+1):2 FTOH. Thus, $CF_3(CF_2)_7(CH_2)_2OH$ is called 8:2 FTOH. Products of telomerization reflect the constitutional arrangement of atoms in the starting telogen material. Therefore, telomerization is often reported to yield only straight-chain products with an even number of fluorinated carbons where the telogen also possesses these qualities[33].

Liquid phase direct perfluorination (LPDPF) is a third method used in industry for perfluorination. However, it only occupies a niche market currently and in terms of production, it is a minor method compared to ECF and telomerization-based production[34]. In the LPDPF method, the hydrogen-containing starting material is dissolved in a perhalogenated liquid medium. Fluorine gas is used to replace hydrogen atoms in the starting material with fluorine atoms. At the end of the 1980s, both ExFluor and 3M patented LPDPF methods for ether and ester perfluorination[35,36]. Products of LPDPF have been found to contain some branched isomer by-products. In a report generated by 3M which was submitted to the USEPA, ^{19}F-NMR studies were performed on some PFCAs produced by LPDPF[37]. In this study, LPDPF PFUnA was found to be 96.4% linear, 2.6% internally mono-methyl branched, and 0.12% terminal isopropyl branched isomer[37]. The percentage of branched isomers in an LPDPF product is significantly less than that of an ECF product.

Table 3.2. Identified constitutional isomers in ECF-derived compounds using PFOA as an example. Table adapted (with permission from 3M) from EPA public docket submitted by 3M[37].

Constitutional Isomer	**Structure**	**% by weight***
Linear	$CF_3(CF_2)_6COOH$	78.0
internal mono-methyl branch	$CF_3(CF_2)_xCF(CF_3)(CF_2)_yCOOH$, where x+y=4 methyl branch can be on any of the internal carbons of the chain	12.5
isopropyl branch	$(CF_3)_2CF(CF_2)_4COOH$	9.0
t-butyl branch	$(CF_3)_3C(CF_2)_3COOH$	0.2
internal gem-dimethyl branch	$CF_3(CF_2)_xC(CF_3)_2(CF_2)_yCOOH$ where x+y=4 dimethyl branch can be on any of the internal carbons of the chain	0.1
alpha branch	$CF_3(CF_2)_4CF(CF_3)COOH$	0.1

* as determined by ^{19}F NMR and ^{1}H NMR

The contrasting outcomes of telomerization and ECF have lead to our hypothesis that the presence or absence of branched isomers of PFCAs in samples from the Arctic may suggest the major process responsible for their delivery to this region. In 1999, 3M, a major user of ECF, announced that it would be phasing out all products involving perfluorooctanyl chemistry, including *N*-MeFOSE, *N*-EtFOSE, PFOSA, PFOS, etc.[38]. However, large-scale commercial production of fluorotelomer alcohols is still in practice via telomerization. Thus it is important to determine the source(s) of PFCAs in Arctic biota because the responsible contaminant species may still be in production and consequently, may currently be emitted which will result in further contamination of these persistent compounds.

The objective of this study was to qualitatively examine the isomer patterns of PFCAs in environmental samples. Although LC/MS/MS is typical for PFCA determination, a GC-based method was chosen to separate the physically similar constitutional isomers because GC-based methods have greater potential for resolution compared to LC-based methods. Another benefit

of GC application is its avoidance of contamination, which sometimes arises with LC instruments containing perfluoro polymer parts[39]. GC determination of PFCAs requires derivatization to volatile analogs[40]. This was accomplished using 2,4-difluoroaniline, which has been used for derivatizing haloacetic acids and more recently, PFCAs[41-43].

Isomer patterns of PFCAs were qualitatively determined in polar bear *(Ursus maritimus)* liver samples from two locations, the south eastern Hudson Bay region of Canada and central eastern Greenland. These samples were part of a larger set of samples quantitatively analyzed for PFOS and PFCAs by Smithwick *et al.*[13] and Martin *et al.*[12]. Polar bears are especially useful samples for several reasons. Firstly, they are from remote and sparsely populated Arctic marine locations, so the transport of contaminants was likely to have been atmospheric. Condensation of contaminants or chemically transformed contaminants then occurs in cold northern waters. These waters then serve as a sink for contaminants with potential for contaminants to be available to marine organisms and subsequent movement through food chains. Polar bears are apex predators that spend most of the year on ice flows and feed almost exclusively on seals. Also high concentrations of PFCAs were found in polar bear liver samples by Martin *et al*. (9 ng/g to 180ng/g wet weight) and Smithwick *et al.* (8 ng/g to 236 ng/g wet weight), consistent with their trophic level, and therefore PFCA isomers would be expected to be readily detected[12,13].

Previously, our research group presented preliminary data on the PFOA and PFNA isomer patterns in Greenland polar bears in support of a proposed tropospheric degradation mechanism of FTOHs[17]. Apart from this, no studies on PFCA isomer patterns in environmental samples have been published in the scientific literature. The number of fluorinated compounds produced from the two major industrial synthetic routes, ECF and telomerization, is vast. Determination of PFCA isomeric profile in environmental samples may provide evidence as to the sources responsible for their presence. This could further fuel investigation of potential precursors as well as limit the emission of those precursor compounds responsible for environmental contamination.

3.2 Experimental

3.2.1 Sample Collection

Livers from 15 individual polar bears from two different locations, Greenland and Canada, were analyzed for this study. Field sampling in Canada has been described by Verreault *et al.* [44]and in Greenland by Dietz *et al.[45]* and was conducted under research licenses/permits from appropriate agencies in each country. In both locations, livers were collected from harvested bears as part of the Inuit subsistence hunt regulated by community quotas. One set of bear samples was obtained in the Ittoqqortoormiit/ Scoresby Sound area of central eastern Greenland from 1999 to 2001. The other set of samples was gathered in February 2002 in a region located in southeastern Hudson Bay, near Sanikiluaq, Nunavut, Canada. Livers were removed from the animals soon after *post mortem* and were stored separately in polyethylene plastic bags. Samples were kept at outdoor temperatures (-20 to -5°C) until storage in a freezer (-20 to -10°C). Tissue samples were shipped by air courier at -20°C and stored in a freezer (-20 to -10°C) until used. For analysis, sub-samples (~1.5g) were accurately weighed (+/- 0.0001g) for extraction and subsequent derivatization.

3.2.2 Standards and Reagents

Perfluorooctanoic acid (99%) was provided by 3M (St. Paul, MN, USA). Perfluorononanoic acid (97%), perfluorodecanoic acid (98%), perfluoroundecanoic acid (95%), perfluorododecanoic acid (95%), tetrabutylammonium hydrogensulfate (TBAS), 1,3-dicyclohexylcarbodiimide (DCC) (99%), and 2,4-difluoroaniline (2,4-DFAn) (99%) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Anhydrous sodium carbonate (99.8%) was obtained from J.T. Baker (Phillipsburg, NJ, USA), while anhydrous sodium sulfate (99%), sodium bicarbonate (99%), and sodium chloride (99%) were from ACP (Montreal, QC, Canada). Hydrochloric acid (36.5%-38%) was purchased from VWR International (Mississauga, ON, Canada) and ethyl acetate (99.9%) and hexane (99.9%) from Fisher Scientific (Nepean, ON, Canada). Methyl-*tert*-butyl ether (MTBE) was purchased from EM Science (99.5%, Gibbsburg, NJ, USA).

3.2.3 Extraction of Perfluorinated Acids from Livers

Extraction of PFCAs from biological samples using an ion-pairing agent into MTBE has been described in previous studies[1]. Approximately 1 to 2 g of homogenized tissue was used for each analysis. The combined MTBE extracts were evaporated to dryness using N_2. The residues were then reconstituted in 50 ml of distilled water.

3.2.4 Derivatization

The derivatization method described here has been reported elsewhere[41-43]. Reconstituted extracts were acidified to pH 1.0 using concentrated HCl. To this, 1.00 g NaCl and 20.00 ml ethyl acetate were added. A catalyst consisting of 1.00 ml 1.1 M DCC in ethyl acetate and derivatizing agent, 1.00 ml 1.0 M 2,4-DFAn in ethyl acetate were then added. The resulting mixture was stirred vigorously for one hour. After dissolving 5.0 g NaCl into the mixture, the aqueous phase of the samples was removed using a separatory funnel. The remaining organic phase was washed with 5 ml 10% HCl, saturated $NaHCO_3$ solution and saturated NaCl solution respectively. This phase was passed through a filter containing Na_2SO_4 and evaporated to dryness using N_2. Residues were dissolved in a 2.00 ml hexane-diethyl ether solvent system (95%/5%). Further clean-up was performed using a silica gel column and elution with 15 ml of the hexane-diethyl ether solution. The eluants were evaporated to 200 µl volumes using N_2.

3.2.5 Gas Chromatographic-Mass Spectrometric (GC-MS) Analysis

2,4-difluoroanilide derivatives of PFOA, PFNA, PFDA, PFUnA, PFDoA, and PFTrA in the tissue extracts were determined by GC-MS using an HP 6890 Series GC System with an HP 5973 MSD (Hewlett-Packard, Palo Alto, CA, United States). 1.00 µl pulsed pressure injections of the samples and standards were performed using an HP 7683 Series Injector. Chromatography of the derivatives was performed using a ZB-35 column (90.0 m x 0.25 mm, 0.50 µm film thickness) (Phenomenex, Torrance, CA, United States) with He carrier gas at 12 psi. The injector was held at 250 °C for the duration of each run. Initial temperature of the oven was 40 °C and held for 2 minutes. The temperature was then increased to 125 °C at a rate of 1 °C /min and then held for 15 minutes. Using the same ramp, the temperature was raised to 140

°C at which point the oven was rapidly heated to 275 °C at 30 °C/min for cleaning. Transfer line and source were kept at 280 °C. All chromatograms presented are based on monitoring of the molecular ions using SIM (shown in Table 3.1) of the derivatized PFCAs. All chromatograms presented are based on monitoring of the molecular ions using SIM (shown in Table 3.1) of the derivatized PFCAs. However, confirmation of PFCA identity was accomplished by monitoring two additional fragments 128 m/z and 156 m/z, which correspond to the 2,4-difluoroaniline fragment and the 2,4 difluoroanilide fragment of the derivatized PFCAs, respectively.

3.3 Results and Discussion

Resolution of constitutional isomers of PFCAs by chromatography was accomplished using an ECF-derived PFOA standard provided by 3M. Detection of its 2,4-difluoroaniline derivative was by MS via the molecular ion. Separation of seven constitutional isomers was achieved as presented in Figure 3.1. In this particular batch of PFOA, 77.0% was determined to consist of the linear isomer based on peak integration of the GC-MS chromatogram. This value closely corresponds to the linear isomer composition (78.0%) of ECF PFOA determined by ^{19}F NMR analysis by 3M[37]; our own ^{19}F NMR analysis is consistent with that reported by 3M. Further studies are necessary to identify the branched isomers identified in Figure 3.1.

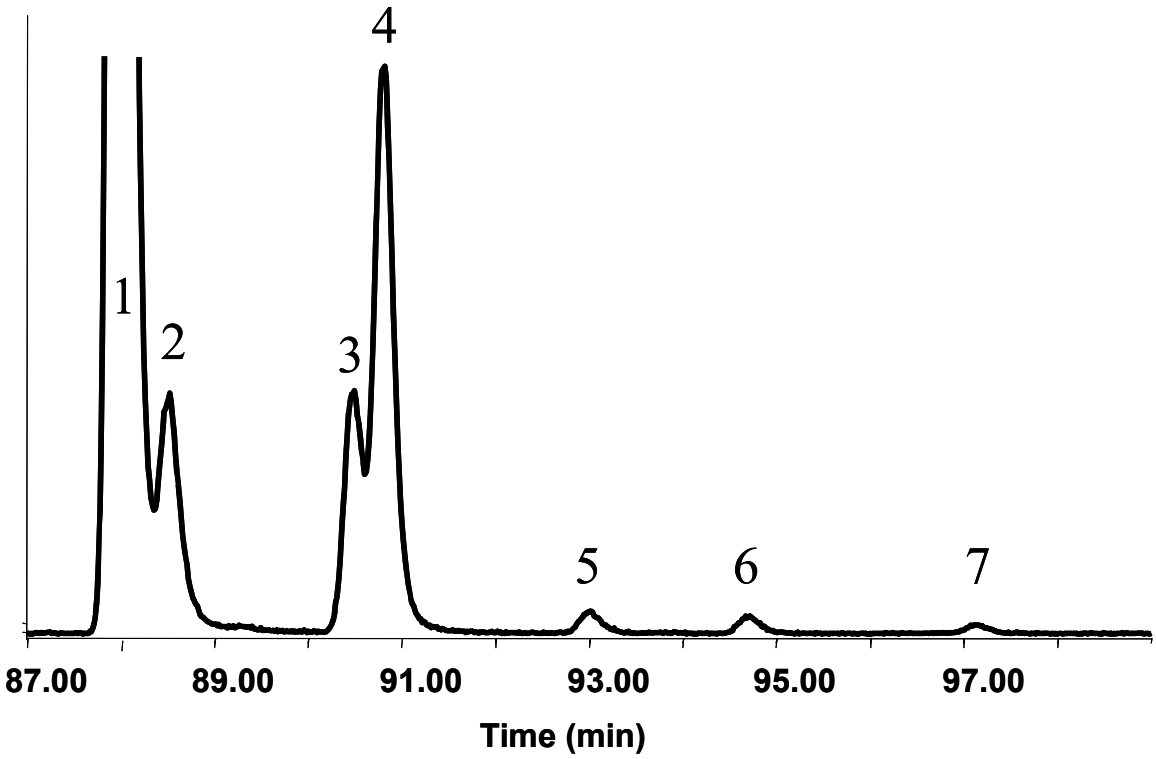

Figure 3.1. GC-MS determination of constitutional isomers of an authentic ECF PFOA standard where peak 1 corresponds to the linear isomer (77% peak area) and peaks 2 through 7 correspond to branched isomers.

The method was then applied to a standard mixture of longer chained PFCAs: PFNA, PFDA, PFUnA, and PFDoA. These were of LPDPF origin because both telomer and ECF derived PFCAs were apparently unavailable commercially. A stacked perspective of the chromatograms is presented in Figure 3.2. The isomer profile in these standards showed at least 97% linear isomer with some minor peaks ascribed to branched isomers, as demonstrated in inset of Figure 3.2. LPDPF products reportedly contain 2-3% structural isomers [46] as evidenced by peaks in the LPDPF PFCA standard chromatograms. Some of these minor components elute earlier than the dominant peak and may be branched isomers, however NMR analysis of the standards is necessary to distinguish isomeric components from impurities.

An interesting feature of the constitutional isomer profiles of these PFCAs is that some of the minor peaks elute earlier than the linear isomer whereas all minor peaks of the ECF PFOA standard elute after the linear isomer. This may be a distinguishing feature of LPDPF products from ECF products.

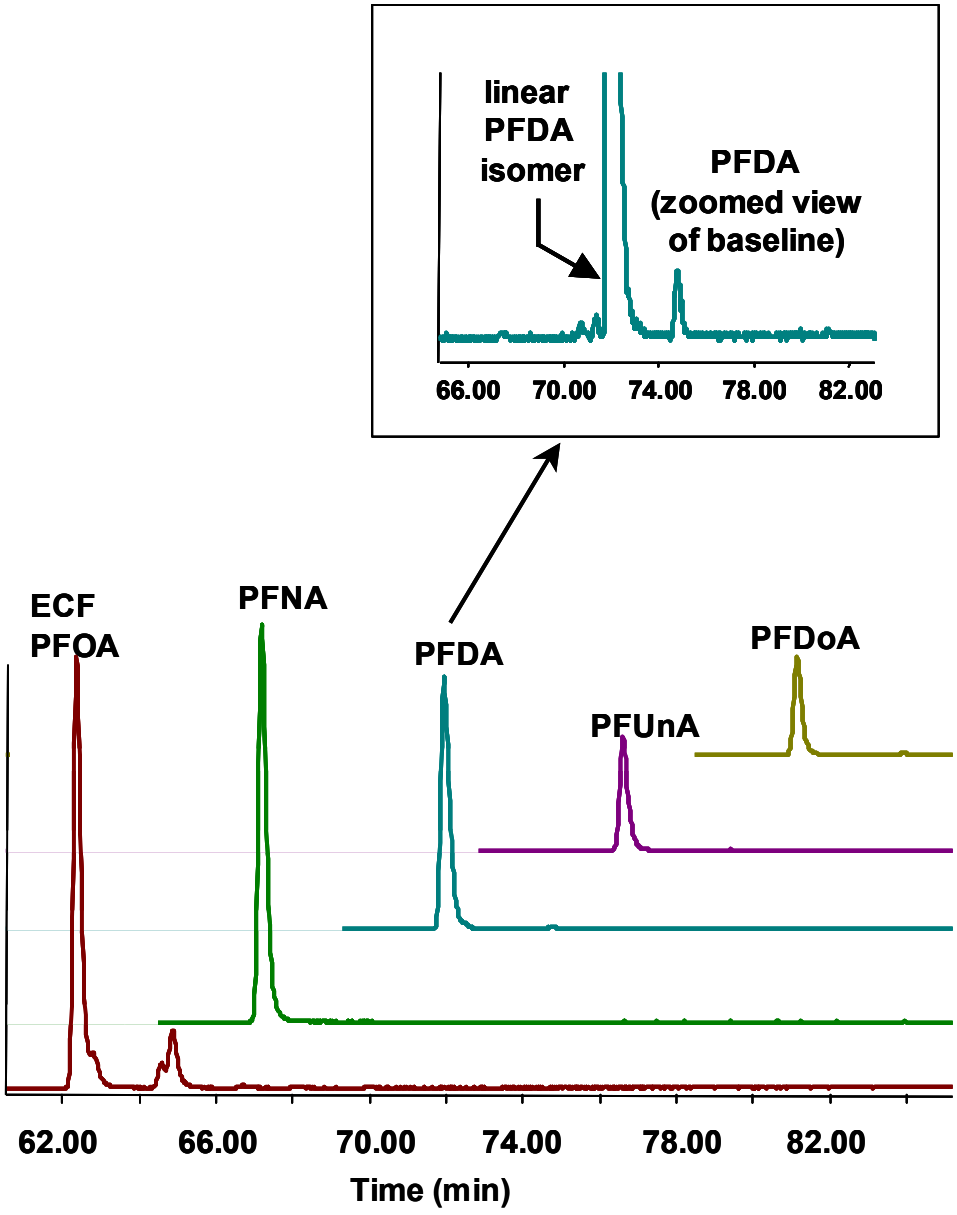

Figure 3.2. GC-MS determination of long chain PFCA standards. All PFCA standards synthesized via LPDPF except for PFOA, which was derived from ECF. Inset shows magnified baseline of PFDA chromatogram.

3.3.1 Resolution of Isomers, Detection Limits, and Precision for GC-MS of Derivatized PFCAs

Resolution(Rs) was calculated for adjacent isomer peaks of ECF PFOA in Figure 3.1 using peak width and retention time. Rs was found to be 0.8 (peaks 1&2), 0.7 (peaks 3&4), 3.0 (peaks 5&6), and 4.1 (peaks 6&7).

Detection limits were determined based on the lowest mass of ECF PFOA injected onto the GC-MS in which the isomer profile depicted in Figure 3.1 could be determined. This was found to be 10 pg. In other words, 2 ng of ECF PFOA was derivatized and concentrated to a 200 ul volume. The least abundant isomer was isomer 7 which, based on chromatographic peak area, comprised 0.2% of the total PFOA. By this, the detection limits for each isomer was 0.02 pg.

Routinely derivatized 50 ml distilled water blanks were determined to be free of PFCAs. An advantage of the derivatization-GC method is the absence of fluoropolymer parts in the GC-MS instrument. Because blanks were clean, blank subtraction from samples was not necessary. Any PFCA contamination present post-derivatization was not a hindrance because PFCA identification was determined via the corresponding 2,4-difluoroanilide derivatives.

Reproducibility on %branched PFCA isomers is represented by standard deviation. Derivatization and GC-MS determinination of PFCAs was performed in triplicate for a polar bear liver sample. Using the corresponding GC-MS chromatograms, % branched PFCA isomers was determined for each PFCA. Based on the three trial, standard deviation was calculated for the %branched PFCA. These values were found to be 0.3%, 0.2%, and 0.1% for PFDA, PFUA, and PFDoA respectively. In addition, triplicate derivatization and analysis of the ECF PFOA standard was performed. The standard deviation on %branched ECF PFOA was determined to be 0.1%.

3.3.2 Constitutional Isomers of PFCAs in Polar Bear Liver Samples

The contamination profile of PFCAs in polar bear livers initially observed by Martin *et al*. was confirmed using this method where PFCAs with an odd number of carbons exceeds that of the even-numbered PFCA preceding[12]. In other words, [PFNA]>[PFOA], [PFUnA]>[PFDA], and [PFTrA]>[PFDoA]. This pattern is consistent both with the atmospheric

degradation mechanism of fluorotelomer alcohols proposed by Ellis *et al.* [17]which yields a homologous series of PFCAs and the bioaccumulation potential reported by Martin *et al.*[47].

As a quality control measure, ECF-derived PFOA was spiked into polar bear liver to ascertain whether the extraction or analysis procedure discriminated amongst isomers. The chromatogram for the spiked sample was compared to that of the derivatized ECF PFOA standard. The results demonstrated that the isomer pattern in the standard was conserved in the spiked tissue sample.

In general, the PFCA isomer profiles in polar bear liver samples were dominated by the linear isomers. This is unsurprising considering both telomerization and ECF yield an abundance of the linear isomer. At least 90% of the isomer distribution for each PFCA in the samples was linear. The distribution of branched isomers of each PFCA is presented in Table 3.3. Branched PFCA isomer composition in the polar bears varied with location. The most striking differences between locations pertained to the PFOA isomer pattern. Compared to other PFCAs, the PFNA isomer profile was quite distinct due to the consistent dominance of its linear isomer. A discussion of the data obtained for PFOA, PFNA, and the remaining PFCAs (PFDA, PFUnA, PFDoA, and PFTrA) follows.

Table 3.3. Distribution of branched isomers of PFCAs in Arctic polar bears of two locations: Greenland and Canada

	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrA
GREENLAND						
Average (%)	5.0	0.3	1.4	2.8	2.1	0.4
Maximum (%)	9.8	1.1	2.5	3.4	4.0	0.9
Minimum (%)	2.8	N/D	N/D	2.3	N/D	N/D
CANADA						
Average (%)	N/D	0.0	2.9	1.2	3.6	N/D
Maximum (%)	N/D	0.4	3.4	1.5	3.9	N/D
Minimum (%)	N/D	N/D	2.3	0.9	3.2	N/D

N/D = no branched isomers detected

3.3.3 Constitutional Isomers of PFOA in Polar Bears.

Application of a derivatization-based GC-MS method for PFCA determination permitted the resolution of constitutional isomers of PFOA in liver samples from Arctic biota. Compared to the subtle variations in branched isomer abundance of longer-chain PFCAs in liver samples between both locations, the PFOA isomer profile showed a marked difference. Branched PFOA isomers were found in all 7 Greenland bear samples. Conversely, the PFOA in all 8 Canadian bears consisted solely of the linear isomer. This contrasting pattern of both locations indicates a difference in source of PFOA. A complete absence of branched PFOA in the Canadian bears suggests a non ECF input of PFOA to this location.

The profile of PFOA isomers in the Greenland bears varied from sample to sample. A chromatogram of the most PFOA isomer-laden bear sample is presented in Figure 3.3. As indicated, the pattern of PFOA isomers in the sample matches well with that of the ECF PFOA standard.

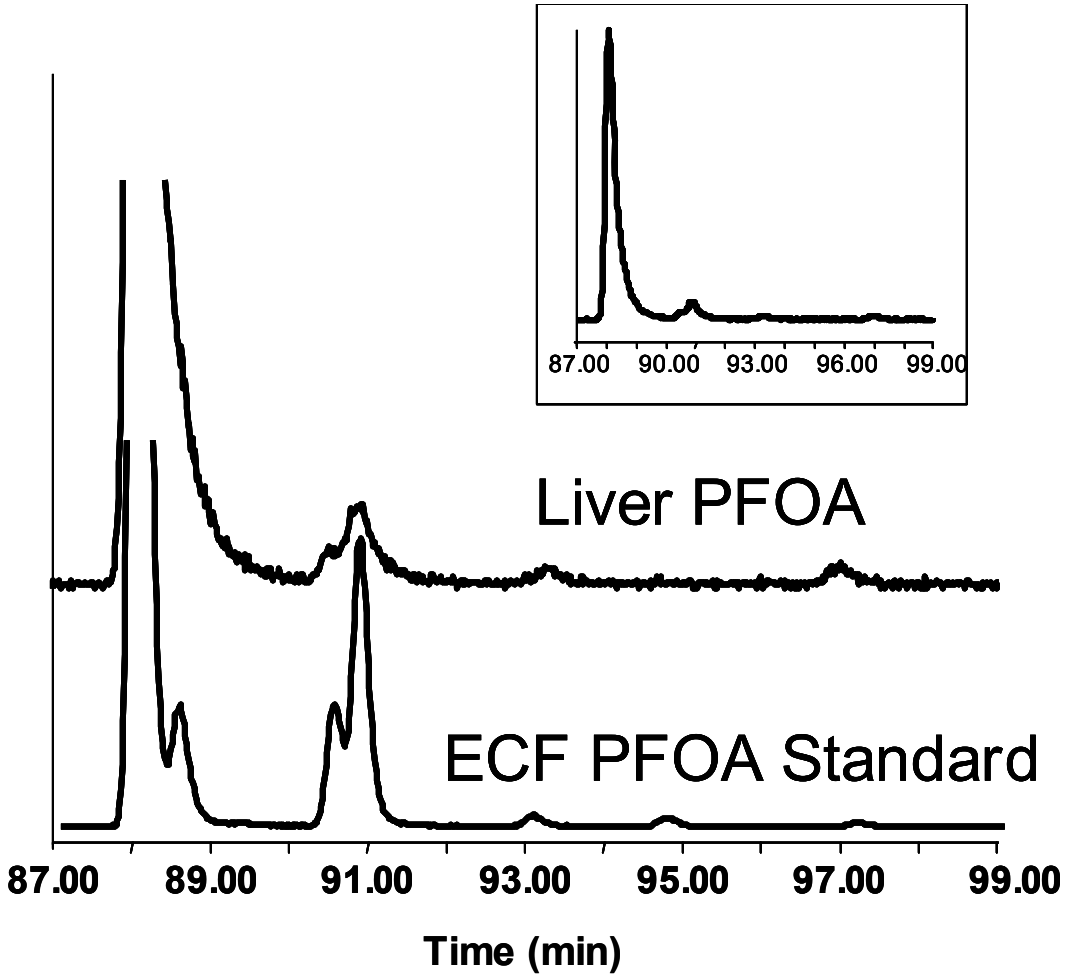

Figure 3.3. GC-MS determination of constitutional isomers of PFOA in polar bear liver sample from eastern Greenland (top) compared to those of an authentic ECF PFOA standard (bottom). Inset displays full view of PFOA isomer profile, normalized to linear PFOA, in polar bear liver sample[17].

In all Greenland bear samples, branched isomers 3 and 4 were present. These two isomers were the most abundant branched isomers in the standard. The absence of isomers 2 and 5 through 8 does not necessarily imply a non-ECF source of the PFCA. The method applied for isomer resolution was developed using a highly concentrated ECF PFOA standard. Although

good resolution between isomers 1 and 2 was obtained with the standard, it is possible that baseline resolution will not be achieved when the relative amount of isomer 2 is significantly less than that of isomer 1. In fact, isomer 1 in the liver samples was broad in shape, which may suggest that the ratio of isomer 2 to the total PFOA in the samples is much less compared to the 5.1 % contribution of isomer 2 to total PFOA in the ECF standard. Similarly, it is likely that detection limit is an obstacle in determining isomers 5 through 7. In the ECF PFOA standard, isomers 5 through 7 accounted for only 2.1% of the total PFOA. The similarity of the pattern of peaks 3 and 4 in the standard to that observed in all 7 Greenland samples suggests that the presence of these two isomers may be diagnostic of identifying an ECF source.

In the Greenland polar bear samples, the average branched PFOA isomer composition was 5.0% of the total PFOA. This value is much less than the 22% found in the ECF standard. Based on this value alone, it appears as though there is an additional source of linear PFOA, which cannot be ascribed to ECF. Telomerization produces only straight-chained compounds (provided starting telogen is also linear). Volatile telomer compounds may be responsible for the additional linear PFOA determined in the samples. One candidate is 8:2 FTOH, which was shown by Ellis *et al.* to undergo hydroxy radical driven atmospheric degradation producing PFOA[17]. By this mechanism, an FTOH will atmospherically degrade to yield two PFCAs in equal yields[17]. The two PFCAs can be distinguished based on the length of the perfluorinated chain. One PFCA has the same number of perfluorinated carbons as the FTOH while the other has one less perfluorinated carbon[17]. Thus, PFOA and PFNA are the major products of 8:2 FTOH. In addition, shorter chained PFCA homologues down to trifluoracetic acid are produced[17].

To investigate the presence of branched isomeric impurities in telomerized compounds, a standard of 8:2 FTOH was analyzed using ^{19}F NMR. The NMR spectra revealed only linear 8:2 FTOH. The presence of branched PFOA isomers in polar bear samples, consistent in pattern with an ECF standard, is significant because to date, no volatile ECF precursor has been determined to biodegrade directly to PFCAs. It has been determined that ECF precursor compounds such as *N*-EtFOSA, PFOSA and *N*-EtFOSE are biologically transformed to PFOS but not to PFCAs[26,27]. However, abiotic degradation of these volatile ECF compounds may prove to be a route to PFCAs in remote locations. This was demonstrated by an indirect photolysis study of *N*-EtFOSE to PFOA by Hatfield, although, these experiments were

performed using OH radical concentrations in gross excess of tropospheric conditions[48]. Other work in our research group is investigating the kinetics and reaction dynamics of volatile ECF compounds including polyfluorinated sulfonamides and sulfonamido-alcohols under tropospheric conditions.

3.3.4 Constitutional Isomer Profile of PFNA in Polar Bears.

The isomer profile of PFNA in the polar bears consisted almost entirely of the linear isomer. Branched isomers of PFNA were only found in 2 of the 7 Greenland bears and 1 of the 8 Canadian bears. In both cases, the linear isomer comprised at least 99% of the PFNA present. No correlation between this observation and specimen gender and age was determined. It is unlikely that the absence of branched PFNA is an issue of detection limit because the total PFNA concentration was at least 5 times the amount of PFOA. It is also unlikely that the absence of branched PFNA isomers is due to limitations in isomer resolution because the observation of branched PFNA in two samples and branched longer-chain PFCAs (discussed below) demonstrates that constitutional isomer separation was achieved for PFCAs based on a method developed using a standard of isomeric PFOA. The much higher proportion of linear relative to any detected branched PFNA suggests that the dominant source of this PFCA in Arctic biota is not from an ECF process. As mentioned, 8:2 FTOH is a candidate precursor for PFNA in remote regions[17].

3.3.5 Constitutional Isomer Profile of Longer-chain (C>9) PFCAs in Polar Bears.

The constitutional isomer profiles of PFOA and PFNA found in polar bear liver tissue were consistent with the atmospheric degradation mechanism proposed for 8:2 FTOH. Both locations were characterized by a dominance of the linear isomer form, >90% linear PFOA and >99% linear PFNA, compared to an authentic ECF PFOA standard (77% linear isomer). Atmospheric degradation mechanism of 8:2 FTOH to PFOA and PFNA observed by Ellis *et al.* is expected to apply to longer-chained fluorotelomer alcohols[17]. Thus, a potential source of PFDA and PFUnA in the Arctic is 10:2 FTOH while 12:2 FTOH could be responsible for PFDoA and PFTrA. The pattern of constitutional isomers for each of these PFCAs was determined to investigate this hypothesis.

Most of the samples from both locations were found to contain branched isomers of PFDA, PFUnA and PFDoA. Like PFOA and PFNA, the linear isomer was much more abundant (>96%) than branched forms, suggesting a dominant non-ECF input. Similar to the isomer pattern of PFNA, in both locations, almost all of PFTrA was in the linear form (>99%). However, 3 of the 7 Greenland samples were found to contain branched PFTrA isomers (average 0.8%).

Subtle differences in branched isomer distribution amongst PFCAs in both locations are apparent in Figure 3.4. There is a contrast in %branched PFCA values between each location. The Greenland samples show the greatest degree of branch isomers in PFOA, PFUnA and PFTrA compared to PFNA, PFDA and PFDoA. The opposite trend is observed in the Canadian samples. It is unknown what the cause of variations between locales. In a recent study by Smithwick *et al.*, perfluoroalkyl substances (PFAs) including PFCAs were quantified in polar bear samples from the same Greenland location reported here[13]. Smithwick *et al.* compared PFCA concentrations in samples from East Greenland with those from southeastern Hudson Bay and other locations in the Canadian arctic, as well as Alaska and Svalbard[49]. The samples from southeastern Hudson Bay and East Greenland had the highest levels of PFCAs of all locations[49]. However, the average concentrations of PFCAs (8 to 13 carbons) were greater in Greenland polar bear liver samples compared to the Canadian polar bear liver samples[49].

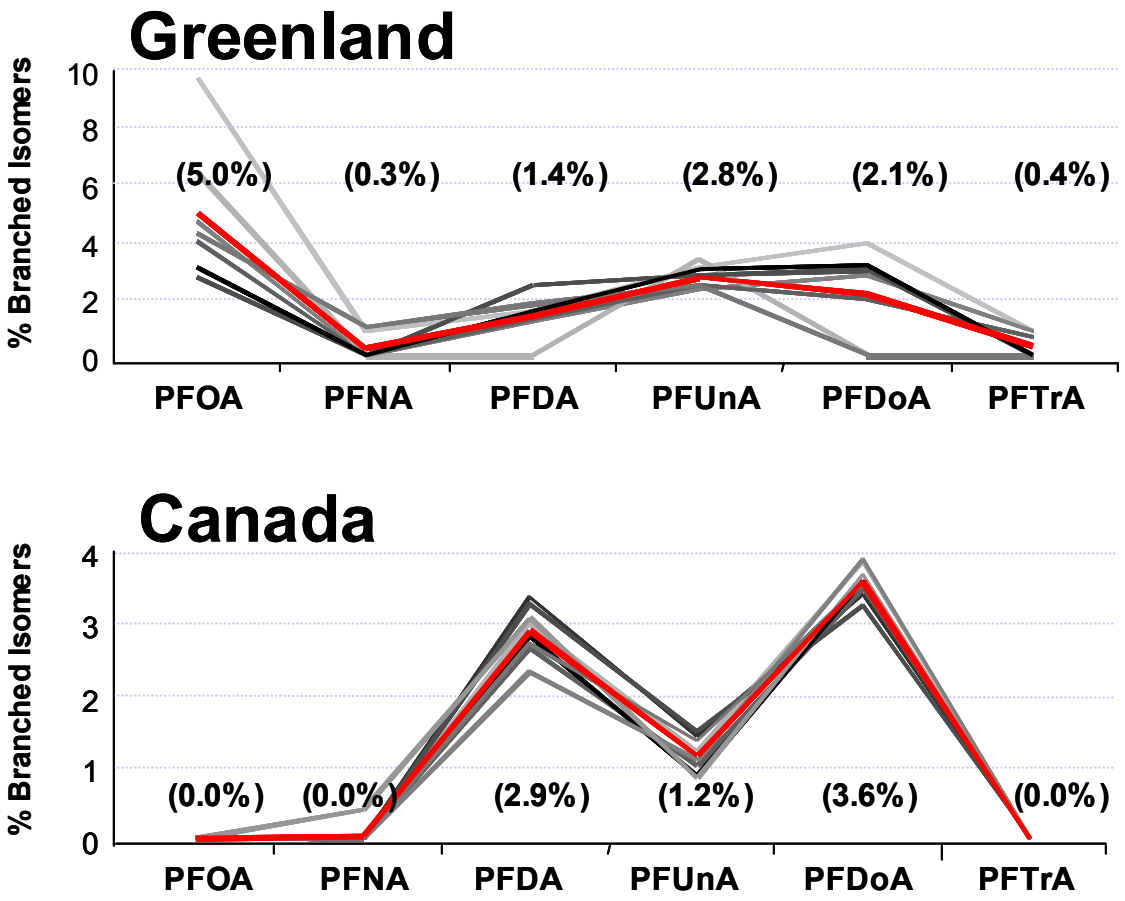

Figure 3.4. Branched isomer distribution of PFCAs in polar bears from Greenland (top) and Canada (bottom). Bracketed numbers refer to average percentage.

It is hypothesized that the source of PFCAs in the Arctic is atmospheric processing of fluorotelomer alcohols, which have the potential for long-range atmospheric transport. This does not necessarily imply a uniform Arctic PFCA concentration or isomer profile. Complex spatial patterns have been observed for many Arctic contaminants [44,45,50-52] as exemplified by chlorinated hydrocarbon profiles in Arctic biota[44,45,53-55]. One factor may be due to air currents. The dominant direction of air flow across eastern North America is north or east[50]. Thus, contaminants in Greenland can reflect contribution from both North America and Europe sources, which may account for the consistent and distinct pattern in PFCA isomer distribution between Canadian and Greenland polar bear samples. This implies that there are differences in production and/or application of fluorinated materials between these two regions.

The source of the branched isomers of PFDA, PFUnA, PFDoA, and PFTrA is of considerable interest. Industrial production of perfluoroalkyl compounds with C>8 would have been minor as it was not apparently pursued commercially[28]. Historical large-scale production of perfluorooctyl compounds using ECF is documented and by-products of ECF are homologous compounds and their constitutional isomers[56]. In the standard of ECF PFOA analyzed here, impurities of PFDA (0.1%) and PFDoA (0.2%) were determined. Therefore, it is possible that the branched isomers found of the longer chain PFCAs in the liver samples are a result of by-products in the production of perfluorooctyl chemicals using ECF. If this is the case, it is curious as to why only one branched isomer peak is present in PFDA, PFUnA, PFDoA, and PFTrA when ECF produces a range of branched isomers as evidenced by the branched isomer profile of PFOA in the Greenland polar bears. It may be that the resolution of branched isomers of PFCAs with increasing chain length will be increasingly challenging as the physical property differences amongst the isomers will correspondingly diminish.

Differences in physical properties will affect not only resolution but transport also. Volatility alone is not sufficient in predicting transport as other physical properties (such as water solubility and octanol-water partitition coefficient) are also relevant. Physical properties of branched versus linear perfluorinated chains are difficult to predict. Smart reported little effect of branching on boiling points for various perfluorinated compounds[57]. However, it is believed that branching will likely yield differential physical and chemical properties and by inference, their respective environmental fate. We are actively pursuing these measurements.

Another possibility is that bioprocessing of the individual isomers varies leading to different isomer patterns in biological samples. For example, there may be preferential biological uptake, accumulation, or excretion of specific PFCA isomers. Similarly, these processes along with metabolism of precursor isomers may also vary. It is hypothesized that bioprocessing is independent of PFCA isomer structure. This is justified based on the contamination profile of PFOA observed in the Greenland bear samples where the ECF isomer pattern of PFOA was conserved. Furthermore, no correlation was observed between % branched isomer distribution and gender and age of the specimens from which the samples were obtained. Further research into the physical properties and biological handling of each constitutional isomer class is underway by the authors.

The presence of branched isomers does not exclude a telomer source since telomerization conserves the geometry of the starting material. A branched telogen, for instance, will result in branched telomer compounds. In fact, a patent describes the deliberate synthesis of an isopropyl branched telogen, heptafluoroisopropyl iodide[58]. Furthermore, a patent details the production and use of terminal isopropyl branched perfluorocarboxylic acids as surfactants[59]. These branched perfluorocarboxylic acids were synthesized from the corresponding telogen, ranging from 5 to 16 carbons, and it is stated that the isopropyl branched acid is better at reducing surface tension of the liquid medium it is employed in than its linear counterpart[59]. Irrespectively, identification of the type of branched isomer observed in the longer chain PFCAs of polar bears is necessary as it may further clarify potential sources. Further, an exploration for branched isomers, within the large suite of telomer-derived materials currently in use, is certainly warranted since there remains the possibility that even minor quantities of these could contribute to what we observe in biological samples.

3.4 Acknowledgements

The polar bear liver samples analyzed in this study were part of a larger set of samples provided to Derek Muir (NWRI, Burlington, Ontario, Canada) for which PFCA data are being reported separately. We would first like to thank the local hunters, field biologists, and other associated personnel and people in Nunavut and Greenland who participated and assisted in sampling collection and processing. Those involved in sampling, cataloguing and summarizing of biological and geographical information included M. Taylor and J. Duchense (Government of Nunavut, Department of Sustainable Development, Iqualuit, Nunavut, Canada). The samples from Greenland were provided by R. Dietz and C. Sonne (Department of Arctic Environment, National Environmental Research Institute, Frederiksborgvej 399, DK-4000 Roskilde, Denmark). We thank Marla Smithwick (NWRI, Burlington, Ontario, Canada) for providing liver samples, Christine Spencer and Brian Scott (NWRI, Burlington, Ontario, Canada) for their assistance and generosity with GC-MS instrumentation. The authors wish to acknowledge the supervision and guidance of David A. Ellis in the research represented in this manuscript. The authors thank Dale Bacon (3M, St. Paul, MN, USA) for the donation of an ECF-derived standard of perfluorooctanoic acid. Funding for this research was provided by the Natural Science and Engineering Research Council of Canada (NSERC).

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CHAPTER FOUR

Isomer Distribution of Perfluorocarboxylates in Human Blood – Potential Correlation to Source

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Contributions – Amila De Silva conducted method development, data collection, interpretation, and manuscript preparation under the guidance of Scott Mabury.

4.1 Introduction

In 2000, the largest global perfluorinated chemical producer at the time, 3M, announced it would voluntarily phase out perfluorooctanesulfonyl fluoride (POSF, $C_8F_{17}SO_2F$) based production, including: perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$), perfluorooctanoate (PFOA, $C_7F_{15}C(O)O^-$), and perfluorooctanesulfonamide derivatives (contain $C_8F_{17}SO_2N$). It has been shown that these chemicals are environmentally persistent, bioaccumulate and may have potential for long term health risks.

Also troubling is the ubiquitous environmental occurrence of PFOS, PFOA, and other perfluoroalkylsulfonates and perfluoroalkylcarboxylates (PFCAs). Both PFOS and PFOA have been found in water[1,2], sediment[3], indoor dust[4,5], and biota[6,7], including non-occupationally exposed humans[8-12]. This latter point has garnered much public attention because it demonstrates that the general human population is exposed to perfluorinated acids. PFCAs are particularly of interest because long chain versions (> 9 carbons) have been detected in biota at significant levels although they have no reported intentional large-scale industrial production[6,13]. Furthermore, bioaccumulation of PFCAs has been shown to increase with chain length in fish[14].

PFOA and PFNA are the only PFCAs that have a significant legacy of intentional industrial production[15]. These were used primarily in an ammonium salt form as an emulsifier in the syntheses of fluoropolymers, mainly polytetrafluoroethylene (PTFE) and polyvinylidine fluoride (PVDF)[15]. Likely indirect sources of PFCAs in the environment are volatile organofluorine precursors that can undergo biotic[16-19] and/or abiotic reactions[20-23] to form PFCAs. Despite these direct and indirect routes, the dominant source of PFCAs in humans remains unknown.

Major industrial synthesis of PFCAs and other organofluorine compounds is accomplished primarily by one of two methods: electrochemical fluorination and telomerization. Products of these two synthetic processes can be distinguished based on their associated structural isomers.

In electrochemical fluorination (ECF), hydrocarbon alkylsulfonyl halides and alkylcarbonyl halides are converted to their corresponding perfluorinated acid halide analogues using anhydrous hydrogen fluoride and an electric current[24]. ECF products are a mixture of structural isomers comprised of predominantly the linear perfluoroalkyl chain (~70-80%) with smaller quantities of branched chain isomers. Perfluoroalkyl acid halides are used to make perfluoroalkylsulfonamides, perfluoroalkylsulfonates and PFCAs[24]. The perfluoroalkyl acid halide is typically not purified for removal of branched isomers prior to reaction; therefore, subsequent products are also a mixture of isomers. Major branched isomers of ECF are the internal mono-methyl branch and the isopropyl branch[25]. The mono-methyl branch geometry consists of a CF_3 group occurring on one of the inner carbons of the perfluoroalkyl chain. For PFOA, there are three possibilities: $CF_3CF_2CF(CF_3)CF_2CF_2CF_2COO^-$, $CF_3CF_2CF_2CF(CF_3)CF_2CF_2COO^-$, and $CF_3CF_2CF_2CF_2CF(CF_3)CF_2COO^-$. Collectively, these three isomers are reportedly ~13% of an ECF PFOA isomer mixture[25]. The major branched isomer is the isopropyl branch (contains $(CF_3)_2$ group on the terminal carbon), comprising ~ 9.0% of ECF PFOA [25]. Up until 2002, 3M was the major manufacturer of ECF PFOA in the USA[15].

The telomerization process involves reaction between tetrafluoroethene (taxogen) and a perfluoroalkyl iodide (telogen) producing a longer perfluoroalkyl iodide (telomer)[24]. Industrially, this polymerization reaction is capped by inserting ethylene to form $F(CF_2)_nCH_2CH_2I$. This is used to prepare fluorotelomer alcohols ($F(CF_2)_nCH_2CH_2OH$), fluorotelomer olefins ($F(CF_2)_nCH=CH_2$), and fluorinated acids[15]. Structural isomers have not been found to be a by-product of telomerization because geometry of the starting materials is apparently conserved in the product[24]. Thus, if both telogen and taxogen have their perfluorocarbons in a linear arrangement, presumably the telomer iodide product will also have this linear arrangement. PFOA and PFNA (ammonium salts) are still employed globally as a processing aid in fluoropolymer manufacturing; however, their current production is understood to be exclusively by telomerization[15].

Based on the differences in isomer composition associated with ECF vs. telomerization products, we hypothesize that the PFCA isomer pattern in humans may be suggestive of sources. For example, the presence of branched PFCA isomers in human tissues may suggest an ECF

source. Conversely, finding linear PFCA isomers in excess may indicate significant contributions from a strictly linear source such as telomer products.

Although many studies have reported PFCA quantitation in human tissues, peer-reviewed isomer distributions have yet to be published. Only two publicly available studies have investigated PFCA isomers in biota. One study was conducted by 3M where 4 pooled human blood serum samples were analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) for PFCAs and PFOS; however, only adequate separation between linear isomers and combined branched isomers was accomplished and the small sample size proved difficult to assess trends in isomer pattern[25]. Previously, we utilized a gas chromatography (GC)-based method for analysis of PFCAs in Arctic polar bear livers[26]. GC has greater resolving power and avoids contamination that has been found with LC systems. In this paper we present a novel method for PFCA quantification and isomer analysis in blood. Investigation of PFCAs in human blood provides an effective and convenient index of exposure because perfluorinated alkyl acids accumulate in liver and blood as opposed to lipids[27]. Sixteen pooled human blood serum samples from North America were analyzed to identify any consistent isomer patterns for possible source elucidation.

4.2 Materials and Methods

4.2.1 Standards and Reagents.

3M Company (St. Paul, MN) provided an authentic ECF PFOA standard (95.2%). Perfluorononanoate (PFNA) (97%), perfluorodecanoate (PFDA) (98%), perfluoroundecanoate (PFUnA) (95%), and perfluorododecanoate (PFDoA) (95%) standards were purchased from Sigma-Aldrich (Oakville, ON, Canada). Isopropyl PFNA ($(CF_3)_2CF(CF_2)_5COOH$) and $^{13}C_4$-perfluorooctanoic acid ($^{13}C_4$ PFOA)internal standard were supplied by Wellington Laboratories (Guelph, ON, Canada). Extraction and derivatization reagents with supplier and purity are listed in Appendix A.

4.2.2 Blood Serum Samples.

Sixteen human blood serum samples were obtained from commercially available sources. All were classified as off-the-clot sera, meaning the blood was allowed to coagulate naturally after collection instead of introducing an anticoagulant agent to defribinate plasma. Fifteen samples, with no overlapping donors, were from Golden West Biologicals (Temecula, CA). Each sample pool consisted of at least 10 individual donors, varying in age (18 to 70 years old), gender, and blood types, collected across the Midwest USA between 2004 and 2005. One human serum sample pooled from 1000-1500 males with type AB blood was purchased from Sigma Aldrich but no further donor information was supplied. By using pooled serum samples, we hoped to obtain representative population-based estimates, diminish the effect of biological and geographical variance, and reduce analytical costs. Furthermore, biosafety risks were lowered by using samples from a commercial supplier that screens for Hepatitis and HIV; however, because individual donor information was not available, we cannot eliminate the possibility of having donors with occupational exposure. In addition, we cannot be certain that source approximation based on these samples is equivalent to regions in North America beyond the Midwest sampling area.

A brief description of the methodology and QA/QC follows. Further details of the PFCA extraction, derivatization, GC method, calibration curves, and spike and recovery data are in Appendix A.

4.2.3 Extraction and Derivatization of PFCAs from Sera.

For each analysis, 4 ml of human blood sera was used. The employed method of PFCA extraction using the ion-pairing agent tetrabutylammonium hydrogen sulfate [8] and derivatization by 2,4-difluoroaniline[26] has been described in earlier studies. Briefly, PFCAs in blood extracts were acidified and derivatized to the 2,4-difluoroanilide analogue by addition of 1,3-dicyclohexylcarbodimide and 2,4-difluoroaniline. After silica gel clean up, the derivatized PFCAs in toluene (1.5% 2-propanol, 1.5% 1-hexanol) eluant were concentrated to a solvent volume of 0.50 ml using a slow stream of N_2 gas.

4.2.4 Gas Chromatographic-Mass Spectrometric (GC-MS) Determination of derivatized PFCAs.

The PFCA derivatives were determined using GC-MS via negative chemical ionization (GC-(NCI) MS). An HP 6890 series GC with an HP 5973 MSD (Hewlett-Packard, Palo Alto, CA) was employed using an HP7683 series injector to make 1.00 ul pulsed pressure splitless injections onto an RTX-35 column (105 m x 0.25 mm, 0.50 um film thickness) from Restek Corporation (distributed by Chromatographic Specialties Inc., Brockville, ON, Canada).

Detection was conducted by single ion monitoring (SIM) of the $[M-20]^-$ ion (PFOA 505 m/z, $^{13}C_4$-PFOA 509 m/z, PFNA 555 m/z, PFDA 605 m/z, PFUnA 655 m/z, PFDoA 705 m/z, and PFTrA 755 m/z) using methane reagent gas. We report, as branched isomers, any peaks appearing at the same m/z as the linear PFCA.

4.2.5 QA/QC.

Linear PFCA isomers were quantified in sixteen pooled human serum samples using a matrix-matched standard calibration curve. Matrix-matched standards were prepared using PFCA standards and human blood serum from Sigma-Aldrich. PFCA standards at masses of 0.5, 1, 2, 5, 10, 20 and 40 ng were spiked into 4 ml human blood serum, extracted and derivatized. As an internal standard, $^{13}C_4$-PFOA was employed. Native amounts of PFCAs (0.93 ng/ml PFOA, 0.17 ng/ml PFNA, and 0.05 ng/ml PFDA) in the Sigma-Aldrich serum serving as matrix for standards, were corrected for in the construction of the calibration curve and represented the limits of quantitation (LOQs) for this method. All of the 15 pooled serum samples from Golden West Biologicals were found to contain PFCAs at levels at least three times as high as those in the Sigma-Aldrich serum. Although the endogenous PFOA, PFNA, and PFDA levels in the Sigma-Aldrich serum restrict LOQ values, it was found that 0.05 ng/ml PFUnA spiked into the control serum gave a relative response factor consistent with the PFUnA calibration curve and its chromatographic peak area could be determined with signal-to-noise ratio (S/N) of 3. Calibration curves for PFNA, PFDA, PFUnA, PFDoA, and the 8 resolved isomers of an ECF PFOA standard demonstrated acceptable linearity (R^2>0.99). Because individual isomer standards were not available, the calibration curves for each isomer of the standard were plotted against x-axis values of 0.5, 1, 2, 5, 10, 20 and 40 ng. Near completion of this project, an

isopropyl PFNA standard became available. External calibration curves for derivatized linear PFNA and isopropyl PFNA branched isomers were constructed and found to yield equivalent detector response.

Recovery of ECF PFOA isomers was investigated in triplicate by spiking 10 ng of ECF PFOA into 4 ml of human serum. Adequate recovery of each isomer was found (mean 88%) with reasonable precision, as judged by RSD<0.1. Precision was assessed by six replicate extractions and derivatization on a single pooled serum sample. Mean linear PFOA was calculated to be 0.93 ng/g with standard deviation ± 0.04 ng/g (RSD 0.04). Also, recovery of longer chain PFCAs at lower levels, 3 ng PFNA, 0.9 ng PFDA, and 0.2 ng PFUnA, from 4 ml of human serum was found to have acceptable mean % recovery: 115% ± 13 RSD, 107%± 15 RSD, and 97 ± 11 RSD, respectively.

In this study, the lowest standard used was an addition of 0.5 ng ECF PFCA to 4 ml Sigma-Aldrich serum. At this level, isomers L, 1, 3-7 (labels correspond to Figure 4.1) could be determined. Without purified isomer standards of individual ECF branched PFOA isomers, it is difficult to report limit of detection (LOD). For isopropyl PFNA, which was available, a 0.05 ng/ml standard could be detected with a S/N ~3. Any peaks with S/N<3 were considered <LOD.

4.3 Results and Discussion

Our GC method was previously optimized to identify PFCA isomer profiles in polar bears[26]. Here we have adapted the GC method to also permit quantitative determination of PFCAs in biological samples. Several measures were taken to reach the lower detection limits. Volatilization of the derivative was avoided by adding a small amount (1.5%) *1*-hexanol to the toluene solvent system. This was also found to decrease chromatographic peak width and enhance isomer resolution. Significant improvement in detection limit was achieved by using NCI detection. Electron impact ionization (EI), positive chemical ionization (PCI) and NCI were tested and detection limits were found to improve by an order of magnitude in the following series: PCI, EI, and NCI. The most abundant ion in NCI mode for each of the 2,4-difluoroanilide PFCA derivatives was $[M-20]^-$, corresponding to the loss of HF and has been reported for PFCA ester derivatives[28].

Resolution of nine structural isomers of an authentic ECF PFOA standard was accomplished using this method. A magnified view of the ECF PFOA chromatogram is shown in Figure 4.1. This resolution is superior to what we previously reported for PFCA isomer separation due to the increased GC column length and adjustments to the solvent system[26]; the resolution substantially exceeds that achievable by current LC-based methods.

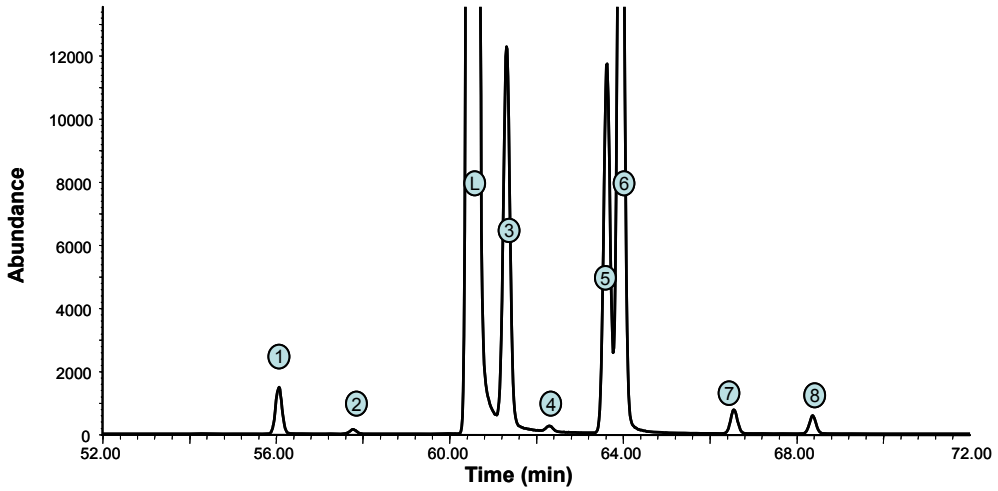

Figure 4.1. Magnified view of GC-(NCI)MS chromatogram of derivatized ECF PFOA standard by SIM of [M-20]. Nine constitutional isomers are resolved and "L" labeled peak represents linear isomer (80% of total PFOA).

4.3.1 Determination of Linear PFCAs in Serum.

In addition to isomer resolution, linear PFCAs were quantified in human serum. In every sample, linear PFOA, PFNA and PFDA were detected and demonstrated the following consistent trends. The dominant PFCA was PFOA (mean 4.4 ng/ml, range 0.9 to 8.6 ng/ml, standard deviation 2.1 ng/ml), followed by PFNA (mean 0.77 ng/ml, range 0.17 to 1.2 ng/ml, standard deviation 0.03 ng/ml) and PFDA (mean 0.17 ng/ml, range 0.05 to 0.25 ng/ml, standard deviation 0.05 ng/ml). Linear PFUnA was detected in two of the samples at 0.048 and 0.067 ng/ml. Although PFDoA and PFTrA were screened, neither was observed above LOD in the available sample set.

Perfluoroalkyl acids in human tissues have previously been reported in the scientific literature[8-12]. Without exception, literature-reported values were acquired using LC-based

methods and the majority of these studies have focused on quantification of the perfluorooctyl acids, PFOS and PFOA, with generally an absence of thorough screening and reporting of longer chain PFCAs. Although our results were acquired with a significantly different method, PFOA levels reported here (mean 4.4 ng/ml) agree well with those found in a study by Olsen et al. who reported mean PFOA concentration 5.5 ng/ml from a population of 645 human samples collected in 6 locations across the USA[9]. The longer chain PFCA concentrations correlate well to the PFCA levels determined in adult human blood samples by Kuklenyik et al.[10] and Guruge et al.[12] from urban populations in the US and Sri Lanka respectively. It should be noted that Kuklenyik et al. reported higher levels in individuals from Atlanta, GA, USA: PFDA (mean 0.67 ng/ml with detection in 75% of the samples), PFUnA (mean 0.80 ng/ml detected in 85% of the samples) and PFDoA (mean 1.35 ng/ml detected in 10 samples)[10], which may correspond to proximity of point source. Stock et al. found elevated concentrations of fluorotelomer alcohols and perfluoroalkylsulfonamide compounds in air samples from Griffin, GA, USA, approximately 60 km away from Atlanta and a major carpet production/treatment location for North America[29].

The PFCA profile in Arctic biota was consistent with a well-described atmospheric degradation pathway of fluorotelomer alcohols[20]. The Arctic data can be compared to the PFCA pattern observed in humans to shed light on potential sources. In Arctic biota, an even-odd pattern of PFCAs was observed where those with an odd number of carbons were more abundant than the preceding even-numbered PFCA i.e. PFNA>PFOA, PFUnA>PFDA, and PFTrA>PFDoA[13]. After taking into account bioaccumulation potentials of each PFCA[14,30], this pattern is consistent with a proposed atmospheric degradation mechanism of fluorotelomer alcohols yielding a suite of PFCAs[20]. Analogous mechanisms have been identified for perfluoroalkylsulfonamides yielding perfluoroalkyl sulfonates and PFCAs[22,23].

Fluorotelomer alcohols (*n*:2 FTOH corresponding to $F(CF_2)_nCH_2CH_2OH$ where *n* is an even-numbered integer) and fluorotelomer olefins (i.e. $F(CF_2)_nCH=CH_2$) are industrially synthesized by telomerization for use as synthetic intermediates for a variety of products including surfactants and polymers[15,24]. Production of PFCAs from fluorotelomer olefins under realistic environmental conditions has not been observed; however, a smog chamber experiment demonstrated potential for atmospheric reactions to form perfluoroalkylaldehyde

$F(CF_2)_nC(O)H$[31]. This would presumably further react according to the mechanism proposed for FTOHs[20] to yield a suite of PFCAs, although further studies are required to confirm this.

Perfluoroalkylsulfonamides are synthesized by ECF and have been used in a wide range of applications including surface treatment and fire-fighting foams. Both FTOHs and perfluoroalkylsulfonamides are volatile, have been detected in the outdoor and indoor air[29,32,33], and have potential for long range transport. Thus, two classes of precursor compounds exist for PFCAs, the fluorotelomers and perfluoroalkylsulfonamides.

In human tissues, PFOA was found to be the most abundant PFCA, in contrast to the dominance of PFNA in Arctic organisms. Furthermore, an opposite even-odd pattern of PFCAs is present in human blood serum with greater prevalence of the even numbered PFCA: [PFOA]>[PFNA] and [PFDA]>[PFUnA] compared to the Arctic biota observations. This suggests that the routes and sources of human exposure are more complex than abiotic degradation of a volatile precursor.

4.3.2 Determination of PFCA Isomer Profiles.

In addition to quantifying linear PFCAs, structural isomer profiles of PFCAs were investigated in the 16 pooled human blood serum samples (Table 4.1). In each sample, the PFCA structural isomer profiles were dominated by the linear isomer. Minor quantities of branched PFOA and PFNA isomers were also observed in a majority of samples. The PFCA isomer profile for a typical blood serum sample is presented in Figure 4.2.

Detection limits were an obstacle for determining branched isomers with confidence in the longer chain acids due to the low levels of linear PFCAs (mean < 0.2 ng/ml). No branched PFDA was detected but in one of the two samples containing PFUnA, a single branched isomer peak was observed representing 2.3% of this PFCA. The GC-MS chromatogram for this particular human blood serum sample is presented in the Appendix A.

In comparing the isomer pattern identified in the ECF PFOA standard, human blood sera was found to contain the following PFOA isomers (as labeled in Fig. 4.1): L, 1, 3, 5, and 6 whereas isomers 2, 4, and 7 were consistently <LOD. As with the ECF PFOA standard, the

branched isomer profile of PFOA was dominated by isomer 6 followed by isomer 3, 5, and 1 respectively in human blood samples (shown in Table 1). Assuming an absence of isomer discrimination, comparison of 97% linear PFOA in human blood (maximum observed) to 80% linear PFOA in ECF standard implies 11% of the PFOA in humans is ECF derived and the remaining 89% is from a solely linear source.

The ability to constrain sources of PFCAs in humans by measuring branched isomers will depend on conservation of the isomer patterns within biological systems. Biological ECF isomer (PFOA and PFOS) discrimination has yet to be reported in peer-reviewed scientific literature. A 3M report from 1998 shows 22% ± 3% branched PFOS in an ECF PFOS standard compared to 41% ± 4% branched PFOS in 55 blood samples collected across the USA in 1998[34] and in a more recent presentation at the 2005 Fluoros Symposium, PFOS isomer signatures in 40 human serum samples from Australia and Sweden were found to be comprised of 30 - 42% branched PFOS compared to ~ 20% branched PFOS in an ECF PFOS standard[35]. However, in a conference abstract by Ehresman et al. [36], it was reported that only linear PFOA was observed by LC-MS/MS in sera of retirees who formerly worked at an ECF fluorochemical manufacturing facility where exposure was to both linear and branched isomers with blood PFOA levels (ranged from 70 000 to 5 100 000 ng/ml) vastly exceeding the general population's mean 5.5 ng/ml[9]. It is unknown whether ECF PFOA isomers behave in an opposite manner compared to ECF PFOS isomers in biological systems. These conflicting results emphasize the need for pharmacokinetic studies of ECF isomers.

Table 4.1. Mean, RSD, range of observed PFCA isomer distribution in 16 pooled human serum samples and ECF PFOA standard.

	% PFOA isomer composition					% branched PFOA (total)	% branched PFNA	% branched PFDA	% branched PFUnA
	Isomer L (linear)	Isomer 1	Isomer 3	Isomer 5	Isomer 6 (isopropyl)				
Pooled human sera (N=16)									
Mean	97.99	0.07	0.28	0.15	1.51	2.1	1.6	-	2.3
RSD	0.01	0.3	0.3	0.3	0.4	0.3	0.3	-	-
Maximum	98.76	0.11	0.41	0.23	2.20	3.0	2.2	-	2.3
Minimum	97.33	0.04	0.17	0.07	0.85	1.2	<LOD	-	<LOD
# samples with isomer > LOD	16	16	16	16	16	16	13	0	1
ECF PFOA standard (N=6)									
Mean	79.6	1.2	4.6	3.9	9.9				
RSD	0.01	0.3	0.08	0.07	0.06				

Note: Mean and RSD calculated using samples with branched PFCAs>LOD. In ECF PFOA standard, mean % and (RSD) for other branched isomers: isomer 2 0.1 (0.04), isomer 4 0.07 (0.3), isomer 7 0.4 (0.2) and isomer 8 0.3 (0.2) determined by 6 replicate derivatizations.

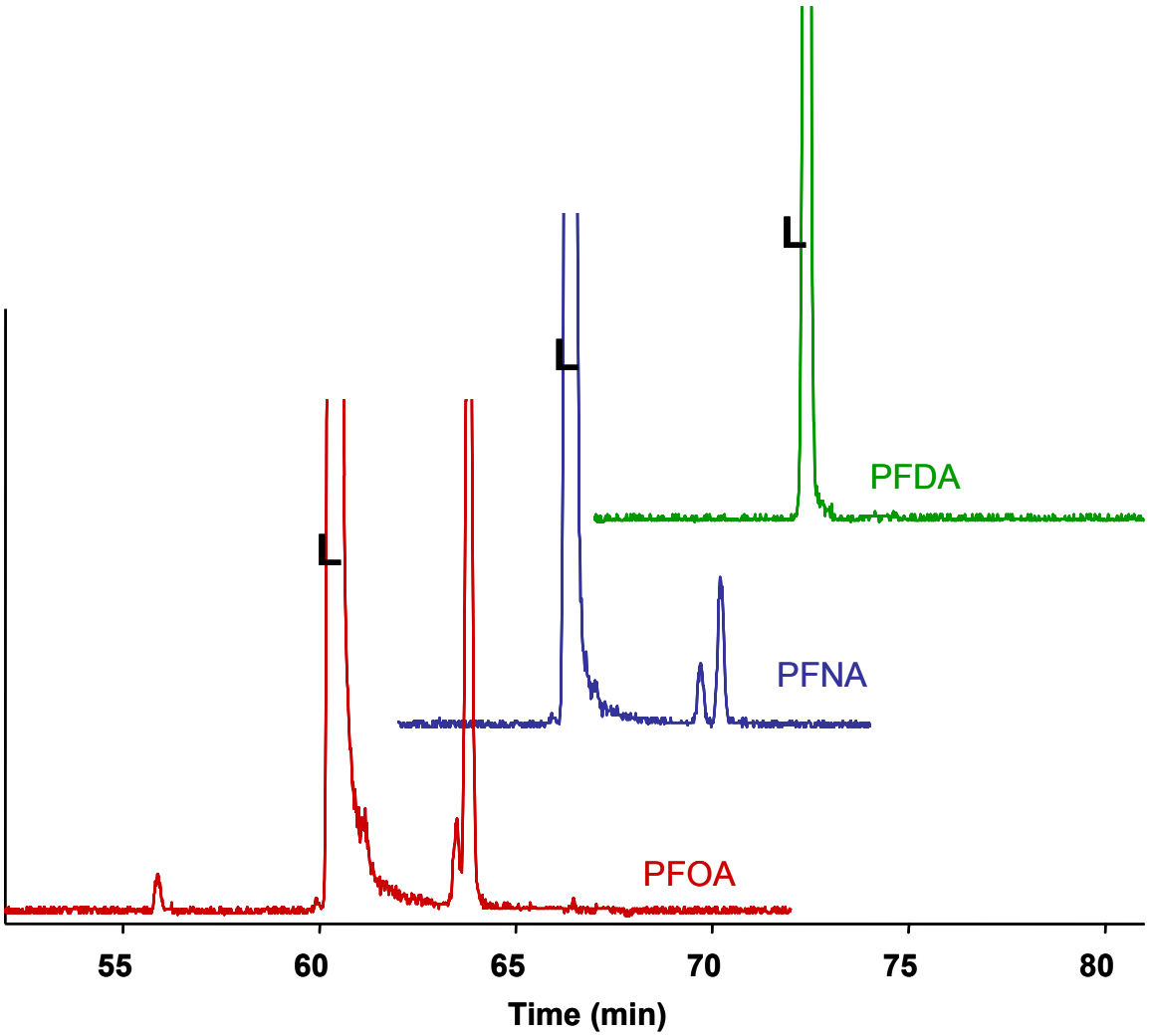

Figure 4.2. PFCA Isomer profile in pooled human blood serum sample. Peaks labeled with "L" correspond to linear isomer and smaller peaks are ascribed to branched isomers. PFCA levels in this sample were found to be 4.53 ng/ml PFOA, 0.98 ng/ml PFNA, and 0.22 ng/ml PFDA. The isomer profile consisted of 2.5% branched PFOA and 1.8% branched PFNA.

Behaviour of chemicals containing a perfluoroalkyl tail is likely related to geometry and branching may lead to differing steric and electronic effects[37]. PFCA chain length has been shown to influence urinary excretion[38], bioaccumulation[14,30] and toxicity in organisms[39]. Shorter chain PFCAs are more rapidly eliminated[38]. Similarly, branched isomers, which have fewer carbons in the linear portion of the chain, may behave differently compared to the linear isomer. If isomer discrimination leads to selective retention of linear PFOA, the structural isomer profiles determined in biological tissues will not allow comprehensive source identification. The presence of four branched PFOA isomers in every human serum sample of this study indicates that complete branched isomer discrimination is not occurring; this trend also appears to hold true for PFNA. This suggests that ultimately we cannot conclude whether isomer discrimination is significant or even occurring for PFCAs.

The branched isomer pattern of PFNA showed similarities to that of PFOA in human blood and the ECF PFOA standard. Two branched PFNA peaks were apparent in each blood sample (that contained branched PFNA>LOD) at 69.83 min and 70.34 min with relatively greater abundance of the later eluting peak. This peak at 70.34 min was determined to be the isopropyl PFNA branched isomer by comparison to its standard chromatogram (in Appendix A). By analogy, PFOA branched isomer 6 presumably corresponds to the isopropyl branched isomer. Further evidence is supported by the 9.9% composition of isomer 6 in the ECF PFOA standard by GC-(NCI)MS which agrees well with the reported composition value (9.0%) for isopropyl PFOA in the certificate of analysis (based on ^{19}F-NMR studies)[25].

The similarities between branched isomer profile in the ECF PFOA standard and human blood sera PFOA and PFNA isomer signatures are indicative of ECF input. We suggest direct exposure to ECF PFOA to be a source of the branched PFOA isomers in humans. 3M has produced PFOA by ECF from 1969 to 2002[40] and was its major manufacturer in the US until production was phased out[15]. From 1992 to 2002, the annual ECF PFOA production was ~ 115 000 kg with more than 95% of this being sold to other companies for fluoropolymer processing[40]. In addition, some exposure to ECF PFOA isomers may occur via perfluorooctanesulfonyl fluoride ($F(CF_2)_8S(O)(O)F$)-based materials, which was made industrially by 3M and is known to contain residual quantities of PFOA[9]. Many fluorochemicals, such as PFOS and perfluorooctylsulfonamides, are derived from this ECF compound. Intentional large-scale industrial production of ECF PFNA has not, to our

knowledge, been documented. It could potentially be a minor byproduct in ECF PFOA; however, no other PFCAs (C>8) were identified in the ECF PFOA standard used in this study and others[25].

Perfluorooctylsulfonamides are not expected to be a significant source of PFOA and PFNA in the general human population. While perfluorooctylsulfonamides are known to undergo biotic reactions producing PFOS, PFCA production by this route has not been demonstrated[41,42]. Smog chamber reactions of perfluoroalkylsulfonamides producing PFCAs are applicable to rural or remote locations with little air pollution (low NO_x levels) [22,23].

The even-odd pattern of PFCAs observed in human sera, coupled with their structural isomer patterns being heavily dominated by the linear isomer, suggest significant contributions from indirect exposure to a linear source. Telomer production (assumed to be linear) is dominated by eight fluorinated carbon chemistry, which makes up 46% of telomerization production; ≥ 10 fluorinated carbon chemistry collectively represents 33% while 4- and 6 fluorinated carbon chemistry together make up 20% of telomer production[43]. Metabolism of 8:2 FTOH is known to produce PFOA[16-19]. In mammals such as rodents, minor quantities of PFNA were also observed, representing an additional minor pathway to odd chain PFCAs in advanced organisms[19]. Therefore, assuming no isomer discrimination, the data is supportive of inhalation or ingestion and subsequent metabolism of the major component 8:2 FTOH to be a significant contributor to the observed linear PFOA (major) and PFNA (minor) observed in humans. Analogously, less abundant 10:2 FTOH in product formulations may contribute to the presence of PFDA and smaller quantity of PFUnA. Fluorotelomer phosphate surfactants, which are used in food packaging coatings as grease and water repellents, also contain the C_8F_{17} moiety[44]. These may enzymatically or through hydrolysis produce FTOHs and consequently be a source of PFOA. A graphical summary of these sources of human exposure are presented in Figure 4.3. Atmospheric production of PFCAs from telomer precursors and perfluoroalkylsulfonamides has been demonstrated abiotically but it is unclear whether this has a measurable impact on the PFCA pattern detected in humans. Also, although this discussion has focused on FTOHs, fluorotelomer olefins (also linear) may play an analogous role. The determination of a significant indirect source of PFCAs is also consistent with the presence of PFOS in humans since it appears unlikely a direct source of PFOS (i.e. aqueous film-forming

foams for fire-fighting procedures) would be responsible for the abundant presence of the compound in human blood[45].

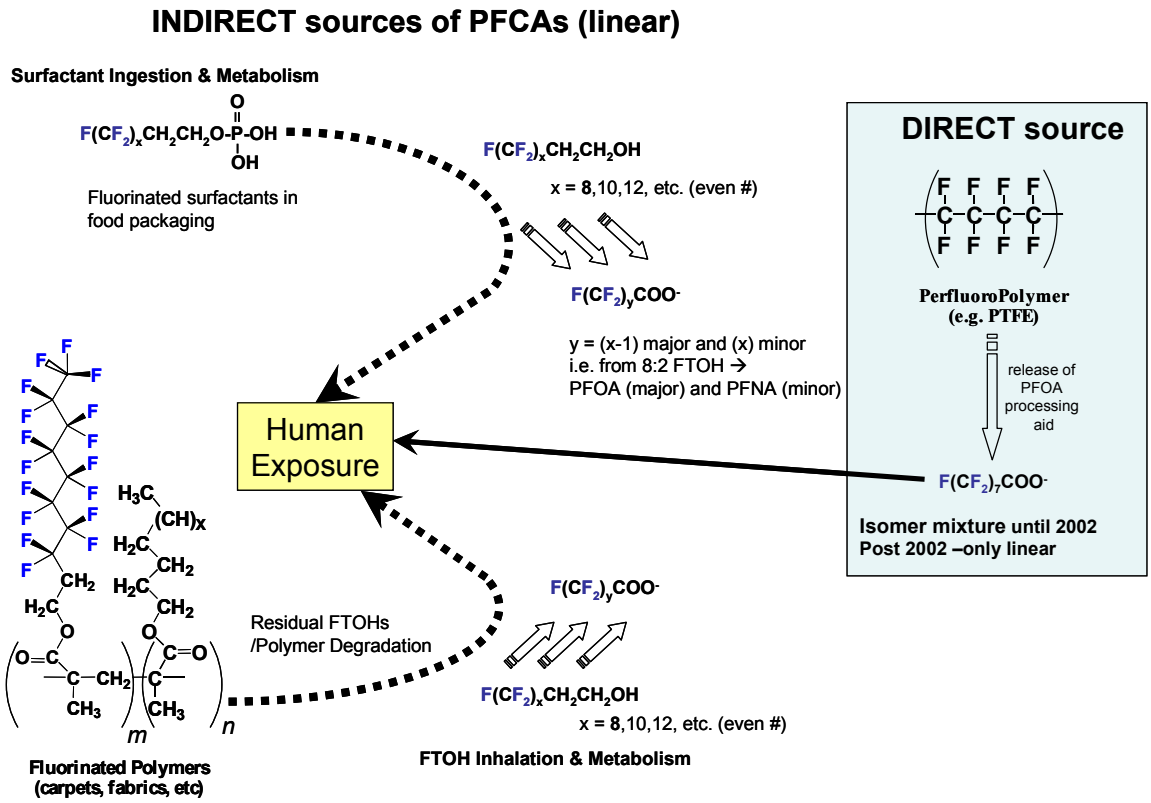

Figure 4.3. Potential indirect and direct sources of PFCAs in humans. Indirect sources may include fluorotelomer phosphates and volatile fluorotelomer precursors (such as FTOHs). Fluorotelomer phosphate surfactants, used in food packaging treatments, may be ingested and undergo hydrolysis or via enzymes produce FTOHs. Fluorinated polymers may yield FTOHs, arising from residual content or polymer degradation, which would be inhaled[46]. Subsequent FTOH metabolism leads to even PFCA (major product) and odd PFCA (minor product). All of these are expected to be linear. Direct sources of PFCAs (PFOA and PFNA) are those used as a processing aid in polymers such as PTFE and PVDF. ECF PFOA, a source of branched isomers was employed until 2002 for this purpose. Post 2002 linear telomer PFOA was used. Telomer PFNA has also been manufactured and employed for polymer treatment [15].

4.4 Acknowledgements

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CHAPTER FIVE

Disposition of Perfluorinated Acid Isomers in Rats; Part II Subchronic Dose

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Contributions – Amila De Silva was responsible for care and handling of animal subjects, preparation of the administered dose, sample acquisition, analysis of perfluorocarboxylate isomers and data interpretation. Jonathan Benskin conducted PFOS isomer determination in samples. Gilles Arsenault, Robert McCrindle, and Nicole Riddell prepared and provided purified isomers of PFOS and PFOA for identification of ECF composition. Each author contributed to the design of the study and provided critical comments to the manuscript prepared by Amila De Silva.

5.1 Introduction

Perfluorooctanoate ($C_7F_{15}C(O)O^-$, PFOA) and perfluorooctane sulfonate ($C_8F_{17}SO_3^-$, PFOS) are two perfluoroalkyl acids (PFAs) with unique properties that have lead to their large-scale manufacture and widespread use. These properties include low surface tension, water and oil repellency, and chemical and thermal stability. 3M (St. Paul, MN) was historically the major manufacturer of PFOA and PFOS using electrochemical fluorination (ECF), but due to environmental concerns, 3M voluntarily phased-out their perfluorooctyl-based chemistries by ca. 2002[1]. Industrial production of PFOA continues today by a separate process, telomerization[1]. In addition, various related fluorochemicals, of telomer or ECF origin, may be environmental precursors to PFAs through atmospheric or biological processes.

Telomer and ECF derived fluorochemicals can be distinguished based on isomeric composition. ECF fluorochemicals are an impure mixture of structural isomers[2], typically, dominated by the *n*-isomer (i.e. linear) and with several branched perfluoromethyl isomers [2]. For example, 3M's ECF PFOS was characterized as having 70% *n*-isomer, 10 – 11% isopropyl isomer, 18.6 % mono-CF_3 branched, 0.15% di-CF_3 branched, and 0.2% *tert*-perfluorobutyl branched[3,4]. Similarly, the isomer distribution of 3M ECF PFOA, which contains one fewer perfluorocarbons than PFOS, reportedly consists of 78% *n*-isomer, 12.6 % mono-CF_3 branched isomers, 9% isopropyl branched, 0.1% di-perfluoromethyl branched, and 0.2% *tert*-perfluorobutyl branched isomers[5]. Several batches of ECF PFOA and PFOS were manufactured by 3M, but isomer characterization of these indicated a high degree of batch-to-batch consistency for ECF PFOA (78% *n*-isomer ±1.5% RSD, N = 18 different lots) and PFOS (70% *n*-isomer ± 1.6% RSD, N = 8 different lots)[6]. Unlike ECF, telomerization does not produce isomer mixtures, and the resulting perfluorocarbons are largely considered to contain linear geometry. In telomerization, a perfluoroalkyl radical starting material (typically linear) adds to tetrafluoroethylene[2], thus telomer compounds retain the geometry of the starting material.

Despite these direct manufacturing sources of PFAs, they are also considered secondary pollutants arising from abiotic and biotic reactions of precursor compounds in the environment [7-15]. Such precursors include ECF perfluorooctyl sulfonamides, fluorotelomer alcohols (FTOHs), and fluorotelomer olefins. Akin to other telomerization products, FTOHs and

fluorotelomer olefins are straight chain (assuming starting materials have linear perfluorocarbon arrangement) whereas ECF perfluorooctylsulfonamides possess a typical ECF signature of linear and branched isomers, analogous to ECF PFOS.

Of the PFAs detected in human blood, the most dominant is PFOS followed by PFOA([16-19], reviewed in[20]). Human exposure to PFOS may arise from a combination of direct and indirect sources. For example, indirect exposure could occur via inhalation or ingestion of perfluorooctylsulfonamides and subsequent metabolism[12,13]. Perfluorooctylsulfonamides were applied as phosphate esters in paper coatings, such as for food packaging and plates, and were also incorporated into polymers for treatment of carpet and home-furnishings for soil and stain-repellence. Perfluorooctylsulfonamides have been detected in outdoor air globally[21-24] and Shoeib et al. reported higher concentrations of these compounds in indoor air[25], likely due to the application of these materials in commercial products for use in the home. Perfluorooctylsulfonamide metabolites have been observed in human blood[26]. In the atmosphere, perfluorooctylsulfonamides undergo oxidation, initiated by ·OH, to form perfluorocarboxylates and PFOS[7,10], representing an indirect source of linear and branched PFA isomers to the environment. Human exposure to directly manufactured PFOS is expected to be of low significance because PFOS was known to be only a minor commercial product with the majority of its application in fire-fighting foams[27]. As such, the majority of PFOS in humans is considered to be via perfluorooctylsulfonamide-based surfactants and polymers[27].

Similar to PFOS, human exposure to PFOA may arise from a combination of indirect and direct exposure. ECF PFOA was synthesized from the 1950s to 2002 for use as an emulsifier in fluoropolymer synthesis[1]. Both PFOS and PFOA have been detected in waterways[28,29] , fish[30] and other components of the human diet[31-33], representing direct exposure sources. Indirect exposure to PFOA may occur via ingestion and inhalation of fluorotelomer precursors. Ingestion of fluorotelomer-based surfactants used in food-contact paper such as perfluoroalkyl phosphates[34] may yield perfluorocarboxylates by metabolism[8,11] in humans. Inhalation and subsequent metabolism of FTOHs as residuals in polymers and surfactants[35] also yield perfluorocarboxylates. FTOHs, like perfluorooctylsulfonamides, have been detected globally in air[21,22] with indoor concentrations exceeding outdoor levels[25].

All sources of PFOS exposure are believed to be of ECF origin, and this is supported by the characteristic ECF isomer signature of human serum PFOS[18,36]. Furthermore, five major isomers, characteristic of an ECF source, were identified in human blood. Using LC-MS/MS, Benskin et al. identified *n*-PFOS, 3*m*-PFOS ($CF_3CF_2CF_2CF_2CF(CF_3)CF_2CF_2SO_3^-$), 4*m*-PFOS ($CF_3CF_2CF_2CF(CF_3)CF_2CF_2CF_2SO_3^-$), 5*m*-PFOS ($CF_3CF_2CF(CF_3)CF_2CF_2CF_2CF_2SO_3^-$) and *iso*-PFOS ($(CF_3)_2CFCF_2CF_2CF_2CF_2CF_2SO_3^-$) as well as three other isomers that could not be elucidated without isomer standards[18]. In contrast, isomer patterns of PFOA in human serum are markedly different than a typical ECF signature. Human blood PFOA isomer profiles are dominated by the *n*-isomer (>95%), but in a much greater proportion than what is present in technical ECF PFOA[18,19]. A preliminary interpretation of this PFOA profile, as presented in our past work[19], was that *n*-PFOA in humans largely reflected exposure to a solely linear isomer source, such as fluorotelomers, with only minor exposure to ECF substances. The default assumption at the time was that the isomer signature in serum reflected the isomer signature of the ultimate exposure source, and this assumption was examined here experimentally.

In the current research, the objective was to investigate the disposition of PFA isomers in a sub-chronic exposure using rats as a surrogate for other mammals. This is a complimentary study to part I of this research involving a single dose exposure for the same test substances[37]. Numerous studies have reported pharmacokinetics of PFOS and PFOA, however these generally focused only on the *n*-isomer, or on all of the isomers analyzed together as a single chromatographic peak (reviewed in [38,39]). One exception to this was where *n*-PFOA was compared to bulk branched isomers[40]. In the present study, male and female rats were exposed to ECF PFOS, ECF PFOA, and two isomers of perfluorononanoate ($C_8F_{17}C(O)O^-$, PFNA) in the diet for 12 weeks. By monitoring isomer patterns in blood samples throughout the exposure period and the subsequent clearance period, differences in accumulation potential and elimination rate were determined among isomers. We hypothesized that differential biological processing of PFA isomers would have an impact on the resulting isomer signature in biota at steady state.

5.2 Method

5.2.1 Dose Preparation

Potassium PFOS (lot 217) and PFOA (lot 332), both synthesized by ECF by 3M (Specialty Materials and Manufacturing Division, St. Paul, MN) were used to dose the rat feed. Both normal-chain and isopropyl PFNA (*n*- and *iso*- respectively) were obtained from Aldrich (Oakville, ON) and Wellington Laboratories (Guelph, ON), respectively, and were included in the dose. All PFAs were dissolved in acetone, mixed with Harlan Rat Chow, and the acetone removed using a rotary evaporator. The diet for the control group was prepared in the same manner but without addition of PFAs. Analysis of the spiked food revealed mean concentrations of 425 ng/g *n*-PFOS (374 – 444 ng/g), 404 ng/g ECF PFOA (397 – 416 ng/g), 536 ng/g *n*-PFNA (519-642 ng/g), and 491 ng/g *iso*-PFNA (486 – 546 ng/g) with the range in brackets corresponding to analyses of 6 independently prepared batches. The acetone-treated control feed contained <4 ppb *n*-PFOA, and <5 ppb *n*-PFOS, while all other PFAs, including branched isomers, were <LOQ (LOQ = 1 ng/g). The resultant PFOS and PFOA isomer profiles in the dosed feed were indistinguishable from the ECF standards.

5.2.2 Analytical Chemistry

Extraction of PFAs from whole blood and other rat tissues was performed by ion-pairing with tetrabutylammonium sulfate (TBAS) and extraction into methyl-*tert*-butyl ether (MTBE), as described by Hansen et al.[17]. Tissues were homogenized in sodium carbonate buffer before addition of TBAS and MTBE. Tissues collected and analyzed in this study were not perfused of blood. To facilitate analysis of perfluorocarboxylate (PFOA and PFNA) isomers by gas chromatography (GC), the extract was taken to dryness and the residue derivatized using 2,4-difluoroaniline and 1,3-dicyclohexylcarbodiimide to yield a volatile 2,4-difluoroanilide[19].

The extent of GC separation of perfluorocarboxylate isomers using derivatization has been presented before[41] and is equivalent to that achieved in this study. Eight isomers of ECF PFOA were separated. Linear PFOA, *n*-PFNA, and *iso*-PFNA were quantified in samples using the *n*-isomer standards from Sigma Aldrich (Oakville, ON, Canada), and *iso*-PFNA from Wellington Labs (Guelph, ON, Canada). Mass labeled *n*-perfluorodecanoate ($C_9F_{19}C(O)O^-$,

$^{13}C_{1,2}$- PFDA, Wellington Labs) was used as an internal standard for quantitation of each perfluorocarboxylate by GC-MS. Standards used for quantification and identification were derivatized and analyzed in the same manner as blood samples. Isomers in ECF PFOA were tentatively assigned (Fig.5.1) using semi pure PFOA isomers obtained from Wellington Labs. The sixth, seventh, and eighth eluting isomers could not be elucidated and are henceforth referred to arbitrarily as PFOA-6, PFOA-7, and PFOA-8. Therefore the order of elution of PFOA isomers in ECF PFOA corresponded to 3*m*-PFOA, *n*-PFOA, 4*m*-PFOA, 5*m*-PFOA, *iso*-PFOA, PFOA-6, PFOA-7 and PFOA-8.

Blood samples from one treated male and one treated female were reserved for PFOS isomer analysis, which is not possible by GC. Dried ion-pair extracts were reconstituted in 0.5 ml of methanol. Liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for PFOS isomer characterization, and quantification was performed using labeled *n*-PFOS with 13C1,2,3,4-PFOS as an internal standard (Wellington Labs). Full details of the instrumental parameters are described in Part I[37]. Structural elucidation was achieved using a combination of retention time, and MS/MS fragmentation relative to semi-pure authentic standards (Wellington Labs). The following MS/MS ion transitions were employed for quantitative analysis of identified isomers, in order of elution: *n*-PFOS (499→80), *iso*-PFOS (499→80), 5*m*-PFOS (499→ 130), 4*m*-PFOS (499→330), and 3*m*-PFOS (499→130), 1*m*-PFOS (499→419), *t-butyl*-PFOS (499→130), B_7-PFOS (499→169), B_8-PFOS (499→130), and B_9-PFOS (499→419). B_7, B_8, and B_9-PFOS could not be identified conclusively using available standards.

5.2.3 Animals and Husbandry

Care and treatment of the rats was in compliance with the guidelines of the Canadian Council on Animal Care, and the protocol was approved by the University of Toronto Animal Care Committee. The following environmental conditions applied throughout the course of the study: 12 air changes per hour, 12-h light/day cycle (lights on at 05:00 h), and an ambient temperature of 21–23 °C with a 50–60% relative humidity. Six male (294-302 g) and six female (267-340 g) Sprague Dawley rats were purchased from Charles River Laboratories (Montreal, QC). Two animals from each gender were randomly selected to serve as controls. A two week acclimation period preceded administration of spiked feed. The rats were housed individually to permit measurement of daily feed consumption. Spiked feed was provided *ad libitum* to the test

group of males and females for 12 weeks. After this period, the spiked feed was replaced with unspiked feed for both control and test subjects. Body mass of all animals was monitored weekly.

Whole blood was collected on days 1, 2, 3, 4, 5, 8, 11, 17, 22, 26, 32, 38, 50, 72, and 84 during the exposure phase and on days 1, 4, 8, 10, 15, 18, 29, 40, 50, 61, 65, and 89 of the depuration phase (females only up until day 65) from the lateral tail vein using disposable heparinized syringes. Blood was kept frozen until analysis in centrifuge tubes. On day 38 (1 male control, 1 treated male, 1 female control, 1 treated female), day 149 (3 treated females, 1 control female), and day 172 (3 dosed males, 1 control male), rats were sacrificed and dissected for tissue collection. After being anesthetized by CO_2 inhalation, rats were euthanized by ex-sanguination and cervical dislocation.

5.3 Results and Discussion

5.3.1 Body weight, feed consumption, and liver somatic index

Male and female rats were exposed for 12 weeks via diet to structural isomers of PFOA, PFNA, and PFOS to determine whether preferential uptake and accumulation of certain isomers occurred. Body weight, feed consumption and liver somatic index were monitored as clinical parameters to assess toxicity. In numerous pharmacokinetic studies with PFAs, high dose exposure has been associated with depressed body weights, diminished feed consumption, and enhanced liver size compared to control populations[42,43]. As such, these parameters were monitored in this experiment to determine whether any toxic response was occurring under the treatment conditions that may have influenced the observed pharmacokinetics.

Based on recordings of body mass increase over time, rat weights were fit to the exponential growth model: ln (mass) = (a·t) + b, where a is the exponential growth rate, b is a constant, t is the time in days, and mass refers to the rat body mass in grams. The range of growth rates, up to day 40 in males, was 0.0077 - 0.0107 g/d for treated and 0.0093 - 0.0102 g/d for controls. In both treated and control males, the growth rate slowed considerably by day 40 when the mean mass (± std dev) reached 560 ± 30 g. From day 40 to day 172, male growth rates were 0.0016 - 0.0027 g/d for treated and 0.0025 g/d for the control rat. A single rate of growth,

from initiation of the experiment to the end of depuration, sufficiently described growth in females with $r^2 > 0.90$. Growth rates ranged from 0.0028 - 0.0040 g/d for treated and 0.0022 to 0.0043 g/d for control females. These results demonstrate that body mass, as a clinical parameter, was not significantly different between control and treated animals of either sex. Specific growth rates for rats in this experiment can be found in the Appendix C.

Mean feed consumption rate per rat was measured during the exposure period and did not vary beyond ± 5 g/d over the first 12 weeks, despite rapid growth of the rats during this period (Appendix C). There was no significant difference among treated and control populations, suggesting the rate of feed consumption was not affected by the treatment conditions. Using daily feed consumption and measured PFA concentrations in the feed, daily PFA intake and cumulative PFA ingestion were estimated (Appendix C). Each male rat consumed between 10 and 14 μg of each PFA (i.e. sum of all isomers) per day (total ca. 50 μg PFA/d). Over the same period, female rats consumed between 8 and 11 μg of each PFA per day (total ca. 37 μg PFA/d). Thus the cumulative total PFA dose for males and females during the uptake phase was approximately 4 mg and 3 mg, respectively. Using the average body mass for males and females during the uptake phase, the dose of total PFA was roughly equivalent between the two sexes, 0.1 mg/kg/d (cumulative 7- 9 mg/kg).

Liver somatic index (LSI) was measured for the rats sacrificed at 5 weeks (1 treated male, 1 treated female, 1 control male, and 1 control female), 21 weeks (3 treated females, 1 control female), and 25 weeks (3 treated males and 1 control female) (Appendix C). LSI is defined as the ratio of liver weight to body weight and can be interpreted as a measure of stress and toxic effects by comparing between control and treated populations. Increases in LSI are usually indicative of hyperplasia or hypertrophy as a result of the liver adapting to detoxify xenobiotics. In this experiment, the LSI of the treated group did not exceed that of the control group.

These results were consistent with observations of other researchers. In a study by Perkins et al., male rats administered a daily dose of PFOA for 13 weeks had no differences in weight gain or feed consumption compared to the control group when the PFOA concentration in feed was 1, 10, or 30 ppm[44]. In the same paper, anatomic pathology based on LSI, along with an absence of clinical signs of toxicity suggested that the administered dose was not inducing any toxicity[44]. Seacat et al. reported elevated LSI and a decrease in food consumption in rats

administered 20 μg PFOS/g feed for 14 weeks, which is approximately 40-fold greater than the feed concentration in the current study[42].

The no observed adverse effect level (NOAEL) has not been determined for the mixture of PFAs used in this experiment but literature toxicological threshold data for individual PFOA and PFOS components are as follows: In male rats, NOAEL corresponded to 7.5 ppm PFOA serum concentrations in a 13 week exposure to 1 ppm dietary PFOA[44]. In a 14-week 5 ppm dietary PFOS dose, the NOAEL translated to 44 and 64 ppm serum concentrations in male and female rats, respectively[42]. In the current research, maximum blood concentrations were close to 1 ppm, thus lower than these NOAELs. Taken together, there was no evidence that any toxicological threshold had been reached under the current treatment conditions.

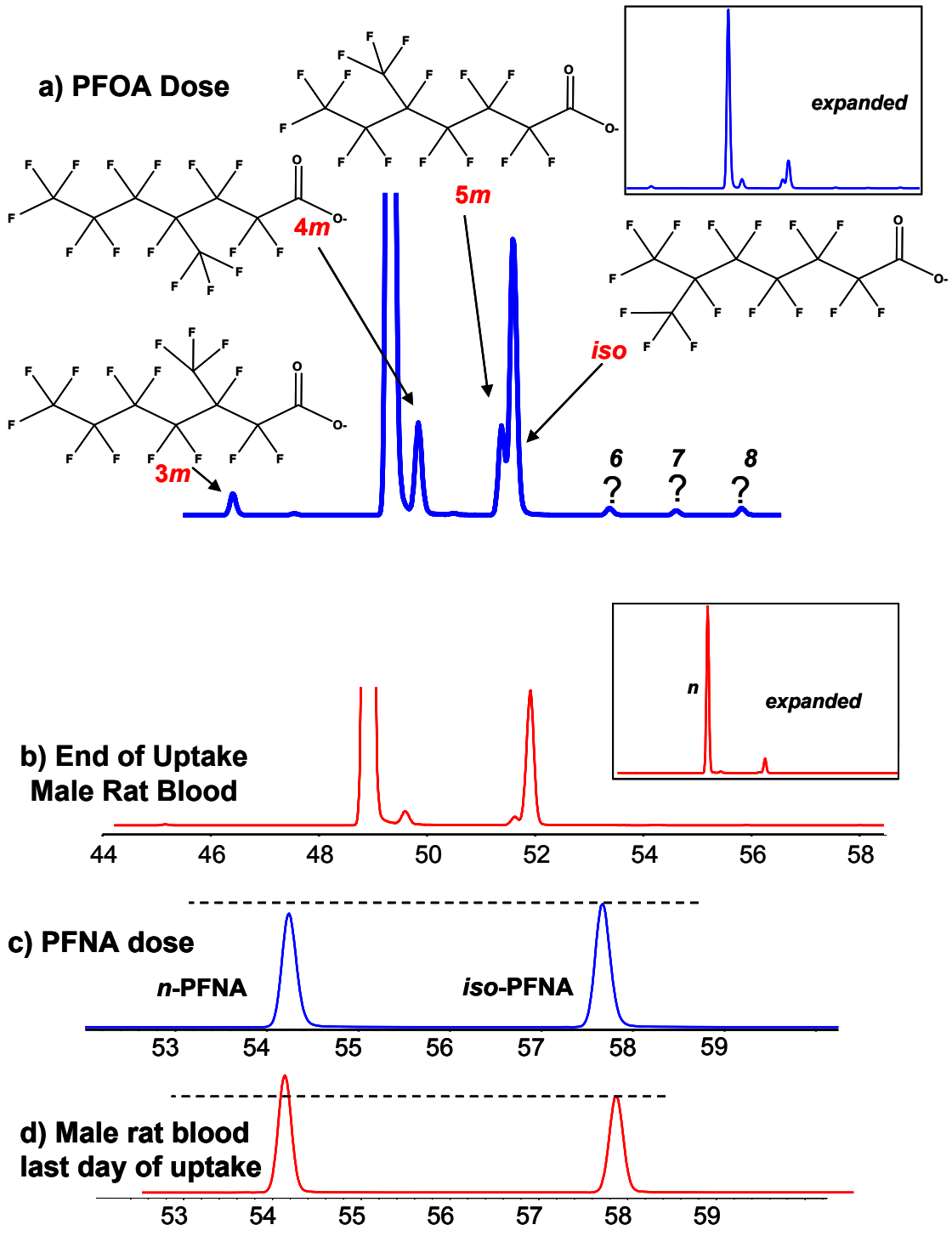

Figure 5.1. PFOA and PFNA isomer profiles in the diet (a and c) and male blood (b and d) at the end of the uptake phase. The dashed lines (c and d) correspond to the height of *iso*-PFNA.

5.3.2 Exposure Phase and Tissue Distribution of PFAs in Male and Female Rats

The time course of concentrations for *n*-PFOS, *n*-PFOA, *n*-PFNA, and *iso*-PFNA in blood during the 12 week exposure phase are shown for treated males and females in Fig. 5.2 and 5.3, respectively. By the end of the exposure phase, perfluorocarboxylate isomers appeared to reach steady state in male blood as judged by a plateau in concentrations between days 50 and 84. On day 84, the last day of exposure, mean (± standard error of the mean, SEM) male blood concentrations were 856 (±326) ng/g *n*-PFOA, 1550 (±43) ng/g *n*-PFNA, 1290 (±127) ng/g *iso*-PFNA, and 1010 ng/g *n*-PFOS. PFOS in large volume blood samples collected at the termination of the experiment revealed *n*-PFOS concentration did not vary greater than 7% relative standard deviation across 3 rats (standard deviation/mean*100 for N=3 per sex, RSD). The time course of concentrations for the other branched isomers of ECF PFOA and PFOS in male rat blood were also recorded during the exposure period (Appendix C). All eight PFOA isomers, and nine PFOS isomers were present in male rat blood at steady state, indicating that each isomer was bioavailable from the diet to some extent.

In female blood, the apparent steady state for *n*-PFNA was achieved earlier than males with the concentrations leveling by day 20 (Fig. 5.3) compared to >60 days in males. Steady state concentrations of *n*-PFOS in blood were not achieved for females (Fig. 5.3). This was consistent with published studies which reported slower time to reach steady state levels of PFOS compared to PFOA in both female and male monkeys[45]. At day 84, the mean (± SEM) *n*-PFNA and *n*-PFOS concentration in female rats was 190 (±33) ng/g and 1490 ng/g, respectively. Time course of branched PFOS isomers in female rat blood can be found in the Appendix C. In general PFOS isomer uptake proceeded similarly in males and females, but not all PFOS isomers appeared to reach steady state in females. For *iso*-PFNA and *n*-PFOA, even when considering female rats on an individual basis, no consistent increasing trends were observed during the exposure period, suggesting relatively rapid clearance of each (Fig. 5.3). In blood, branched PFOA isomers were <LOD (LOD = 0.3 ng/ml) in females, and as such the extent of uptake and depuration kinetics could not be determined. The intermittent detection of *n*-PFOA and *iso*-PFNA was likely due to short half-lives as discussed further below.

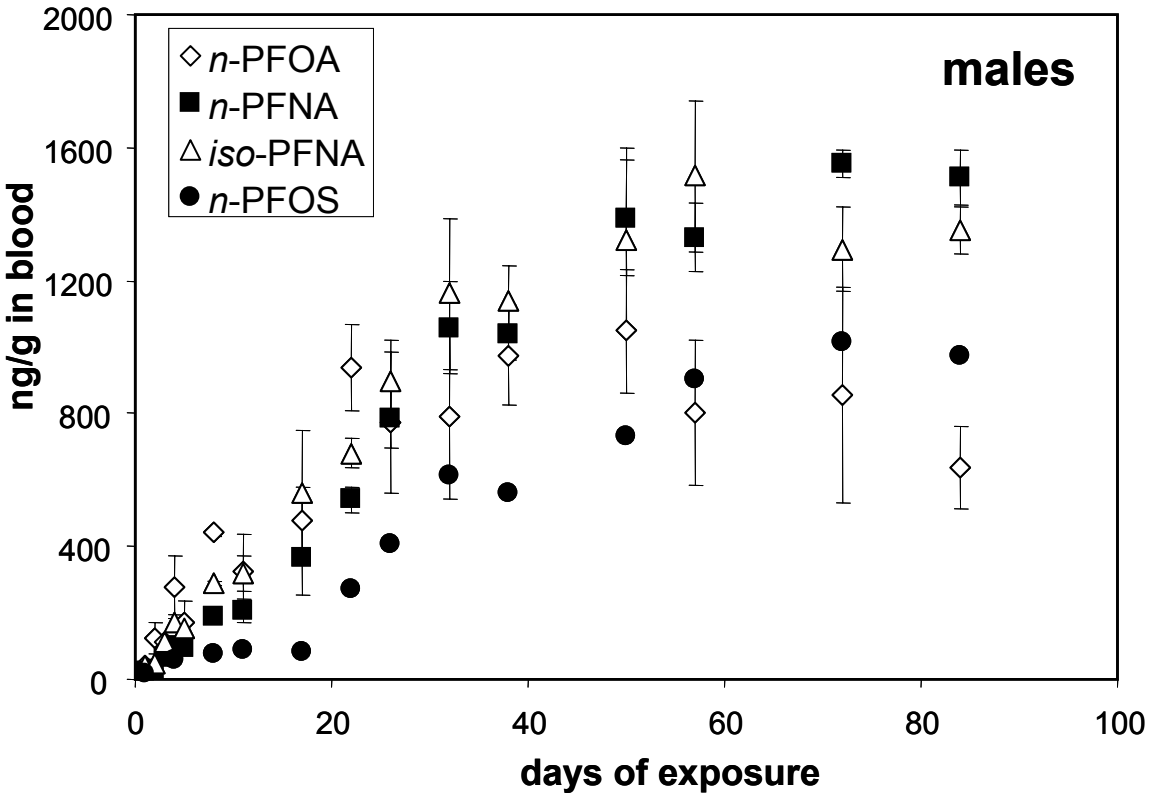

Figure 5.2. Concentrations of *n*-PFOA (◇), *n*-PFNA (■), *iso*-PFNA (△), and *n*-PFOS (●) in male rat blood during the 12 week treatment period. Error bars represent standard error of the mean, SEM. For PFOA and PFNA, each data point is the mean of 4 rats up to day 38 at which point one treated rat was sacrificed and the remaining points represent the mean concentration in 3 rats. PFOS analysis was conducted on separate samples by LC-MS/MS using blood samples from one individual (hence, no error bars).

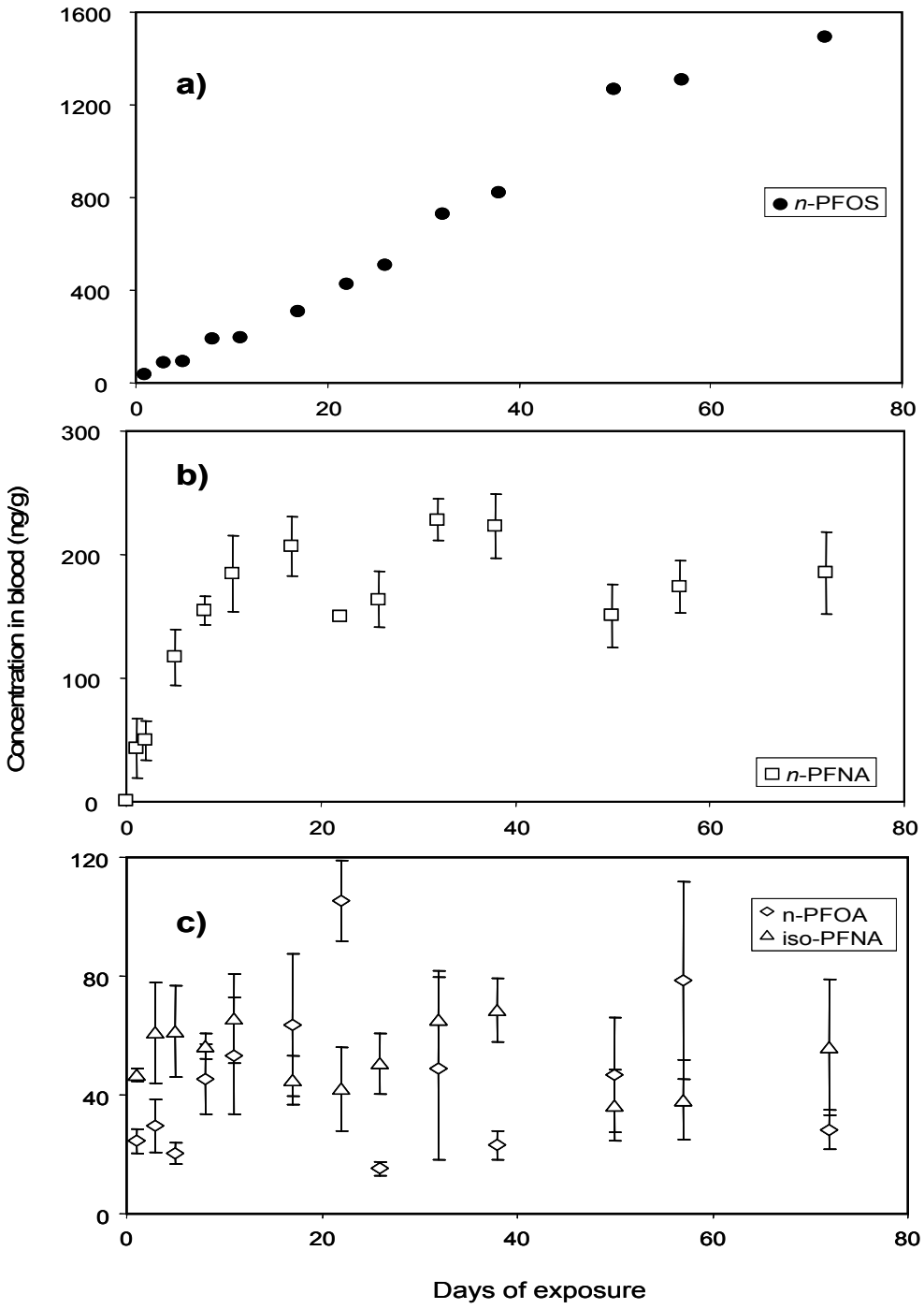

Figure 5.3. Concentrations in female rat blood during the 12 week uptake period for (a) *n*-PFOS (b) *n*-PFNA and c) *n*-PFOA and *iso*-PFNA. Error bars represent SEM. PFOS measurements were only made in one rat.

The relative extent of PFA isomer accumulation was assessed in day 35 tissue samples, and at the end of the exposure phase on day 84. We did not have pure standards with which to quantify the absolute accumulation potential of each isomer, thus we calculated a relative

accumulation coefficient (C_{ra}) to quantify the relative propensity of each branched isomer to accumulate relative to the *n*-isomer. This term was defined as the relative response of the branched isomer (B_x) to the *n*-isomer in blood, divided by the relative response of the branched isomer to the linear isomer (Equation 1).

$$C_{ra} = \frac{B_{x(\text{blood})} / n\text{-isomer}_{(\text{blood})}}{B_{x\,(\text{dose})} / n\text{-isomer}_{(\text{dose})}}$$

Equation 1.

Thus, for a particular branched isomer, $C_{ra} > 1$ indicates preferential accumulation potential relative to the *n*-isomer, whereas $C_{ra} < 1$ indicates diminished accumulation compared to the *n*-isomer. The C_{ra} term was calculated on day 35 in various tissues (n=2 rats?), and on day 84 (the last day of the exposure phase) in blood for PFOA (Fig. 5.4), PFNA (Fig. 5.5), and PFOS (Fig. 5.6).

Accumulation of all PFOA isomers was greatest in the liver. In males, *n*-PFOA tissue concentrations at day 35 corresponded to 32 ng/g in fat, 61 ng/g in muscle, 90 ng/g in gonads, 190 ng/g in spleen, 230 ng/g in kidney, 290 ng/g in lungs, 280 ng/g in heart, and 1800 ng/g in liver. Accumulation in liver and diminished partitioning into fat tissue was not surprising given the ability of PFAs to undergo enterohepatic circulation and their unique lipophobic physical properties, respectively. In blood on day 84, as steady-state was achieved or approached, the C_{ra} values were much less than 1 for each branched PFOA isomer indicating that these branched isomers were not as accumulative as *n*-PFOA. In male blood at day 84 among branched isomers, *iso*-PFOA was most accumulative with a C_{ra} of approximately 0.6. The least accumulative was PFOA-7, whereas 3*m*, 4*m*, 5*m*, PFOA-6 and PFOA-8 all had similar mean C_{ra} values ranging from 0.1 to 0.2. In females rats, C_{ra} values in blood at day 84 were much less than in males. In females, the C_{ra} corresponding to 4*m*, 5*m*, and *iso*-PFOA were less than 0.05 and PFOA-7 was <LOD in blood. PFOA-6 and PFOA-8 were most accumulative with C_{ra} values similar to what was observed in male rats.

Isomer profiles were examined in tissues at day 35 but do not likely represent steady state. For example, comparing PFOA C_{ra}'s in blood at day 35 and day 84 (Fig. 5.4) it is clear that the branched isomers continue to be diminished in the latter half of the exposure period, relative to *n*-PFOA. However, the trend in relative isomer accumulation is similar among these two time points for blood. Other tissues at day 35 do not show any remarkable differences from

tissue to tissue in relative accumulation in comparison to blood. The low concentration of PFOA isomers in female tissues and blood, suggests that PFOA was excreted, rather than pseudo-elimination to deep storage sites. In females, the kidney was the only tissue in which the majority of PFOA isomers were observed which could suggest urinary clearance. In earlier studies, urinary excretion was confirmed as the primary elimination route for *n*-PFOA in females and males, but much faster in females[46,47]. Therefore, the lack of substantial accumulation for PFOA (Fig. 5.3c) and the mechanism of preferential branched-isomer excretion (Fig. 5.4b) may both be attributed to rapid urinary elimination in females.

In male rats, relative accumulation of *n*-PFNA versus *iso*-PFNA was not as pronounced as it was for PFOA. At steady state in blood (day 84), the C_{ra} of *iso*-PFNA was approximately 0.8. However, for tissues including blood at day 35, $C_{ra} > 1$. This suggests that day 35 isomer profiles did not represent steady-state conditions and also indicates a faster uptake rate for *iso*-PFNA compared to *n*-PFNA. Although we did not measure tissue concentrations on day 84, based on the relative blood profile on day 84 compared to the blood profile on day 35, it is also anticipated that *n*-PFNA concentrations would be higher than *iso*-PFNA in tissues on day 84.

In tissues of males at day 35 of the exposure period, the greatest site of accumulation of PFNA isomers was the liver. On day 35, concentrations of *n*-PFNA were 64 ng/g in fat, 113 ng/g in muscle, 74 ng/g in gonads, 370 ng/g in spleen, 430 ng/g in kidney, 550 ng/g in lungs, 420 ng/g in heart, and 9900 ng/g in liver. The concentrations of *iso*-PFNA were 67 ng/g in fat, 100 ng/g in muscle, 62 ng/g in gonads, 360 ng/g in spleen, 310 ng/g in kidney, 590 ng/g in lungs, 430 ng/g in heart, and 6300 ng/g in liver. In females, as with males, that the propensity for *iso*-PFNA to accumulate was less than *n*-PFNA (Fig. 5.5 b). For example on day 35 (i.e. at steady-state for PFNA), *n*-PFNA and *iso*-PFNA in female liver tissue corresponded to 1300 ng/g and 370 ng/g, respectively.

Previous research has shown that female rats eliminated *n*-PFCAs faster than males, but within each gender there was a consistent tendency for increasing retention and diminished urinary elimination with perfluoroalkyl chain length among *n*-isomer homologues[47]. These differences between the sexes were ascribed to urinary excretion because, regardless of chain length and gender, one study demonstrated only 2 to 5% of an *n*-PFOA dose was excreted in feces in rats[47]. These results were corroborated in Part I,[37] such that urinary elimination

accounted for 91-96% *n*-PFOA, 32-35% *n*-PFNA, and 14-30% *n*-PFOS of the total excreted product, with the remainder being eliminated in feces.

In blood at the end of the exposure phase, C_{ra}'s for PFOS were similar between sexes (Fig. 5.6). A number of branched PFOS isomers, including *iso*, 5*m*, 1*m*, and B7-PFOS had a C_{ra} exceeding 1, indicating greater accumulation than *n*-PFOS. However, over-interpretation of the C_{ra} measurements should be avoided for PFOS considering that there was no strong evidence for steady state being reached in males or females for any isomer in blood by day 84. In most tissues at day 35 and in blood on day 84, 1*m*-PFOS had the greatest propensity to accumulate based on its high C_{ra} in females and males, and consistent with its slow elimination half-life (discussed below). These results are comparable to the single dose exposure study in which 1*m*-PFOS was preferentially taken-up and retained relative to *n*-PFOS, whereas the major branched PFOS isomers (3*m*, 4*m*, 5*m*, and *iso*) had similar uptake compared to *n*-PFOS [37]. Furthermore, branched PFOS isomers, with the exception of 1*m*-PFOS, were more prevalent than *n*-PFOS in urine[37].

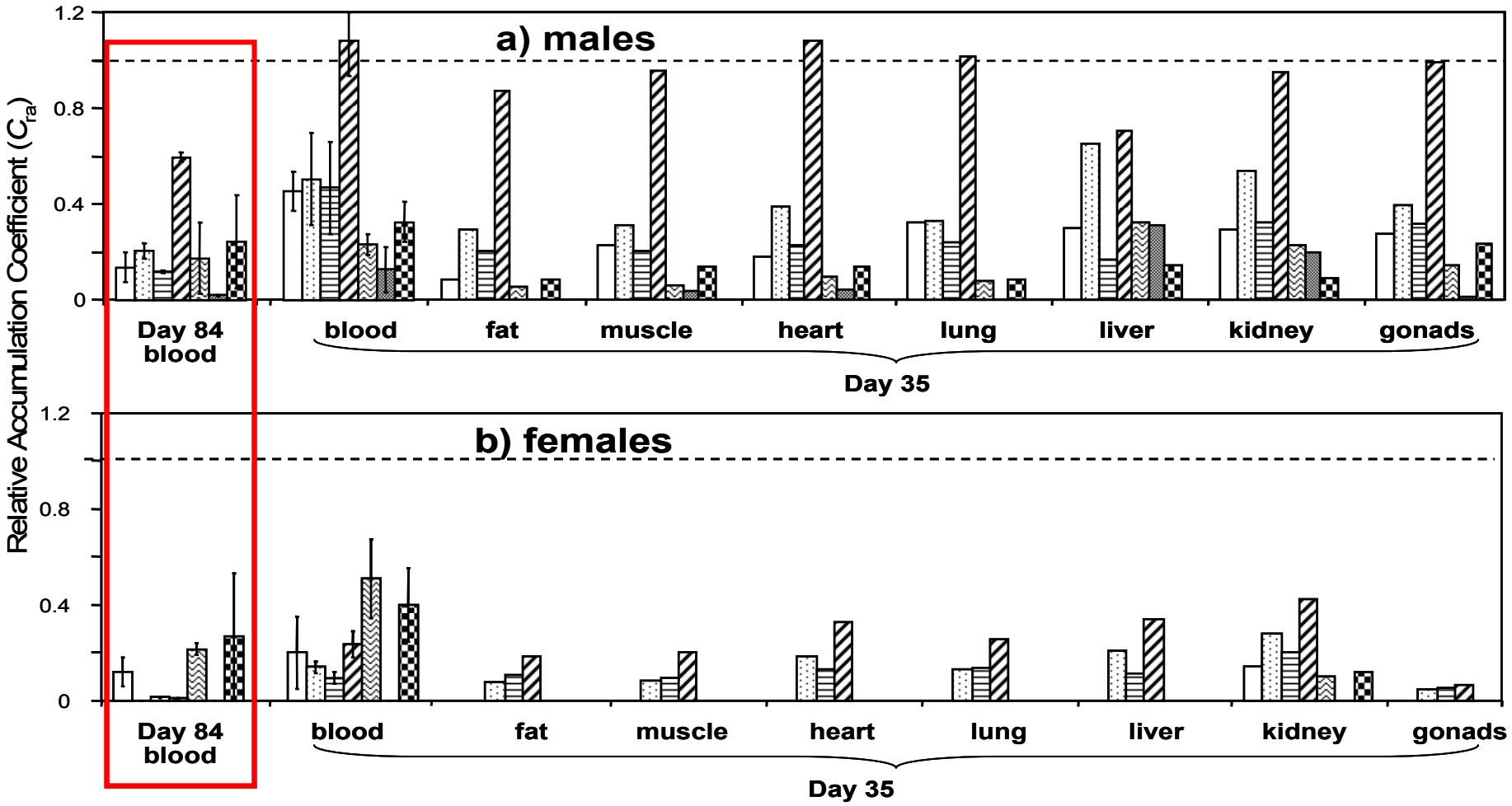

Figure 5.4. ECF PFOA isomer C_{ra} in blood at end of exposure phase (day 84) and in tissues on day 35 in a) male and b) female rat. By definition, C_{ra} for *n*-PFOA is 1 (dotted line). Legend: 3*m*-PFOA, 4*m*-PFOA, 5*m*-PFOA, *iso*-PFOA, PFOA-6, PFOA-7, PFOA-8. Error bars on day 35 and 84 correspond to observed range in 2 rats.

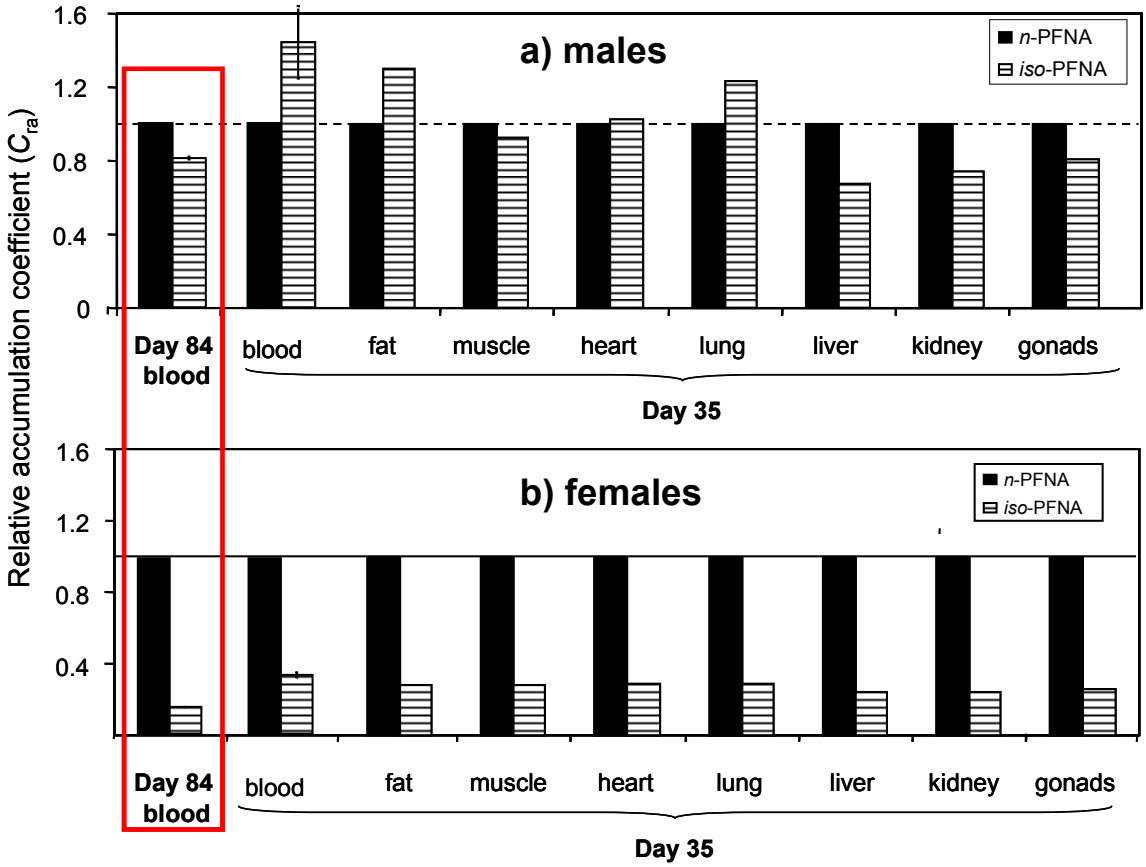

Figure 5.5. PFNA isomer C_{ra} in blood at end of exposure phase (day 84) days of exposure and other tissues on day 35 in a) male and b) female rats. By definition, C_{ra} for *n*-PFNA is 1 (dotted line). Error bars on day 35 and 84 correspond to observed range in 3 rats.

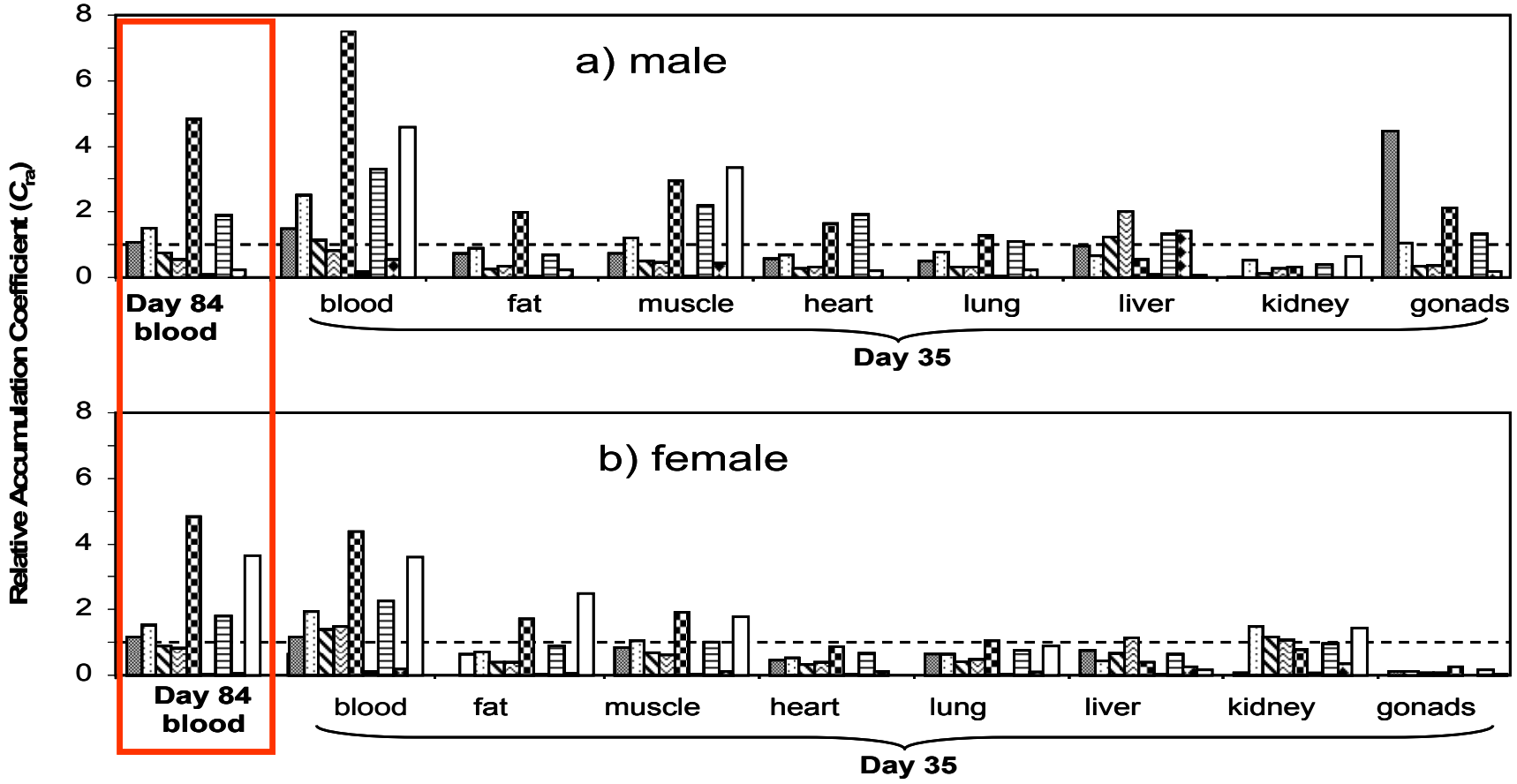

Figure 5.6. PFOS C_{ra} in blood on last day of exposure phase (day 84) and in tissues on day 35 in a) male and b) female rats. By definition C_{ra} for *n*-PFOS is 1 (dotted line). Legend: *iso* ▩, 5*m* ⬚, 4*m* ▨, 3*m* ▨, 1*m* ▩, *t*-butyl ■, B_7 ▤, B_8 ▩, B_9 □.

5.3.3 Elimination of PFAs in Male and Female Rats

Isomer discrimination was further evident once rats were switched to a clean diet and the clearance of PFA isomers monitored. First-order elimination kinetics were noted (r^2 >0.80) and depuration rate constants (k_d) (Table 5.1 - PFOA and PFNA isomers, Table 5.2 - PFOS) using linear regression (Systat, Ver 9.01, Systat Software, Richmond, CA, USA) and elimination half-lives (i.e. $\ln(2)/k_d$) were calculated for PFOA, PFNA and PFOS isomers (Fig. 5.7 a, b, c, respectively). Elimination kinetic parameters were calculated using blood PFA concentrations corrected for growth dilution effects by multiplying each concentration by a factor of $(1+a \cdot t/b)$, where a and b were derived from the exponential growth equation for each particular rat during the elimination period.

Among PFOA isomers, *n*-PFOA, PFOA-6, and PFOA-8 had the slowest rates of clearance from male rat blood. The latter two isomers had half-lives nearly double that of *n*-PFOA, and half-lives of PFOA-6 and PFOA-8 were indistinguishable from each other based on overlapping 95% confidence intervals (CI). However, half-lives for PFOA-6 and PFOA-8 were not as long as those of *n*-PFNA or *iso*-PFNA, suggesting that both the number of perfluorocarbons and their geometry are factors determining elimination kinetics. PFOA-7 had the highest elimination rate constant, and within 3 days of the clearance phase PFOA-7 was <LOD in male blood. Accordingly, this isomer also had the lowest C_{ra} in tissues at day 35 of the uptake phase. Interestingly, in the PFOA isomer profile of Canadian Arctic polar bears, PFOA-7 was the only isomer in the ECF mixture that was not detectable in liver[41].

Isomer geometry could be a key determinant for selective retention of PFOA-6 and PFOA-8, and the preferential elimination of PFOA-7. Based on chromatographic peak area, these isomers represent less than 0.5% of the ECF PFOA mixture. We were able to rule out the identity of *t*-butyl PFOA and 4,4m_2-PFOA for PFOA-6 and PFOA-8 by LC-MS/MS. The peak corresponding to PFOA-6 may be 2,2m_2-PFOA or 3,3m_2-PFOA, and the observation of its long half-life determined by LC-MS/MS in the single dose experiment[37]. Standards for these isomers were not available. It is possible that PFOA-8 corresponds to 2*m*-PFOA (alpha branch isomer) for which a standard was unavailable. This would be consistent with the

PFOS results where 1*m*-PFOS (also the alpha branch isomer) was characterized as having a long half life in both subchronic and single dose exposure[37]. This may be due to the alpha branch shielding the hydrophilic carboxylate moiety.

Table 5.1. Elimination rate constants (k_d) from ln concentration vs. depuration day for PFOA and PFNA isomers in male and female rat blood (growth corrected).

PFA Isomer	mean k_d ± std error ($days^{-1}$)	k_d ($days^{-1}$) (95% CI range)	Elimination ½-life (days) (95% CI range)	r^2
Males				
ECF PFOA				
3*m*	0.14 ± 0.01	0.11 - 0.17	5.0 (4.1 – 6.2)	0.888
n-	0.076 ± 0.08	0.060 - 0.092	9.1 (7.5 – 11.6)	0.852
4*m*	0.16 ± 0.02	0.12 - 0.21	4.3 (3.3 – 5.8)	0.857
5*m*	0.12 ± 0.02	0.092 - 0.16	5.6 (4.5 – 7.5)	0.812
Iso	0.11 ± 0.007	0.095 - 0.12	6.3 (5.6 – 7.3)	0.933
PFOA-6	0.033 ± 0.005	0.022 - 0.043	21.2 (16 -31)	0.712
PFOA-7	0.26 ± 0.03	0.12 - 0.39	2.7 (1.8 – 5.7)	0.971
PFOA-8	0.043 ± 0.005	0.033 - 0.054	16.0 (12.8 – 21)	0.817
PFNA				
n-PFNA	0.015 ± 0.001	0.012 - 0.018	47.5 (39.5 – 59.9)	0.801
iso-PFNA	0.022 ± 0.002	0.018 - 0.026	32.1 (27.1– 39.3)	0.837
Females				
n-PFNA (up to day 18 depuration)	0.30 ± 0.03	0.26 - 0.34	2.31 (2.02 – 2.69)	0.903
iso-PFNA (up today 8 depuration)	0.80 ± 0.08	0.62 - 0.97	0.87 (0.72– 1.11)	0.914

Table 5.2. Growth corrected elimination rate constants (k_d, d^{-1}) for PFOS isomers in male and female rat blood.

PFOS Isomer	mean k_d ± std error (d^{-1})	k_d (d^{-1}) (95% CI range)	Elimination ½-life (d) (95% CI range)	r^2
Males				
n-PFOS	0.0085 ± 0.0009	0.0065 - 0.0105	82 (66 – 107)	0.921
iso-PFOS	0.0106 ± 0.0018	0.0064 – 0.0148	65 (47 – 108)	0.839
5*m*-PFOS	0.0127 ± 0.0013	0.0096 - 0.0158	55 (44 - 72)	0.930
4*m*-PFOS	0.0154±0.0022	0.0102 - 0.0205	45 (34 – 68)	0.875
3*m*-PFOS	0.0112 ± 0.0012	0.0083 - 0.0141	62 (49 – 84)	0.922
1*m*-PFOS	0.0068±0.0018	0.0024 - 0.0111	103 (63 – 287)	0.660
t-butyl – PFOS	0.0233±0.0030	0.0163 – 0.0303	30 (23 -43)	0.898
B_7 – PFOS	0.0129±0.0012	0.0100 – 0.0158	54 (44-69)	0.942
B_8 – PFOS	0.0343 ± 0.0054	0.0215 - 0.0471	20 (15-32)	0.851
Females				
n-PFOS	0.0087±0.0005	0.0076 - 0.0098	80 (71-91)	0.983
iso-PFOS	0.0178±0.0031	0.0110 - 0.0246	39 (28-63)	0.750
5*m*-PFOS	0.0221±0.0034	0.0145 - 0.0297	31 (23 – 48)	0.791
4*m*-PFOS	0.0281±0.0029	0.0217 – 0.0345	25 (20-32)	0.894
3*m*-PFOS	0.0213±0.0036	0.0133 – 0.0293	33 (24-52)	0.757
1*m*-PFOS	0.0018±0.0033	0.0053 – 0.0090	381	0.028
t-butyl - PFOS	0.0919±0.0146	0.0574 - 0.1264	8 (5-12)	0.850
B_7 - PFOS	0.0143±0.0032	0.0072 - 0.0214	49 (32-96)	0.641

Similar to the trend for *n*-PFOA and *iso*-PFOA, clearance of *iso*-PFNA was faster than *n*-PFNA. In fact, for both PFOA and PFNA in male rats, the half-life of the *n*-isomer was 1.4-fold greater than the corresponding *iso*-isomer. The PFNA isomer profile at steady state did not reflect this in male rats (Fig. 5.5a) in which near steady-state accumulation of both *n* and *iso* was similar. This is because steady-state concentrations are a function of both uptake and elimination, and thus we can surmise that uptake of *iso*-PFNA was more efficient than *n*-PFNA; thus almost nearly compensating for its faster elimination rate. These results are consistent with Part I[37] wherein for a single dose the half-lives of *n*-PFOA, *n*-PFNA, and *iso*-PFNA overlapped at the 95% CI level. These findings are also in agreement with

Ohmori et al. who reported half-lives of 5.6 and 30 days for *n*-PFOA and *n*-PFNA in male rats, and 0.08 and 2.4 days in females[48], and with Kemper et al. who reported a half-life of 8.4 days for *n*-PFOA in male rats[46]. In the current work, a sex-related difference in pharmacokinetics was again observed whereby half-lives of *n*-PFNA and *iso*-PFNA were 20 and 37 times lower, respectively, in females (Fig. 5.7).

Compared to PFOA and PFNA isomers in male and females, half-lives of PFOS isomers were much longer. The elimination half-life of *n*-PFOS was approximately 80 d for both sexes. Although the mean k_d for each major branched isomer (i.e. *iso*, 3*m*, 4*m*, and 5*m*) was higher than the *n*-isomer, 95% CIs overlapped, thus their elimination kinetics could not be deemed statistically different from the *n*-isomer. Elimination kinetics of one of the structurally unidentified PFOS isomers, B_8-PFOS, was also indistinguishable from *n*-PFOS based on 95% CIs. Two other isomers, B_7-PFOS and B_9-PFOS, had faster elimination than all other isomers. In contrast, one isomer, 1*m*-PFOS, had a significantly slower elimination half-life than the other PFOS isomers, approximately 103 days. The depuration period in this experiment was shorter than this estimated half-life, thus the r^2 (0.660) from its elimination plot was poorer than for other isomers. The slow elimination of 1*m*-PFOS was consistent with its large C_{ra} in male and female tissues at day 35 of the uptake phase, and also with Part I[37].

Overall, the same relative PFOS isomer trends were noted in Part I, the single-dose study[37], but the magnitude of the k_d's was smaller than in the present study. In general, half-lives in the sub-chronic exposure were at least double those determined in the single-dose study. The extended persistence following subchronic exposure may be due to increased partitioning of PFOS from other tissues into the blood. In addition, rats in the single dose study had less body mass (mean mass 434 g ± 6 g) compared to those of the current study (570-654 g at the start of depuration). The 80 d half-life of *n*-PFOS determined in the current study is close to the value of 100 days reported by Johnson et al for total PFOS in male rats in a single dose exposure[49]. Branched PFOS isomers were generally cleared more rapidly in females than in males (Fig. 5.7). The exceptions to this were *n*-PFOS and 1*m*-PFOS, for which elimination rate constants were not significantly different between

sexes. As in males, 1*m*-PFOS was very slowly eliminated from female rats. In fact, virtually no elimination occurred, leading to a poor r^2 value (0.028) in the k_d determination.

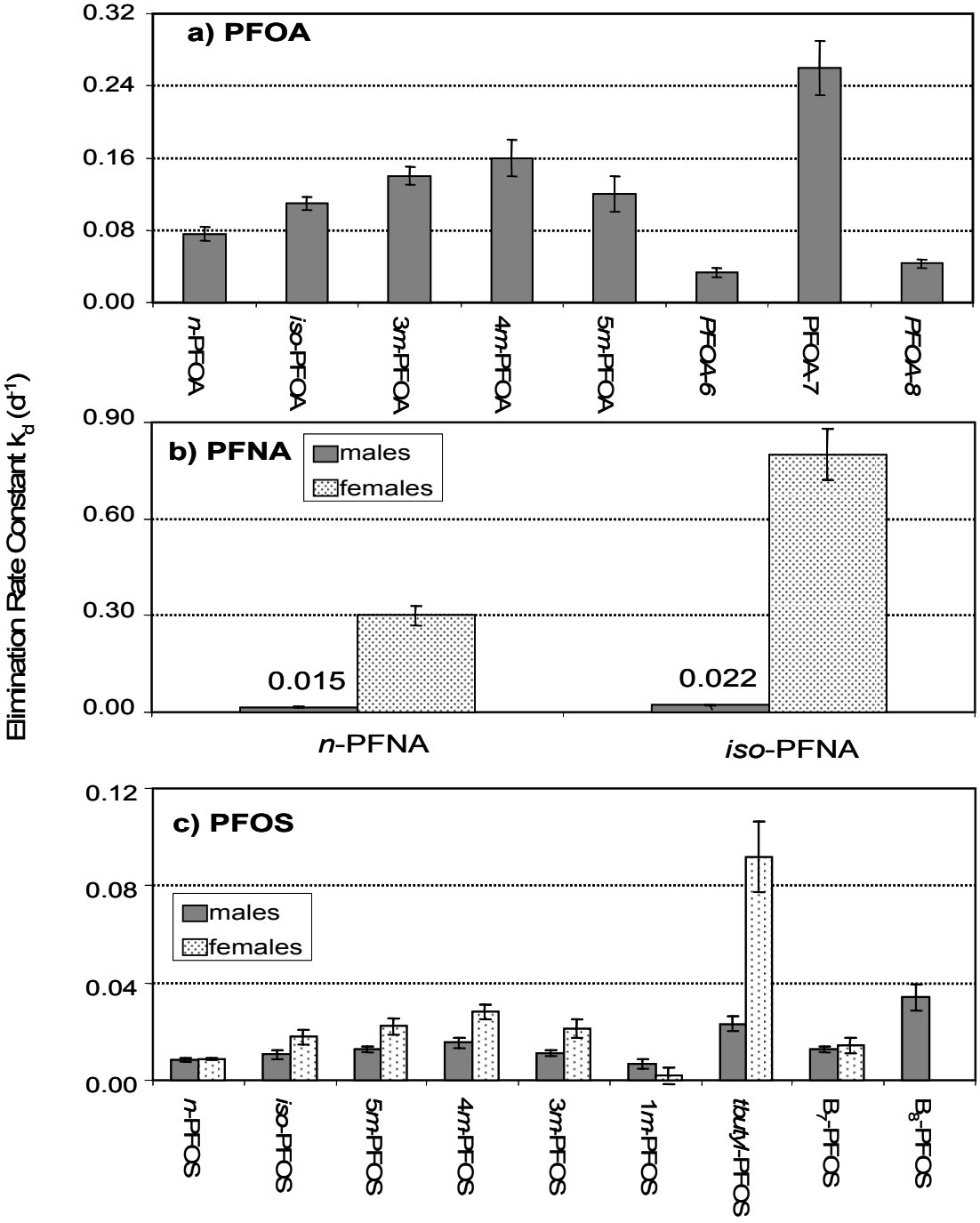

Figure 5.7. First order elimination rate constants in male and female rat blood. Error bars represent standard error. PFOA isomer elimination rate constants from females could not determined due to rapid elimination. B_8- and B_9-PFOS could not be determined in female rat blood (and B_9-PFOS in males) due to instrumental difficulties.

Given the gender-specific differences in pharmacokinetics observed in this study and others[42,43,48], a sex-related transport mechanism must be mediating elimination. Straight chain isomers of PFOS and PFOA bind efficiently with serum proteins such as albumin and beta-lipoprotein[50,51]. However, these serum proteins are not expected to vary greatly by gender. Urinary elimination is a significant clearance route of PFOA and PFOS from rats [46,52] and has been hypothesized to be impeded by binding of the perfluorochemical to specific organic anion transporter proteins (OATPs)[53,54]. These transporters may be involved in resorption of PFAs from the urinary filtrate back into the blood (saturable renal resorption uptake). Gender-specific elimination in rats may be accounted for by such a mechanism because OATP profiles are regulated by sex hormones and are thus different among male and female rats[53,55]. The longer half-life of PFOS, compared to PFOA, has previously been interpreted as a higher affinity and higher capacity of PFOS for transporter proteins[45].

Compared to the linear isomers, albeit with a few exceptions, faster elimination of most branched perfluorocarboxylate isomers occurred. Akin to the accelerated clearance of *n*-isomers from females, branched isomers were also less persistent in females. For example, the k_d values for *n*-PFNA and *iso*-PFNA were 20 and 36-fold larger in females compared to males, respectively. Gender differences were less pronounced among PFOS isomers. The k_d for *iso*-PFOS, 5*m*-PFOS, 4*m*-PFOS, and 3*m*-PFOS were all 1.7 to 1.9-fold larger in females compared to males. An exception was B7-PFOS which was eliminated 5-fold faster in females.

5.3.4 Environmental Significance

A major objective was to validate whether PFA isomer signatures in biological samples could be used as a tracer to quantify the relative extent of exposure to PFAs from telomer and ECF sources. In male and female rats, we observed more rapid depuration of the major branched isomers, relative to linear, in technical ECF PFA mixtures. Thus, assuming rats are a reasonable model for humans and other wildlife, direct comparison between the

isomer signature in an organism to a technical ECF standard would result in over-estimation of telomer -derived PFA exposure if both sources of exposure are present.

In our previous work, linear and various branched PFOA isomers were observed in human blood[19]. Specifically, the detected branched isomers consisted of 3*m*-PFOA, 4*m*-PFOA, 5*m*-PFOA, and *iso*-PFOA[19], comprising 1 – 3% of the overall PFOA response by GC/MS[19]. In itself, the detection of several branched isomers implies ECF PFA exposure, but given the current results, a quantitative assignment of telomer versus ECF exposure is difficult.

Assuming that the relative pharmacokinetics (i.e. isomer rank-order) observed in rats hold true in humans, then the absence of PFOA-6 and PFOA-8 in human blood profiles is curious, given their relatively long half-lives. The absence of PFOA-6 and PFOA-8 in human blood analyzed may be suggestive that rats do not entirely capture pharmacokinetics of PFA isomers in humans, albeit knowledge of the isomer signature in human exposure sources (e.g. food, water, air particulate, etc.) would be necessary to make this conclusion.

It is clear that the rat model does not entirely capture pharmacokinetics in humans for this class of compounds. Humans have much longer half-lives of PFOA (3.8 years) and PFOS (5.4 years)[56] compared to any laboratory animal, including monkeys[39], which was postulated to be due to OATPs involved in mediating urinary excretion and renal resorption of PFAs[45]. In rats, OATPs appear to be critical to PFA pharmacokinetics based on differences between sexes since expression of some OATPs in rats are sex-hormone regulated and result in contrasting OATP profiles in female and male rats. In male rats, the longer half-lives of *n*-isomers appear to be related to renal excretion in which enrichment of branched isomers in urine was observed in our single dose study[37]. Although female rats eliminated PFAs more rapidly, isomer discrimination also occurred, consistent with male rats. Thus although rats are not a perfect model for humans, the fact that isomer descrimination occurred in both male and female rats, despite their vastly different biological handling, provides confidence that PFA isomer discrimination could also occur in other mammalian species.

Another mechanism for preferential *n*-isomer retention could be that the branched isomers are less bound to serum proteins, rendering more facile glomerular filtration or passive diffusion. The specific proteins involved in PFA clearance by humans are currently not known. However, each species and gender may have a characteristic profile of proteins that pertain to PFA retention. This is evident in the research of Loveless et al. in which male rats and mice were administered ECF PFOA (77% *n*-PFOA). Preferential retention of *n*-PFOA occurred in both mice and rats but the linear isomer was more pronounced in mice[40]. It should be noted, however, that those results were obtained using a non-isomer specific analytical method in which branched isomers co-eluted together but were separated from *n*-PFOA[40]. The findings of the current study suggest that the majority of branched PFOA isomers are likely eliminated faster than the *n*-isomer, however, further research into the mechanisms involved in PFA clearance in humans is warranted in order to ascertain the degree to which isomer discrimination in rodents translates to humans.

The research presented by Loveless et al. highlighted not only the influence of species but also of dosage on PFA isomer selectivity[40]. PFOA isomer studies in rodents have shown that retention of the *n*-isomer was increasingly dominant with dose[40]. For example, at 30 mg/kg/d, mice and rats had 90-92% *n*-PFOA in blood serum whereas, at a lower dose of 0.3 mg/kg/d the *n*-isomer contributed only 80% in mice and 84% in rats[40]. Typical environmental PFA concentrations are quite low and it is uncertain what impact this would have on human PFA isomer discrimination. In this study, we administered a lower dose (0.1 mg/kg/d of total PFA; 0.02 mg/kg/d of ECF PFOA) than Loveless et al. and clearly observed isomer discrimination. Nonetheless, the resulting PFA blood concentrations in our study (approximately 1 ppm per PFA) and in that of Loveless et al. (20 ppm in rats exposed to 0.3 mg/kg) were significantly higher than what is reported in humans and other organisms, with the exception of polar bears[20,57]. Employees at an ECF PFOA production plant had elevated serum PFOA concentrations (mean 691 ng/ml)[56] due to occupational exposure compared to that of the general human population (1.5 to 3.5 ng/ml in 2005)[16]. Interestingly, only *n*-PFOA was observed in employees in spite of exposure to the ECF isomer signature[56]. The PFOS isomer pattern in these employees was not reported.

The single dose study[37], and the current sub-chronic study both suggested that PFOS isomer discrimination is not as pronounced as for PFOA. The major branched isomers of PFOS (*iso* and internal methyl branch) had overlapping half lives with the *n*-isomer. However, in the single dose study, urine was enriched with branched PFOS isomers. This indicates that some isomer discrimination may occur due to preferential renal elimination of branched isomers[37], and possibly that significant differences in the blood pharmacokinetics may have been borne-out if a longer elimination period had been used. Albeit, the overlapping elimination rate constants among the majority of PFOS isomers is consistent with repeated observations that non-occupationally exposed humans have PFOS isomer signatures in blood that are very similar to ECF PFOS standards[18,36]. The entire story becomes less clear, however, considering that metabolism of PFOS precursors, such as the perfluorooctylsulfonamides, are thought to be a major source of indirect PFOS exposure to humans[16], and a recent study demonstrated significantly different biotransformation rates among various perfluorooctylsulfonamide isomers[18]. Therefore, further research is warranted on perfluoroalkyl isomer disposition**.**

5.4 Conclusions

In this experiment we probed the pharmacokinetics of linear and branched PFA isomers to examine for biological discrimination of PFA isomer signatures in a repeated exposure. Considerable discrimination occurred in rats, with the majority of branched PFCA isomers eliminated faster than the corresponding linear isomer. PFOS isomer discrimination also occurred, but to a lesser extent than PFOA or PFNA. These results were consistent with a companion single dose study[37] and those of Loveless et al.[40]. In addition, PFA isomer clearance in female rats was faster than in males, providing further evidence of gender-related differences in transport. In order to quantitatively extrapolate rat pharmacokinetics to humans and other organisms, further research is necessary to determine whether rats and humans share similarities in PFA transport mechanisms. Isomer analysis of PFAs in abiotic samples may therefore have a greater potential for designation of telomer or ECF exposure sources.

5.5 Acknowledgements

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CHAPTER SIX

Uptake, Accumulation and Elimination of Perfluorocarboxylate Isomers in Rainbow Trout

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Contributions **–** Care of animal subjects, sample collection, and preparation for analysis was by undergraduate student Pablo Tseng, under the guidance of Amila De Silva. Amila De Silva was also involved in sample preparation, analysis, and interpretation. All versions of the manuscript were prepared by Amila De Silva with critical comments provided by Pablo Tseng and Scott Mabury.

6.1 Introduction

Perfluorocarboxylate acids (PFCAs) are persistent organic pollutants found ubiquitously in the environment including water[1,2], snow[3], precipitation[4], biota ([1], reviewed in [5]), and sediment[6]. The major industrial use of PFOA and PFNA are as emulsifying ingredients in fluoropolymer synthesis[7]. Perfluoroalkyl sulfonates were primarily used in aqueous film forming foam (AFFF) employed in fire-fighting measures[7] and there is some evidence that PFOA may have been a minor component of these formulations[7]; however, it is unclear whether PFOA was intentionally added or merely a byproduct of perfluorooctyl sulfonate (PFOS) synthesis. In water samples collected near an AFFF spill Moody et al. found PFOA to represent 0.4 to 1.4% of the PFOS concentration[8]. PFCAs are also considered secondary pollutants because they are degradation products of fluorinated precursor compounds, including FTOHs [9-12], FTOs [13], and perfluorooctylsulfonamides ($F(CF_2)_8SO_2NRR'$)[14-17]. These precursors are building block intermediates used in synthesis of surfactants and monomers for polymer production. Final fluorochemical sales products may still contain residual (unbound and in the raw form) precursors[18,19]. Recent research from our group demonstrated biotransformation of fluoromonomers including 2(perfluorooctyl)ethyl acrylate and 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl methacrylate[20], and fluorosurfactants such as fluorotelomer polyfluoroalkyl phosphates[21] yield FTOHs which ultimately form PFCAs via biological reaction pathways[12,22].

Perfluorooctylsulfonamides, PFOA, and PFOS were manufactured until 2002 primarily using ECF. In ECF, *n*-octanesulfonyl fluoride or *n*-octanoyl fluoride is dissolved in anhydrous hydrogen fluoride and an electric current is passed through the solution, replacing each hydrogen of the starting material with fluorine[23]. The major product is the perfluorinated analog of the starting material (*n*-$C_8F_{17}SO_2F$ or *n*-$C_7F_{15}C(O)F$) and minor products are isomers with branched arrangement of the perfluoroalkyl moiety [23]. The pattern of isomers associated with ECF products can be considered characteristic as there appears little variation between batches[24]. Industrially, technical ECF perfluorooctanesulfonyl fluoride was used to synthesize perfluorooctylsulfonamides and PFOS whereas PFOA was acquired by reaction of ECF perfluorooctanoyl fluoride[25]. These products are isomer mixtures akin to the starting materials. Atmospheric reactions of perfluorooctylsulfonamides form PFOA, shorter PFCAs, and PFOS[14,15]. Presumably the isomer composition of these products would be analogous to

that of ECF precursor. Biologically, PFOS appears to be the dominant fate of perfluorooctylsulfonamides[17]; PFCAs have not been shown to be metabolites of perfluoroocylsulfonamides.

The major constituent of ECF PFOA is *n*-PFOA (~ 78%), followed by 9% isopropyl PFOA ($(CF_3)_2CF(CF_2)_4C(O)O^-$, *iso*-PFOA) [26]. Collectively the internal perfluoromethyl branched PFOA isomers, in which a CF_3 group is on an inner carbon, constitute 13 % of the mixture[26]. Minor branched isomers are the alpha CF_3 branch PFOA ($F(CF_2)_5CF(CF_3)C(O)O^-$, 0.1%), *tert*-perfluorobutyl branched PFOA ($(CF_3)_3C(CF_2)_3C(O)O^-$, 0.1%)[26], and di-CF_3 branched PFOA (two CF_3 branches on the same carbon (*gem*), adjacent carbons (*vic*), or otherwise), which collectively comprise 0.1%[26,27]. Shorter and longer PFCA isomer homologues are also impurities in ECF PFOA[25]. For example, ECF PFOA contains perfluorohexanoate (1%), perfluoroheptanoate (4%), PFNA (0.16%), and C-10 to C-12 PFCAs (together <0.002%) [24].

Unlike ECF, telomerization does not produce isomer mixtures and is believed to consist predominantly of linear perfluorocarbon geometry. Telomerization has widely replaced ECF for synthesis of PFOA post 2002[7] and is also used for the manufacture of FTOHs, FTOs, and *n*-PFNA[7]. This is a radical process in which *n*-perfluoroethyl iodide (telogen) repetitiously adds to perfluoroethene (taxogen), thereby increasing the length of the telogen in units of CF_2CF_2 [25]. Upon termination of the reaction, the perfluoroalkyl iodide product is an assortment of linear even-numbered chain lengths[25]. The mixture is further reacted to produce analogous FTOHs or FTOs[7]. The abiotic and biotic fate of FTOHs are PFCAs[10,11]. The environmental fate of FTOs has been less interrogated but atmospheric reactions are expected to produce PFCAs[13].

Both telomer (PFOA, FTOH, FTO) and ECF (PFOA, perfluorooctylsulfonamides, PFOS) sources are plausible contributors to environmental PFOA. Previous research suggested perfluorinated acid isomer analysis in environmental samples could be used to elucidate source[28,29]. Isomer analysis of PFCAs, including PFOA, appear predominantly linear with a minor (<5%) fraction consisting of branched isomers in Arctic polar bears and human blood[28-30]. In contrast, the PFOS isomer profile in human blood, which is only of ECF origin, is very similar to technical grade PFOS[30,31]. Whether this indicates telomer-based products are a

significant source of PFCAs requires investigation of the potential for biodiscrimination to influence the observed isomer patterns.

It is necessary to explore differential environmental processes that could alter the isomer pattern between source and sink. These include abiotic and biotic factors that influence transport and partitioning. The objective of this study was to assess biological influence on PFCA isomer patterns in fish. Previously toxicokinetics of *n*-isomers of perfluorinated acids in rainbow trout (*Oncorhynchus mykiss*) were assessed with food- and water-borne exposure[32,33]. In the dietary exposure study, elimination half life ($T_{½}$) from carcass was determined to be 3.0±0.4 days for *n*-PFOA with increasing $T_{½}$ observed for longer chain lengths[32]. PFCAs have been monitored in trout from the natural environment [34,35], rendering them a suitable species for study. In addition, rainbow trout are a carnivorous species occupying a high trophic level in the freshwater food chain and thus PFCA exposure may occur via a diet of smaller forage fish[35]. PFCA isomer dynamics in fish are also likely to have an impact on other fish consumers. Falandysz et al. observed a correlation in *n*-PFCA serum concentrations of humans and the fish in their diet [36].

In the current research, immature rainbow trout were fed a fixed dose of PFCA isomers for 36 days, followed by an elimination phase during which non-dosed feed was supplied for 40 days. During both the uptake and elimination phase, fish were periodically sacrificed and tissues harvested for isomer composition analysis. The dose consisted of the ECF PFOA isomer mixture, *n*-PFNA, and isopropyl perfluorononanoate ($(CF_3)_2CF(CF_2)_5C(O)O^-$, *iso*-PFNA). The influence of dose magnitude on isomer discrimination was sought in a second experiment in which fish were exposed to a lower dose of ECF PFOA.

6.2 Materials and Methods

6.2.1 Standards and Reagents

An authentic standard of ECF PFOA was obtained from 3M Co. (St. Paul, MN). This standard consisted of a mixture of isomers: 78% *n*-PFOA, 9.0% *iso*-PFOA, and 12.5% internal CF_3 branched PFOA isomers. Linear PFOA and PFNA isomers were purchased from Sigma

Aldrich (Oakville, ON). Wellington Laboratories (Guelph, ON) supplied *iso*-PFNA (93% pure, 7% *iso*-PFOA). Reagents used for sample preparation were described in our earlier work[29].

6.2.2 Food Preparation

Approximately 150 g of commercial fish food (Martin's floating feed, size 3, Martin Mills, Elmira, ON) were placed in a 1000 mL round bottom flask. Small volumes (<10 mL) of ECF PFOA, *n-PFNA*, and *iso*-PFNA standard stock in acetone were introduced along with 120 mL of acetone. The flask was capped with a glass stopper and undisturbed for 30 min. Using a rotary evaporator the bulk of the solvent was removed. The dosed feed was spread on aluminum foil in the bottom of a Pyrex dish and left for at least 72 hours in the fumehood. Control feed was prepared in precisely the same manner without the addition of the PFCA isomers. Concentrations of PFCAs in fortified and control feed were determined. Analysis of 4 batches indicated dose concentrations to be 458 ng/g PFOA (410 – 472 ng/g), 91 ng/g *n*-PFNA (83 – 104 ng/g), 73 ng/g *iso*-PFNA (66 – 83.2 ng/g). Although these ranges indicated some variability between batches, the isomer pattern was consistent. Of the PFCAs monitored in control feed, only *n*-PFOA was observed and ranged from 1 to 4 ng/g.

6.2.3 High Dose Experiment

Juvenile rainbow trout were purchased from Rainbow Springs Trout Hatchery (Orangeville, ON). At the start of the experiment, initial mean and standard deviation (N= 8) mass and length were 30.69 g ± 10.33 g and 15.3 cm ± 1.2 cm. Fish were evenly distributed among four tanks (21 fish/ tank). Each tank was a 475 L fiber glass flow-through system. Tanks received dechlorinated, carbon-filtered tap water at a rate of 85 - 90 mL/min, maintained at 18 ºC. Fish were subjected to a daily 12 h photoperiod.

One tank was designated as the control tank to which unfortified feed was given and the remaining three received PFCA-dosed feed for 36 days. The daily feeding rate for the duration of the experiment was 1.5% body weight, adjusted throughout this period for increasing body mass. This corresponded to a mean daily dose of 6.9 µg/kg PFOA, 1.4 µg/kg *n*-PFNA, and 1.1 µg/kg *iso*-PFNA. In the depuration phase that followed, fish received regular feed for 40 further days. Sacrifice of 3 treated and 1 control fish occurred on days 1, 1.3, 2, 5, 9, 17, and 36 of the

uptake phase and days 1, 2, 3, 5, 8, 12, 17, 26, and 40 of the depuration phase. Mean growth over the entire experiment was 18 g per fish as evidenced by the mean and standard deviation mass and length on the last day of the experiment 48.39+/-10.23 g and 16.2 cm +/- 1.4 cm.

Fish were euthanized using a 4 g/L solution of tricaine methanesulfonate (MS-222), buffered to pH 7 using sodium bicarbonate. Whole blood samples (approximately 500 µl) were obtained by cardiac puncture using a heparinized syringe. Each fish was dissected to collect liver, heart, spleen, and kidney. All samples were stored in a freezer at -20 ºC.

6.2.4 Low Dose Experiment

To investigate the influence of dose level on isomer discrimination, a second experiment was conducted in which a tank of 20 fish were exposed to a PFCA dietary low dose consisting of 81.1 ± 6.5 ng/g ECF PFOA (based on N = 6). This translated to a dose expressed per body weight of 1.2 µg/kg, approximately the same level as each PFNA isomer in the high-dose experiment. Administration of the diet and environmental conditions were consistent with the High Dose treatment. Uptake phase progressed for 33 days followed by a 28-day depuration. Fish were handled as described above and sampled on days 13 (N=3), 20 (N=2), and 33 (N=5) of the uptake phase and days 2 (N=3), 14 (N=5), and 28 (N=2) of the depuration phase. Care and treatment of the fish were in compliance with the guidelines of the Canadian Council on Animal Care and protocol was approved by the University of Toronto Animal Care Committee.

6.2.5 Sample Extraction, Derivatization, and Instrumental Analysis

Extraction of PFCAs from tissues was conducted as previously described via ion-pair formation with tetrabutylammonium sulfate into methyl *tert* butyl ether[29,37]. PFCAs were then derivatized to 2,4-difluoroanilide analogs for quantitation and isomer analysis by gas chromatography with negative chemical ionization mass spectrometry (GC-(NCI)MS) according to the procedure outlined previously[29].

6.2.6 Data Analysis

Growth rates of fish were determined by fitting temporal fish mass data to an exponential model ln (mass) = a•t+b where b is a constant, a is the growth rate, mass of the whole body fish is in grams and t represents time is in days. PFCA concentrations in blood and liver were corrected for growth dilution in order to assess elimination kinetics. PFCA Depuration rates (k_d) from blood and liver were calculated by fitting the growth-corrected depuration phase data to a first order elimination curve where $\ln(C_{fish}) = k_d \bullet t + d$, where C_{fish} is the growth-corrected blood or liver PFCA concentration, d is a constant, time (t) is in days and k_d is the in inverse days (d^{-1}). Quantitation could only be performed where purified standards were available, which were limited to *n*-PFOA, *n*-PFNA and *iso*-PFNA. For the other branched PFOA isomers, an estimate of concentration was obtained from the relative response factor of the isomer divided by the sample mass. Relative response factor was defined as the peak area corresponding to the PFOA isomer divided by the peak area of the internal standard. At the end of the uptake period, isomer profiles in tissues were described using accumulation ratio (AR), defined as the response of the isomer per g of tissue mass divided by the response of the isomer in dosed feed per g of feed (i.e. $AR = tissue_{PFCA\ isomer} \div feed_{PFCA\ isomer}$). In this regard, AR is similar to a bioaccumulation factor where the numerator of the fraction is proportional to tissue concentration and the denominator to dose concentration. Detector response of individual isomers may not be equivalent, however, this was unlikely to bias the data as AR is a ratio of response in tissue to dose and elimination kinetics were deciphered using slope, which is independent of concentration.

6.3 Results and Discussion

To investigate biological discrimination of PFCA isomers, juvenile rainbow trout were administered a mixture of PFOA isomers, *n*-PFNA, and *iso*-PFNA through diet for 36 days. Only food-borne exposure was assumed because of the flow-through conditions of the tank and rapid ingestion of food. Growth rates and liver somatic indices between control and treated fish populations were not significantly different (Table 6.1), implying the dose was not toxicological significant, consistent with other reports[32,33,38]. Liver somatic index is a measure of liver enlargement, a consequence of increases in cell number or cell size as a response by the liver to detoxify by enzymes. No mortality was observed in either the control or treated population after commencement of the experiment.

Unlike most traditional persistent organic pollutants, lipid-rich adipose tissue is not a target site of accumulation for PFCAs. This is due to lipophobicity of the perfluorinated chain and hydrophilicity of the polar head. PFCAs have the ability to bind to blood serum proteins such as albumin[39,40]. This enables them to distribute and reside in the body by binding to other proteins in tissues and accumulate in blood, liver and kidneys[41]. Accumulation in liver may also be enhanced by enterohepatic circulation of PFCAs, the circulation of bile from the liver to the small intestine[42].

Table 6.1. Liver somatic index (mass of liver ÷ body mass • 100%) and growth rates of high-dose treated and control populations. Triplicate sampling of treated population per time point permitted calculation of standard deviation.

	Treated	Control
Liver Somatic Indices (± standard deviation)		
Liver somatic index at day 17 of uptake	1.37 ± 0.23	1.40
Liver somatic index at day 36 of uptake	1.43 ± 0.25	1.25
Liver somatic index at day 17 of depuration	1.41 ± 0.17	1.44
Liver somatic index at day 40 of depuration	1.62 ± 0.29	1.84
Fish Body Mass (± standard deviation) and Growth Rate		
Initial mass (g) over first 25 hours post exposure	32 ± 13, N = 9	33 ± 5, N = 3
Mass at day 40 of depuration (g)	47 ± 6, N = 6	55 ± 11, N = 3
Growth rate equation ln fish mass = at + b,	a = 0.0162	a = 0.0146
fish mass is in g and t is in days (first 36 days)	(± 0.0065)	(± 0.0062)
standard error in brackets for a	b = 3.539	b = 3.628

High concentrations and prolonged $T_{½}$ of PFCA isomers in hepatic tissues such as blood and liver enabled toxicokinetic measurements. Eight PFOA isomers were separated using previously developed methodology[28]. A GC-MS chromatogram of the PFCA isomer profile in the dose is given in Fig. 6.1. Each PFOA peak was numbered according to elution order. PFOA-2 and PFOA-5 were identified as *n*-PFOA and isopropyl PFOA (*iso*-PFOA), respectively, by comparison of retention times to standards. PFOA isomers 1 through 8 constituted 0.68, 80, 4.4, 3.9, 10, 0.29, 0.22, and 0.26% of the total PFOA isomer signature as determined in the ECF standard.

Seven out of the eight isomers in ECF PFOA appeared to reach steady state in blood by the end of the uptake phase (Fig. 6.2). PFOA-7 was present in the dose but never observed in any tissues collected from the fish, suggesting it was either rapidly eliminated or unable to partition into the tissues analyzed. This is consistent with a similar study with rats in which, PFOA-7 had the most rapid elimination rate (blood $T_{½}$ = 2.7 days) compared to other ECF PFOA isomers [43]. At day 36, the concentration of *n*-PFOA, *n*-PFNA, and *iso*-PFNA in blood corresponded to 79, 55, and 27 ng/g and in liver, 69, 45, and 40 ng/g. In liver, PFNA isomers did not appear to have reached steady state by day 36, which may be a reason for the similarity *n*-PFNA and *iso*-PFNA concentrations in liver.

PFOA isomer profiles in blood, liver, and other tissues collected on day 36 were enriched in *n*-PFOA relative to the dose. In the dose, *n*-PFOA represented 80.0 % of the total chromatographic area and this percentage increased to 88, 89, 90, 92, and 94% (± 0.3 to 0.8% standard deviation, N=3) of PFOA in the blood, kidney, liver, heart and spleen, respectively, at the end of the uptake phase. In the dose, *n*-PFNA represented 55% of the PFNA, the remaining 45% comprised of *iso*-PFNA. Composition of PFNA isomers corresponded to 58, 65, 67, 75, and 78% *n*-PFNA in liver, kidney, blood, heart and spleen with standard deviations of 7.6, 0.6, 1.1, 7.5, and 3.9%, respectively, indicating preferential accumulation of *n*-PFNA. Clearly, the degree of preferential *n*-isomer accumulation is not equivalent in all tissues. Heart and spleen were more enriched in *n*-isomers than the liver, blood, and kidney. The reason for this variance is unknown.

Generally, most branched PFOA isomers constituted a smaller proportion of total PFOA in the tissues compared to dose. The exceptions were PFOA-6 and PFOA-8, which were not

significantly different in any of the tissues at day 36 compared to the dose. The distribution of PFOA and PFNA in tissues was in accordance with observations by Martin et al. who reported highest concentrations in blood, followed by kidney and liver for PFOA, and other PFCAs, in which the daily dose to rainbow trout was 0.0063 mg PFOA/ kg body weight [33] compared to this study with a daily *n*-PFOA dose was 0.0069 mg/kg.

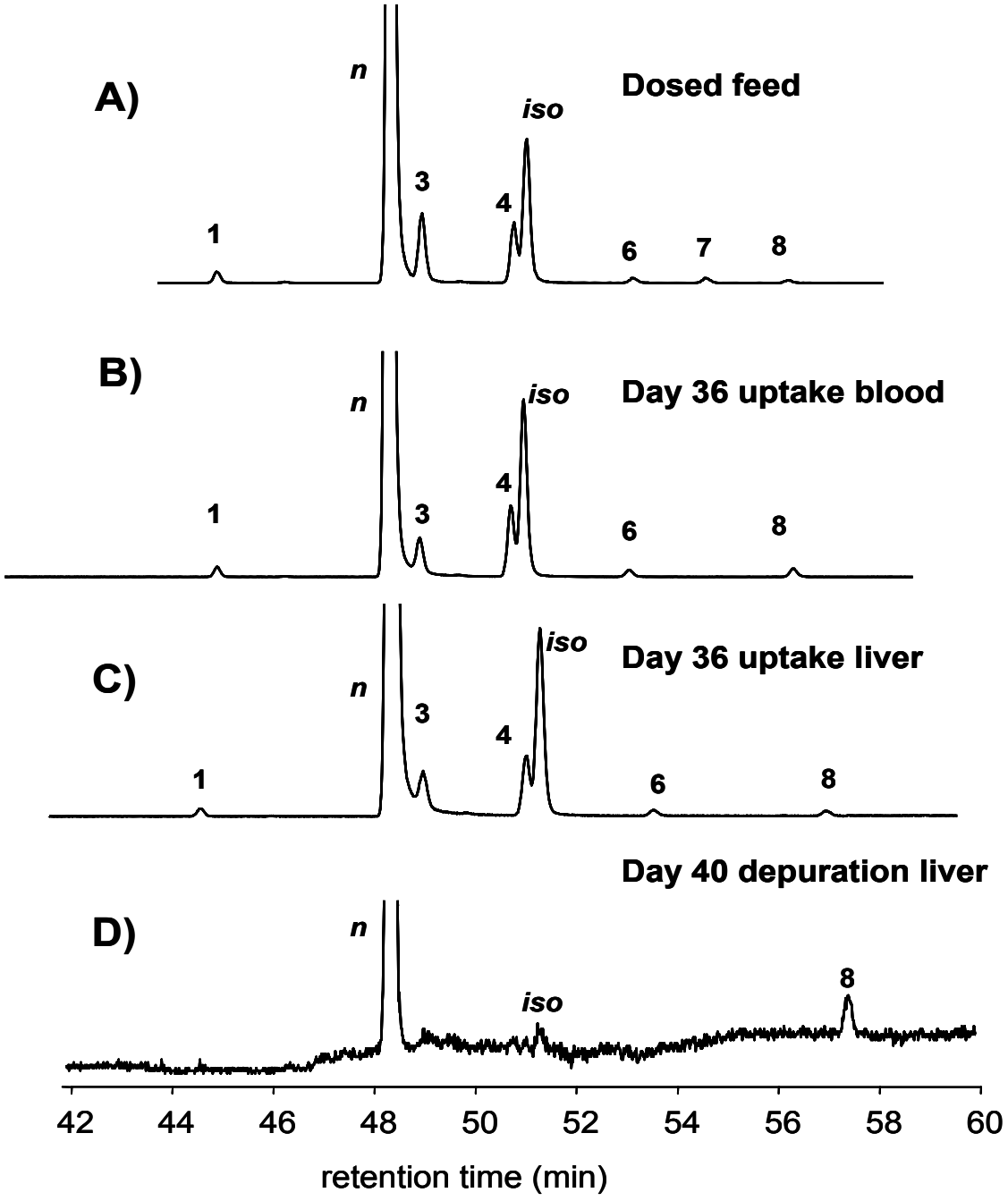

Figure 6.1. Chromatograms of PFOA isomers in A) dosed feed, B) fish blood sample on day 36 of uptake phase, C) fish liver sample on day 36 of uptake phase, and D) fish liver sample on day 40 of depuration phase.

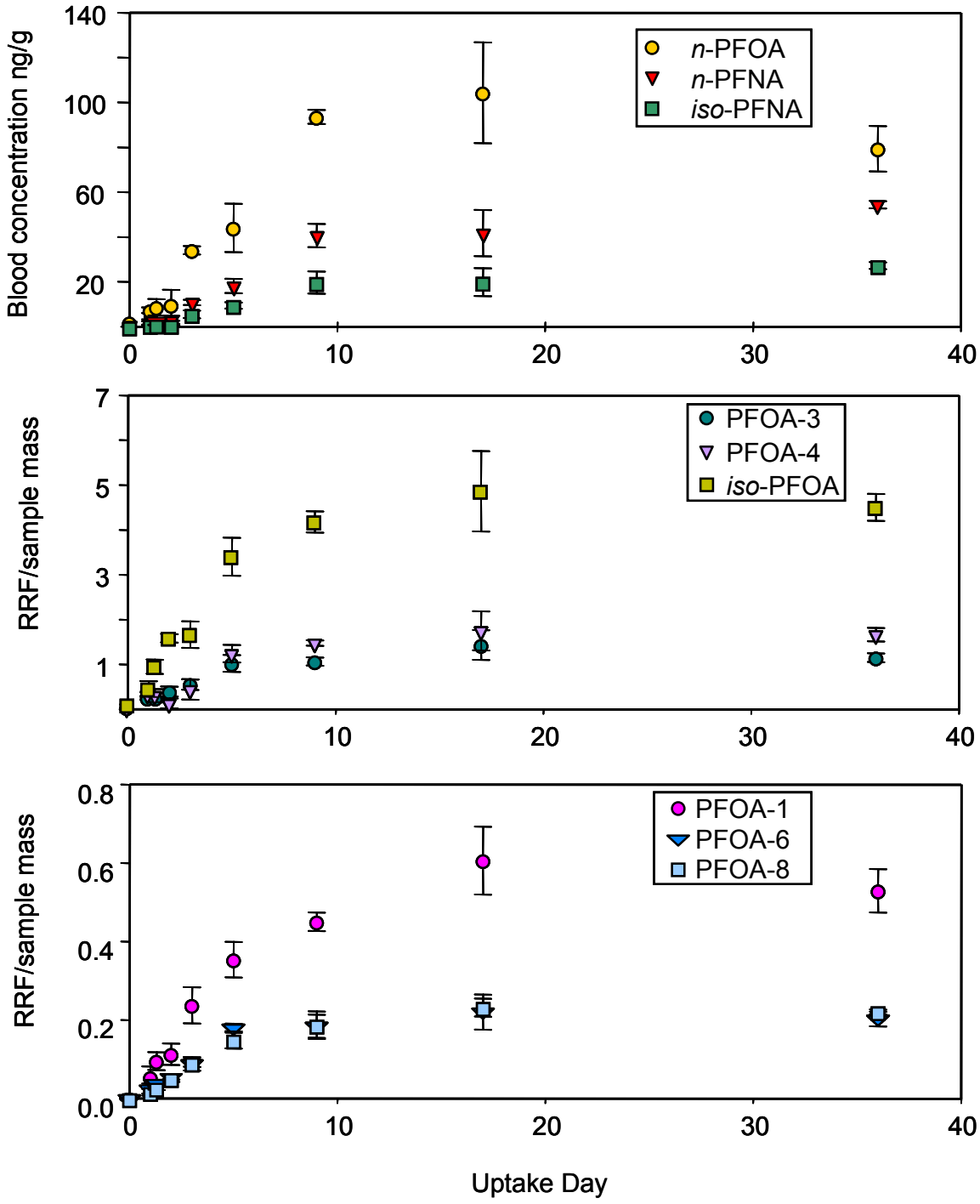

Figure 6.2. Uptake of ECF PFOA isomers in rainbow trout blood. PFOA isomers are grouped together according to scale. Each point represents mean (N=3) and standard error of mean, indicated by error bars.

Whole body carcass was not analyzed in this investigation and as such, assimilation efficiency could not be calculated. A closely related term is the accumulation ratio (AR) which is the isomer response in tissue divided by the isomer response in dosed feed (both terms

expressed per g of tissue or per g food) at day 36. This ratio represents the propensity for each isomer to accumulate in fish tissue (Fig. 6.3, hatched bars). The AR values of *n*-PFOA and *n*-PFNA corresponded to 0.13 ± 0.05 and 0.48 ± 0.15 in liver, respectively. The liver AR for PFOA in this study overlaps that reported previously for rainbow trout (0.091) but our n-PFNA depicted a higher AR than expected based on the value of 0.35 measured for *n*-PFDA by Martin et al.[32]. In that study, the fish were considerably smaller (2 – 5 g) and *n*-PFNA was not administered in the dose[32]. As such, the livers for that experiment ranged from 0.038 to 0.070 g whereas in this experiment livers ranged from 0.68 to 1.18 g. Larger samples may lead to more accurate determination of concentration and greater accumulation. Liver-based AR may not be predictable with chain length based on previous research by Martin et al. [32]who reported liver-based AR for perfluoroundecanoate, perfluorododecanoate, and perfluorotetradecanoate as 0.36, 0.23, and 0.23. In that same study, carcass-based AR calculations correspondingly increased with PFCA chain length[32].

Of all the ECF PFOA isomers, PFOA-8 and *n*-PFOA displayed the highest ARs, accumulating more efficiently than other branched PFOA isomers (Fig. 6.3). This is different than the AR distribution for ECF isomers in rats in which *iso*-PFOA was the most accumulating branched isomer[43] which may suggest different elimination mechanisms between fish and mammals. AR values for *n*- versus *iso*- isomers of PFOA were compared to *n*- and *iso*- PFNA to determine whether any chain length dependency existed (Fig. 6.4). With PFOA, ARs were 1.6 to 3.8x greater for *n*-isomer compared to *iso*-isomer. Similarly, AR of *n*-PFNA was 1.6 to 3.0x higher than that of *iso*-PFNA in all tissues except the liver in which the AR was only 1.1 times higher. The trend of isopropyl isomer being discriminated against and the *n*-isomer preferentially retained may not hold with longer chain lengths for which a single branch may not be as distinct. For example, a single CF_3 branch on an inner carbon of perfluorotridecanoate would probably not have markedly different physical properties compared to the *n*-isomer but the same branch on perfluorobutanoate could be much more distinctive than the *n*-isomer.

PFCA isomer composition at day 36 likely represents different isomer elimination rates, as judged by first order elimination rate constants (k_d). Temporal analysis of blood and liver over the 38-day depuration phase revealed first order elimination kinetics ($r^2>0.7$) (Table 6.2). Blood and liver $T_{½}$ were calculated using $\ln 2/k_d$. For some isomers, the concentration

diminished to <LOQ. For these isomers, only concentrations up to and including the previous time point were used.

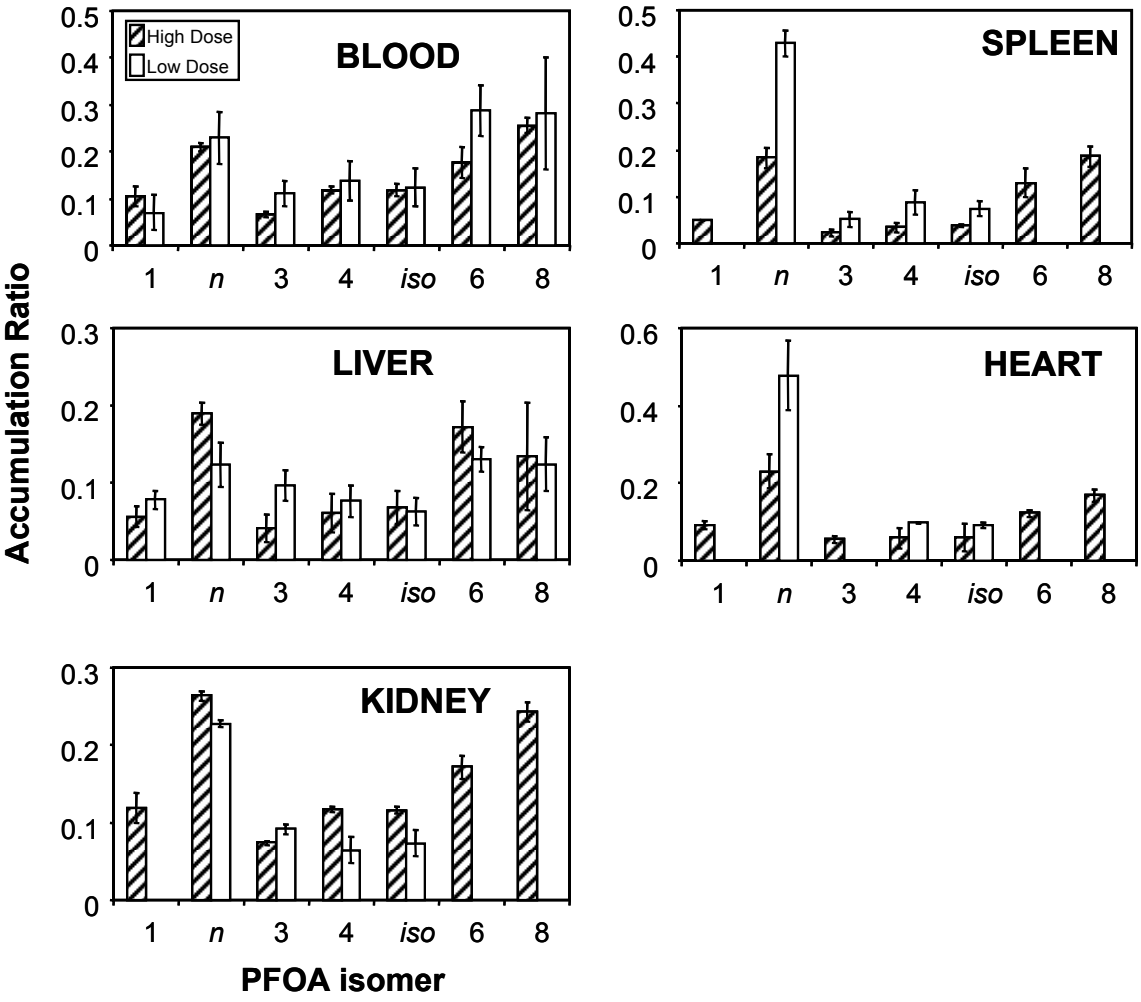

Figure 6.3. Accumulation ratio (PFOA $isomer_{tissue}$÷PFOA $isomer_{dose}$) for each PFOA isomer in fish tissues for high dose treatment (▨) and low dose treatment (□) at the last day of the uptake phase. Error bars are standard deviation for N = 5 for low dose and N = 3 for high dose. No bar represents <LOD for low dose treatment.

$T_{½}$ values of *n*-PFOA and *n*-PFNA corresponded to 3.7 (3.2 to 4.4) days and 6.0 (5.2 to 7.1) days in liver, respectively and are similar to those by Martin et al. for *n*-PFOA and *n*-PFDA, 5.2 and 14 days, respectively[32]. In our study, the trends in k_d and $T_{½}$ in liver were also observed in blood. The chromatogram of a fish liver sample from the last day of depuration is depicted in Figure 6.1 D in which *n*-PFOA and PFOA-8 are clearly visible. Thus as expected, the longest $T_{½}$ (and slowest k_d) corresponded to PFOA-8, 6.4 days in blood, but based on the 95% confidence interval, it was non-distinct from *n*-PFOA ($T_{½}$ =5.6 days). $T_{½}$ values of the

remaining isomers were shorter ranging from 2.5 to 3.8 days, in most cases with overlapping 95% confidence intervals. PFOA-6 did not overlap with all of the other branched isomers and could be considered to have an intermediate residence time between *n*-PFOA & PFOA-8 and the other branched PFOA isomers. Consistent with observations at day 36 in tissues, the $T_{½}$ of *n*-PFNA was 1.3 to 1.5x longer than *iso*-PFNA in blood and liver. The depuration rate constants and standard error were compared for each isomer using a one-way ANOVA and *post hoc* Tukey test. These results indicated that PFOA-7 was statistically different from PFOA-1, PFOA-3, PFOA-4, and *iso*-PFOA. PFOA-6 was also found to be eliminated slower statistically compared to PFOA-3, PFOA-4, and *iso*-PFOA. The major branched isomer peaks PFOA-1, PFOA-3, PFOA-4, and *iso*-PFOA all demonstrated a statistically larger k_d compared to *n*-PFOA.

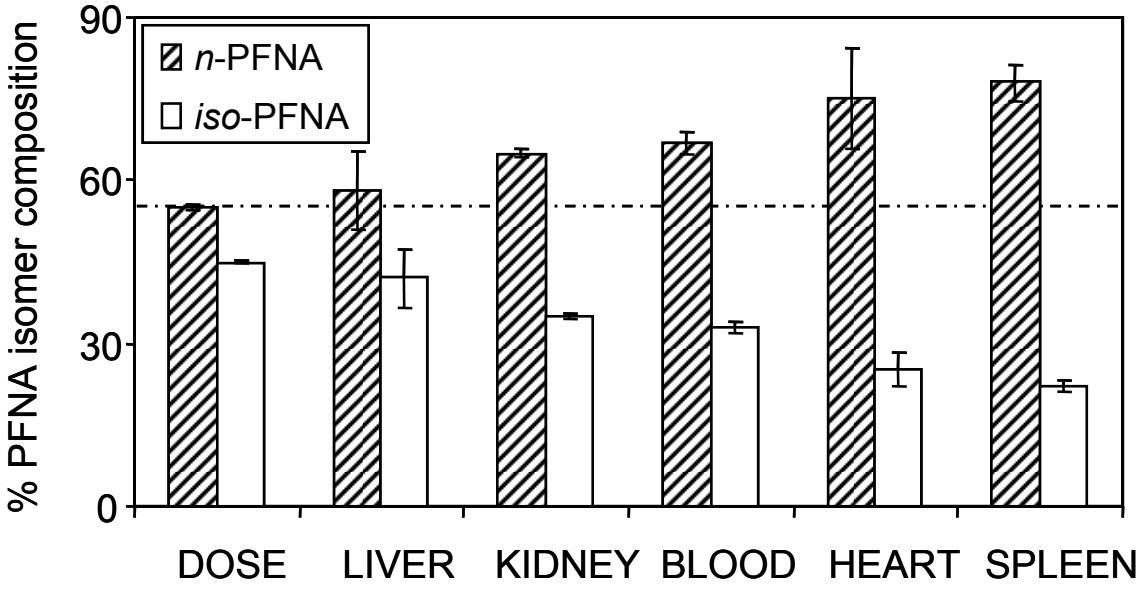

Figure 6.4. Composition of PFNA isomers in dose and tissues at end of uptake phase (day 36). Standard deviation for N=3 indicated by error bars represent. Dashed line corresponds to dose composition.

Table 6.2. Elimination kinetics (ln concentration vs. depuration day) for blood and liver in high dose treatment.

	BLOOD			LIVER		
	k_d ± std error (95% CI range) ($days^{-1}$)	**Elimination ½-life (95% CI range) (days)**	**R^2 ln(c) vs. day**	**k_d ± std error (95% CI range) ($days^{-1}$)**	**Elimination ½-life (95% CI range) (days)**	**R^2 ln(c) vs. day**
PFOA-1	-0.228 ± 0.023 (-0.276 to -0.179)	3.0 (2.5 – 3.9)	0.8365	-0.220 ± 0.022 (-0.265 to -0.175)	3.2 (2.6 - 4.0)	0.8461
n-PFOA	-0.123 ± 0.010 (-0.143 to -0.104)	5.6 (4.8 – 6.7)	0.8726	-0.188 ± 0.01 (-0.218 to -0.157)	3.7 (3.2 - 4.4)	0.8763
PFOA-3	-0.275 ± 0.022 (-0.322 to -0.228)	2.5 (2.1 – 3.0)	0.9123	-0.216 ± 0.020 (-0.256 to -0.175)	3.2 (2.7 - 4.0)	0.8523
PFOA-4	-0.264 ± 0.017 (-0.300 to -0.229)	2.6 (2.3 – 3.0)	0.9195	-0.239 ± 0.017 (-0.274 to -0.205)	2.9 (2.5 - 3.4)	0.9058
iso-PFOA	-0.273 ± 0.016 (-0.306 to -0.240)	2.5 (2.3 – 2.9)	0.9343	-0.253 ± 0.017 (-0.289 to -0.217)	2.7 (2.4 - 3.2)	0.9052
PFOA-6	-0.183 ± 0.016 (-0.216 to -0.151)	3.8 (3.2 – 4.6)	0.8664	-0.188 ± 0.013 (-0.215 to -0.161)	3.7 (3.2 - 4.3)	0.9094
PFOA-8	-0.109 ± 0.015 (-0.140 to -0.077)	6.4 (4.9 – 9.0)	0.7094	-0.181 ± 0.013 (-0.208 to -0.153)	3.8 (3.3 - 4.5)	0.8963
n-PFNA	-0.044 ± 0.004 (-0.051 to -0.035)	15.9 (13.5 – 19.6)	0.8392	-0.116 ± 0.009 (-0.134 to -0.097)	6.0 (5.2 – 7.1)	0.8692
iso –PFNA	-0.067 ± 0.004 (-0.075 to -0.059)	10.3 (9.2 – 11.8)	0.9222	-0.147 ± 0.012 (-0.171 to -0.122)	4.7 (4.0 - 5.7)	0.8513

In rats and mice, urinary elimination is a major route of PFOA excretion and research supports that this renal excretion is governed by specific organic anion transporter proteins (OATPs)[44,45]. For some species such as rats, the expression of certain OATPs are gender-specific, which may account for sex-dependent toxicokinetics[46]. In this study, the sex of individual fish was not identified and as such, gender-related elimination could not be examined. Oakes et al. did not find significant differences in toxicity endpoints associated with PFOA exposure between male and female fathead minnow (*Pimephales promelas*)[38]. The underlying mechanisms responsible for retention of PFCAs in fish have not been deciphered.

In other research, toxicity of linear and branched perfluoroalkyl compounds suggested that branched isomers are less potent. Loveless et al. determined branched PFOA isomers were less toxic than *n*-PFOA in rats and mice when considering peroxisomal β-oxidation, body weight gain, feed consumption, and a variety of serum lipid parameters[47]. Peroxisome proliferation is a biological effect of perfluorinated acids and involves binding to a specific nuclear receptor (PPAR), which exist in several isoforms: α, β, and γ. Peroxisome proliferation activity also appears to be dependent on perfluoroalkyl geometry. Binding of a ligand such as *n*-PFOA to a PPAR is the first step in a complex chain of events resulting in peroxisome proliferation[48]. Vanden Heuvel et al. demonstrated that *n*-PFOA readily bound to all three PPAR receptors whereas though branched-PFOA binded to human PPAR-α, it did not have as strong agonist activity compared to the *n*-isomer[48]. Metabolism rates of ECF precursors also vary with geometry as demonstrated by differing metabolism rates in isomers of N-ethyl perfluoroctanesulfonamide[30]. Together, research from these studies support the hypothesis the perfluorocarbon arrangement is critical to biological behaviour. Elimination of branched PFOA isomers may be faster than for *n*-PFOA across gill membranes. The relative importance of partitioning related to hydrophobicity versus carrier-mediated transport for PFCAs has not been reported in fish. The structure of PFOA-8 is unknown but given its minor presence in ECF PFOA, it may be a di-methyl or t-butyl branched isomer.

Active transport relies on binding to a mobile agent (such as OATPs or serum proteins). It is thus plausible that at some dose, binding sites on transporter proteins become saturated, resulting in markedly different toxicokinetics at a high dose compared to a lower dose[45]. To

investigate this, we repeated the experiment using an ECF PFOA dose of 0.0012 mg/kg/d, which was approximately 6x lower than 0.0069 mg/kg/d dose first implemented. At the end of the uptake phase (day 33) *n*-PFOA concentrations in blood, liver, kidney, heart and spleen were 18.63 ± 4.58, 10.07 ± 2.31, 18.50 ± 0.34, 38.82 ± 7.32, and 34.84 ± 2.27 ng/g. The most abundant branched isomer observed was *iso*-PFOA with concentrations in blood, liver, kidney, heart and spleen corresponding to 1.29 ± 0.43, 0.65 ± 0.18, 0.77 ± 0.18, 0.97 ± 0.07, 0.77 ± 0.17 ng/g. Concentrations in heart and spleen were higher than in blood for *n*-PFOA and *iso*-PFOA.

At the end of the uptake phase for the low-dose treated fish, the % *n*-PFOA in the low dose tissues followed that of the high dose tissues closely where the greatest enrichment of the *n*-isomer was observed in the heart and spleen (both 96% *n*-PFOA). AR in liver and blood were similar but for other tissues, some distinctions were noted (Fig. 6.3, white bars). The high AR of *n*-PFOA in spleen and heart of low dose fish suggested selectivity for this isomer in these tissues at this dosage. It was expected that the selective accumulation would be observed in a high dose scenario where competitive binding to proteins may be prevalent. It is unclear why the opposite effect occurred. Values of k_d could only be calculated for *n*-PFOA in blood due to reduced response of other isomers and relatively low concentrations in liver. First order elimination was apparent (R^2 0.763) albeit with fewer time points. The k_d was 0.135 d^{-1} ± 0.031 d^{-1} (standard error), corresponding to a $T_{½}$ of 5.2 days agreeing well with $T_{½}$ of the high dose treatment, 5.6 days. Yoo et al. also did not observe any significant difference in elimination kinetics of PFOA in chickens when dose was increased by a factor of 5[49].

Tissue concentrations in the low dose group were of lower magnitude than in the high dose treatment but still greater than fish from the natural environment. For example, biomonitoring of freshwater fish suggested typical liver PFOA concentrations range from <1 ng/g to 5 ng/g.[1,5] and in the current study, steady state PFOA concentrations in blood and liver corresponded to 16-18 ng/g and 5-8 ng/g, respectively. Katakura et al. found the distribution of PFOA in male rats to depend on dose magnitude. At higher doses, typical of most PFOA toxicokinetic studies, there was greater distribution to other tissues including blood, kidney, intestine, testes, spleen, fat, lung, heart, and brain[45]. At lower doses, greater accumulation in the liver occurred as evidenced by liver/plasma PFOA concentration ratios ~ 2 at the lowest dose

of 0.041 mg/kg as opposed to the liver/plasma PFOA ~ 1 at the highest dose of 16.56 mg/kg[45]. The lowest dose resulted in male rat liver concentrations of 558 ng/g PFOA which was nearly 10x greater than the fish liver concentrations observed in this study, 60.9 ppb in the high dose treatment group[45]. Renal clearance in the rats did not appear to be affected by dose level and as such, it is unclear what effect on toxicokinetics would ensue considering this is the major route of elimination in rats[45].

The lengthy $T_{½}$ of PFOA-8 has important implications for biomonitoring and source identification. Although fluorochemicals produced by ECF have a characteristic isomer signature, this isomer profile is not conserved in some biological samples based on the faster elimination for most of the branched isomers. The most dominant branched isomer in the ECF mixture is isopropyl which is also the most frequently identified branched isomer in environmental samples[28-30]. Isopropyl PFCAs are problematic for source identification as there is some evidence that there may be additional sources beyond ECF[28]. Therefore, source designation based on *n*-isomer and isopropyl isomer patterns may not be feasible due to confounding sources. Instead PFOA-8 may be considered more diagnostic of ECF exposure. Due to its similar or longer $T_{½}$ compared to the *n*-isomer, perhaps the ratio of these could be used to quantify ECF contribution to the environmental isomer patterns. An obstacle is the relatively small contribution of this isomer in the ECF mixture. In our sample of ECF PFOA, PFOA-8 represented 0.26% of the total PFOA isomers peak area and as such may present an analytical challenge. PFOA-8 was identified in a polar bear liver sample from Eastern Greenland and comprised a much higher percentage of total PFOA compared to the additionally identified isomers - 4, *iso*, and 6[28], suggesting that even in a mammalian apex predator, PFOA-8 could have a long residence time.

PFOS arises from ECF-based materials. Thus, it is expected that in the absence of biological isomer discrimination, the PFOS isomer profile should match that of technical ECF PFOS. This was supported in human blood from Australia, Sweden and Canada[30,31]. However, given that the half-life in humans is 4 years coupled with the continued exposure through dust, food and water borne PFOS, it could be that human biological discrimination of PFOS isomers may be difficult to assess. Selective retention of *n*-PFCA over *iso*-PFCA and

other branched isomers noted in our research may not translate to PFOS. Overall bioaccumulation of PFOS isomers is expected to be higher than PFNA isomers [32]. The dietary bioaccumulation presented in this paper is but one of many aspects contributing to the overall perfluorinated isomer pattern in the environment. Further study is warranted on potential differences in activity of isomers in bioconcentration from water and sorption to sediment.

6.4 Conclusion

Fish exposed to PFOA and PFNA isomers through diet showed selective retention of *n*-isomer compared to the majority of branched isomers. Liver, blood, kidney, heart, and spleen tissues all showed enrichment in the contribution of *n*-PFOA and *n*-PFNA relative to most branched isomers when compared to the dose. Elimination appeared first-order and k_d were calculated for each isomer. The longest $T_{½}$ was found for *n*-PFNA followed by *iso*-PFNA. The major ECF PFOA isomer *n*-PFOA and one of the least abundant PFOA isomers, PFOA-8 both shared the longest $T_{½}$ of all PFOA isomers. In fact, PFOA-8 in blood after a 33-day repeated exposure did not show an appreciable difference in tissues compared to the dose. Accumulation efficiency of PFOA-8 was comparable to that of *n*-PFOA which may suggest that PFOA-8 may be diagnostic in identifying and quantifying ECF exposure based on analysis of PFOA isomer patterns in environmental samples. Kinetics may depend on binding and thus it must be ensured that toxicokinetics do not reflect saturation of binding sites. Although conducting the current experiment with a lower dose of ECF PFOA isomers showed similar results to the high dose study, a more exhaustive investigation of dose is warranted to determine whether the low dose and high dose levels implemented in this study resulted in saturation of processes involved in transport. As such, it is possible that toxicokinetics of PFCA isomers at environmentally realistic levels may deviate from the results of this study.

6.5 Acknowledgements

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CHAPTER SEVEN

Distribution of Perfluorinated Carboxylate Isomers in the North American Environment

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7.1 Introduction

Two perfluoroalkyl acids (PFAs), perfluorooctane sulfonate ($F(CF_2)_8SO_3$-, PFOS) and perfluorooctanoate ($F(CF_2)_7C(O)O$-, PFOA), have known industrial production and are ubiquitous in the environment. Both have been detected in aquatic organisms including marine mammals, rodents, terrestrial mammals including humans, fish-eating birds, insectivore/herbivore birds, and reptiles[1-4]. Sediment, air particles, marine water, fresh water, precipitation, snow are also demonstrably contaminated with PFOS and PFOA[5-9]. The breadth of monitoring not only encompasses a wide variety of samples but also geographical locations ranging from urban to remote, temperate to polar.

Longer chain perfluorocarboxylates ($F(CF_2)_xC(O)O^-$, PFCA) ($x > 8$) are also present in the environment. PFCAs from PFOA to perfluorotetradecanoate (PFTA, $x = 13$) were first reported in fish of an urban waterway[2]. This was surprising given PFOS and PFOA were the only PFAs with known intentional industrial production[10]. Even more curious was the observation of these relatively nonvolatile acids, PFOS, PFOA, and long chain PFCAs in Arctic biota, sediment, glacial snow, and waterways far from production and usage[3,6,9].

Two potential transport mechanisms have been proposed to account for the presence of PFCAs in remote regions and the relative importance of these pathways is a current subject of debate. One view is that directly emitted industrially synthesized PFCAs containing longer chain PFCA impurities, by-products during manufacture, are transported via ocean currents[11-13]. Another hypothesis is that PFCAs in remote regions are mainly the result of long range atmospheric transport and subsequent degradation of volatile perfluoroalkyl-containing chemicals[14,15]. Identified PFCA precursors with relevant production volumes are perfluorooctylsulfonamides ($F(CF_2)_8SO_2NRR'$), fluorotelomer alcohols ($F(CF_2)_yCH_2CH_2OH$, *y*:2 FTOHs), and fluorotelomer olefins ($F(CF_2)_yCH_2{=}CH_2$, *y*:2 FTOs). Industrial FTOHs and FTOs are of mixed chain length consisting of even-numbered perfluorinated carbons from $y = 4$ to 12. These volatile percursors have been detected globally in air including urban[16-18], rural[16-19], and remote[9,20,21] regions.

Industrial synthesis of perfluoroalkyl-containing compounds is conducted largely by electrochemical fluorination (ECF) and telomerization. From 1947 to 2002, the majority of

PFOS, PFOA, and perfluorooctylsulfonamides was produced by ECF[10]. The nature of the ECF reaction is one whereby a mixture of structural isomers are produced, varying in the perfluorocarbon arrangement[22]. Feedstock for ECF are comprised of linear alkyl geometry and therefore, the ECF product is predominantly the *n*-perfluorocarbon isomer (70-80%) with branched isomers (20 – 30%) as well as shorter and longer chain homologues[23,24].

Since 2002, PFOA production has been based primarily on telomerization, an industrial synthetic pathway used to generate FTOHs and FTOs since the early 1970s. Industrial telomerization is known to use tetrafluoroethene (taxogen) and *n*-perfluoroethyl iodide (telogen) as starting materials. During the reaction, the perfluoroethyl radical is repetitiously added to the taxogen thereby lengthening it in units of CF_2CF_2[25,26]. When chain propagation is terminated the product is a mixture of *n*-perfluoroalkyl iodides with even-numbered carbon chains. Further reactions are employed to produce FTOHs, FTOs, and PFOA from the perfluoroalkyl iodide mixture.

These precursors can be converted to the longer-lived PFAs via abiotic and biotic reactions. Perfluorooctylsulfonamides undergo biodegradation or metabolism to form PFOS[27,28]. Atmospheric oxidation of perfluorooctylsulfonamides yields PFOS, PFOA and shorter chain PFCAs[29,30]. Atmospheric oxidation of *y*:2 FTOHs and *y*:2 FTOs results in the formation of a series of PFCAs comprised of *y*+1 carbons to trifluoroacetate (*x*=1, TFA)[14,31]. For example, atmospheric oxidation of 6:2 FTOH forms perfluoroheptanoate (*x*=6), Perfluorohexanoate (*x*=5), perfluoropentanoate (*x* = 4), perfluorobutanoate (*x*=3), perfluoropropionate (*x*=2), and TFA. Biological transformation of *y*:2 FTOHs proceeds by a beta-oxidation pathway from which the PFCA containing *y* carbons is produced[32-34]. Alpha oxidation represents a minor pathway in FTOH metabolism producing the PFCA with *y*+1 carbons[34].

Previously we developed a method for isomer resolution of PFCAs in environmental samples[35]. Using this method, separation of eight peaks (isomers) of an authentic ECF PFOA sample with the peak corresponding to the *n*-isomer accounting for 78 to 80% of the total peak area. In human blood serum samples collected from across the Midwest in the United States, PFOA and PFNA isomer patterns qualitatively resembled the isomer pattern associated with ECF[36]. However, quantitatively, the *n*-isomer was far more prevalent in blood in comparison

to ECF PFOA[36]. This may suggest greater telomer input than ECF to the human PFOA isomer profile, assuming a lack of biodiscrimination. The PFOA isomer profile was similar in polar bears from the Arctic, consisting of 5% branched PFOA and 95% *n*-PFOA. Using these observations, we postulated that while both polar bears and humans were exposed to an ECF source, the major contribution to PFCAs was from a linear source such as fluorotelomers. The observation of ECF PFNA isomers in blood was attributed to ECF PFNA impurities in ECF PFOA. A recent presentation by 3M measured PFNA to be 0.2% by weight of ECF PFOA[37]. Based on the absence of branched PFNA isomers in polar bears, we suggested that branched PFOA isomers were due to atmospheric oxidation of perfluorooctylsulfonamides.

The contrasting isomer profiles between polar bears and humans highlights the use of isomer analysis as a tool to discern source exposure. In the current project, structural isomer profiles of PFCAs, from PFOA to PFDoA in a variety of environmental samples is presented with the objective of PFCA source elucidation.

7.2 Materials and Methods

7.2.1 Standards and Reagents.

An authentic standard of ECF PFOA was provided by 3M (St. Paul, MN). Based on ^{19}F-NMR analysis, the isomer mixture contained: 78% *n*-PFOA, 9.0% *iso*-PFOA, 12.5% internal CF_3 branched PFOA isomers, 0.2% *t*-butyl ($C(CF_3)_3$) branch, 0.13% dimethyl branch, and 0.1% alpha CF_3 branch. Linear PFOA and PFNA isomers were purchased from Sigma Aldrich (Oakville, ON). Wellington Laboratories (Guelph, ON) supplied *iso*-PFNA (93% pure, 7% *iso*-PFOA). The major isomers in ECF PFOA were tentatively identified using purified isomers provided by Wellington Laboratories and comparison of molecular ion and retention time on chromatograms. Acronyms for these isomers correspond to 3*m*-PFOA ($F(CF_2)_4CF(CF_3)CF_2C(O)O$-), 4*m*-PFOA ($F(CF_2)_3CF(CF_3)CF_2CF_2C(O)O$-), 5*m*-PFOA ($F(CF_2)_2CF(CF_3)CF_2CF_2CF_2C(O)O$-) and *iso*-PFOA ($(CF_3)_2CF(CF_2)_4C(O)O$-). Reagents used for the extraction and derivatization are described in a previous publication[36]. Details of QA/QC are in the Appendix E.

7.2.2 Sample Collection.

Ringed Seals

Livers from Ringed seals (*Phoca hispida*) analyzed in this study were part of a larger sample set analyzed by Butt et al.[38]. The livers were collected by local subsistence hunters and trappers from Resolute Bay, Nunavut (79 42 N, 94 49W) in 1993, 2000 and 2004. All seals sampled for this study were male and between 6 and 16 years of age. The samples from 1993 were generous contributions from the National Wildlife Research Center, Canadian Wildlife Service Specimen Bank, Ottawa, ON, Canada.

Dolphins

Plasma samples from Bottlenose dolphins (*Tursiops truncatus*) residing in the Gulf of Mexico and Atlantic Ocean were previously analyzed[39] and a subset from that study were used here. The Sarasota Dolphin Research Program, a collaborative initiative by the Chicago Zoological Society and Mote Marine Laboratory, conducted blood sampling from the fluke vein of live capture free-range bottlenose dolphins in the summer of 2003 in Sarasota Bay (27.3 N, 82.5 W) on the west coast of Florida. Also during the summer of 2003, dolphin blood sampling from two additional sites was conducted by the Harbor Branch Oceanographic Institution and the National Ocean Service of NOAA in Indian River Lagoon (27.6 N, 80.4 W) on the east coast Florida and Charleston, South Carolina (32.8 N, 80.0 W). Blood samples were rapidly centrifuged to isolate plasma and kept frozen until analysis.

Humans

Previously we reported the distribution of PFCA isomers measured in human blood serum[36]. Those blood serum samples were pooled from 10 donors each, of mixed age, gender and blood type and collected across the Midwest in 2005. That data is revisited in the current research for comparison to other samples.

Precipitation

PFCAs in rainwater collected at 9 sites across North America were presented in an earlier study[8]. Samples from two of the Canadian sites were re-analyzed here for isomers. Rainwater (600 to 650 ml) was obtained from semi-rural Egbert (44.2 N, 79.8 W, at the CARE facility) and urban Toronto (43.7 N, 79.4’ W) by the Canadian Atmospheric Precipitation Monitoring

Network from June to December 2003 using polyethylene bags with care to avoid fluoropolymers. Each sample was concentrated down to 50 ml using a rotary evaporator. The 50 ml water concentrate was then subject to derivatization (described below).

Lake Ontario

Biological samples from Lake Ontario in this study were previously analyzed for *n*-PFCAs[40,41]. The following samples were processed as composites of whole individual: alewife (mean fish mass 26.9 g, 2 fish/composite), rainbow smelt (mean fish mass 9.0 g, 5 fish/composite), and slimy sculpin (mean fish mass 9.7 g, 3 fish/composite). All were collected in September 2002 offshore in Lake Ontario, near Niagara on the Lake (43 3' N, 79 2' W). At the same location, a pooled sample of the invertebrate amphipod *Diporeia* was obtained. In September 2001, a pooled *Mysis* sample was taken from an offshore site near Coburg (43 7' N, 78 3' W) using an epibenthis sled towed at 50 to 80 m depths. Both Diporeia and Mysis were processed separately as bulk composites. With a 0.5 m diameter Wisconsin net (103 – 187 μm mesh) towed at 1 m subsurface, two grabs of plankton were caught on July 17, 2006 and July 6, 2004 at Station 13 (43 25' 00' N, 79 24' 00' W) and Station 40 (43 43' 00' N, 78 01' 36' W) which are in the Niagara basin and central area of Lake Ontario, respectively. Trout samples were provided by the Great Lakes Fisheries Specimen Bank and each represented an individual male (3 – 5 years) caught overnight near Port Credit (43 4' N, 79 6' to 79 9'W) using nylon gillnet at 30 to 40 m depth in September 2002. Each fish was processed as individual whole body homogenate.

Sediment cores were collected from the CCGS Limnos vessel using a 10 cm diameter large box corer. These sediment samples subsampled according to depth. For this study, two samples were analyzed: Station 40 on June 26, 2002 and Station 1014 (Rochester basin, 43 21 43 N, 77 30 02 W) on June 8, 1998. Large volumes of surface water at 4 m depth were also sampled from station 40, 13 in June 2002 and from station 41 (Mississauga Basin, 43 38'57'' N, 77 54'01' W) in August 2001. The water samples were centrifuged to remove the particle bound fraction.

Arctic Sediment

An earlier study details the profile of PFCAs in air, water, and sediment in the Canadian Arctic[9]. For the current research, sediment and water samples were obtained from Char Lake

and Amituk Lake which are both on Cornwallis Island, Nunavut. A sediment core from Char Lake was sampled on July 10, 2004 from its deepest point (approximately 10 m). The core was section and dating performed in an earlier study revealed 0 - 0.5, 1 - 1.5 and 1.5 – 2 cm subsamples corresponded to 1997 – 2004, 1990 – 1996, and 1983-1989[9]. Surface waters (4 m depth) were taken from Char Lake and Amituk Lake in August 2003.

7.2.3 Sample Extraction

A. Whole fish composite homogenate, dolphin plasma, ringed seal liver homogenate, invertebrates, and human blood sera.

Extraction of PFCAs from the tissues (1.5 to 2.3 g samples for Lake Ontario biota samples, 1.1 to 1.4 g for ringed seal livers, 2.00 ml for dolphin plasma) was conducted as previously described[42] using $C^{13}_{1,2}$-perfluorodecanoic acid (Wellington Laboratories, Guelph, ON) as an internal standard[36]. Liver, fish homogenate, and invertebrate samples were homogenized with tetrabutylammonium sulfate ion-pairing agent in sodium carbonate solution. Methyl *tert*-butyl ether (MTBE) was shaken with the aqueous homogenate and then centrifuged to isolate the organic phase. A second aliquot of MTBE was combined with the homogenate, shaken, centrifuged and added to the first extract. Extracts were taken to dryness using a slow stream of N_2. PFCAs were then derivatized to 2,4-difluoroanilide analogs for quantitation and isomer analysis by gas chromatography with negative chemical ionization mass spectrometry (GC-(NCI)MS) according to the procedure outlined previously described[36].

B. Sediment.

Each subsample of sediment was extracted by shaking 7 – 10 g of wet sediment with 4 ml sodium carbonate solution, 1 ml TBAS and 15 ml MTBE. After centrifuging and removing the MTBE phase, a second 15 ml aliquot of MTBE was added, shaken, centrifuged and the two organic phases were combined. PFCA concentrations in sediments are expressed as ng PFCA per g of dry sediment. To determine the dry sediment weight, wet sediment was heated in an oven to remove moisture at 100 °C for 1 week and then re-weighed dry.

C. Rainwater and Surface water.

Sample handling and processing for precipitation in this study was described in an earlier publication[8]. Each sample was a composite of event rainfalls, collected over two weeks. The

500 ml composites were concentrated by rotary evaporator to 50 ml. Each surface water sample was extracted analyzed in duplicate. All water samples (Arctic 1.5 to 1.6 L and Lake Ontario 1.5 – 3 L) were concentrated to 20 ml using a rotary evaporator. Each concentrated precipitation and surface water sample was derivatized. HPLC grade water (1 – 3 L) was treated in the same manner and served as method blanks.

7.2.4 Instrumental Analysis

Derivatized extracts were analysed for PFCA isomers using GC-(NCI)MS, described previously[36]. Separation of analytes in rainwater, human blood, dolphin plasma, and seal liver was conducted using a 105 m Rtx-35 stationary phase (Restek, Bellefonte, PA). Due to degradation of the original stationary phase, sediment and Lake Ontario biota were analyzed using a 60 m ZB-WAX (0.25 mm ID, 0.25 μm film thickness, Phenomenex, Torrance, CA) column. Details of the oven program using the 105 m Rtx-35 was reported[36]. The oven program for the 60 m ZB-WAX began at 80 °C and was held for 1.5 min, ramped by 20 °C/min to 110 °C and held for 4 min, followed by a 0.3 °C/min ramp to 120 °C and finally a fast 25 °C/min climb to 220 °C. Separation of 8 isomers of ECF PFOA was achieved using both stationary phases (Fig. 7.1). Of these eight, five isomers were identified using purified standards.

Table 7.1. Samples analysed for PFCA isomers.

Sample Subject	Location	Year	Specifications of Sample	N
Dolphin	Indian River Lagoon, FL, USA	2003	Plasma	2
	Sarasota Bay, FL, USA	2003	Plasma	2
	Charleston, SC, USA	2003	Plasma	2
Ringed Seals	Resolute Bay, NU, Canada	1993	Liver	3
	Resolute Bay, NU, Canada	2000	Liver	3
	Resolute Bay, NU, Canada	2004	Liver	3
Diporeia	Lake Ontario	2002	Composite	1
Mysis	Lake Ontario	2002	Composite	1
Zooplankton	Lake Ontario	2004, 2006	Composite	2
Trout	Lake Ontario	2001	Whole Body Individual	3
Sculpin	Lake Ontario	2002	Whole Body Composite	3
Smelt	Lake Ontario	2002	Whole Body Composite	3
Alewife	Lake Ontario	2002	Whole Body Composite	3
Sediment	Char Lake, Cornwallis Island, NU, Canada	Collected 2004	0 – 1 cm	1
			1 – 1.5 cm	1
			1.5 – 2.0 cm	1
Sediment	Lake Ontario, Station 1014	Collected 1998	0 – 3cm	1
	Lake Ontario, Station 40	Collected 2002	1 -2 cm	1
Precipitation	Toronto, ON, Canada	June - July and Oct - Nov 2003		3
		Aug 2004		1
	Egbert, ON, Canada	Oct – Dec 2003		2
Surface water	Lake Ontario station 40	June 2002	4 m depth	2
	Lake Ontario station 13	June 2002	4 m depth	2
	Lake Ontario station 41	Aug 2001	4 m depth	1
	Char Lake	Aug 2005	4 m depth	2
	Amituk Lake, Cornwallis Island, NU, Canada	Aug 2005	4 m depth	2
Human Blood sera [36]	Midwestern States	2004 – 2005	Each is a composite from 10 donors	16

7.3 Results and Discussion

Biological samples in this study consisted of dolphin plasma, Arctic ringed seal livers, Lake Ontario food web (invertebrates, forage fish, and top predator trout), and human blood serum. Abiotic samples were surface sediment and water from the Canadian Arctic and Lake Ontario as well as precipitation collected from urban Toronto and semi-rural Egbert. Human blood samples and polar bear livers reported here were analyzed for PFCA isomers previously [35,36] and are compared here to other environmental isomer profiles. Similarly, concentrations of PFCAs in the dolphin plasma[39], ringed seals[38], sediment from Lake Ontario [43] and Arctic Lakes[9], Lake Ontario trout[41], and North American precipitation[8] were detailed previously using larger sample sets, from which PFOA to PFDoA concentrations are presented in Table 7.2. With the exception of human blood and polar bear livers, isomers were not resolved in these earlier studies and quantitation was based on *n*-isomer calibration. Thus, the concentrations in Table 7.2 likely correspond to both *n*- and branched isomers.

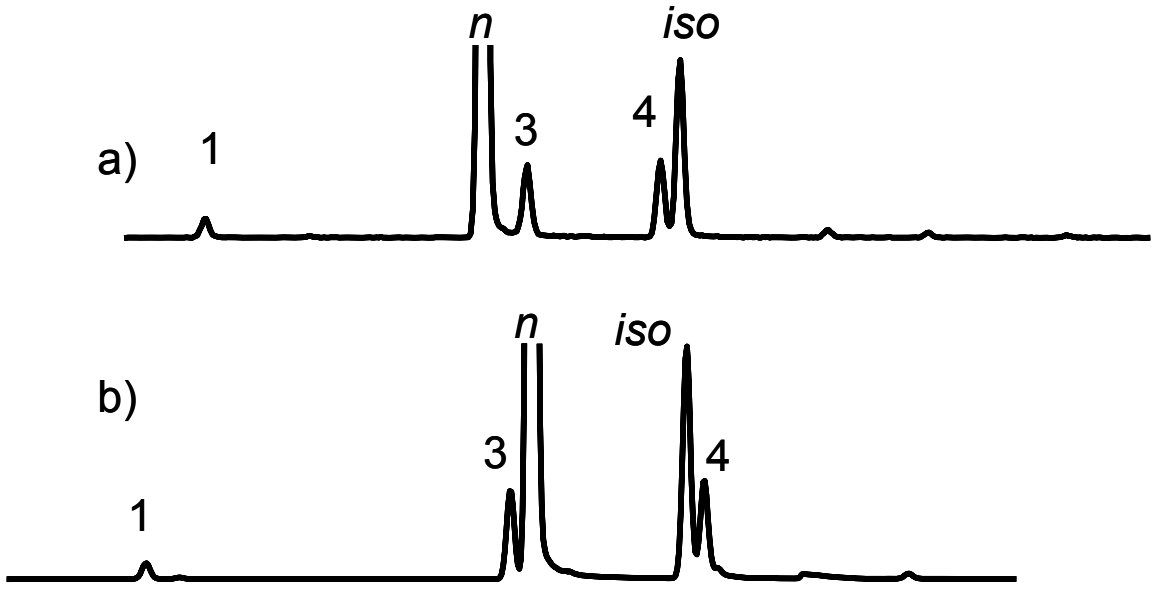

Figure 7.1. GC-MS chromatograms of ECF PFOA using a) Rtx-35 column and b) ZB-WAX column. Peaks were numbered according to elution order on Rtx-35. Isomers 1, 3, 4, and *iso* correspond to 3*m*-PFOA, 4*m*-PFOA, 5*m*-PFOA and *iso*-PFOA. The ratio of 3*m*, 4*m*, 5*m*, and *iso*-PFOA to *n*-PFOA corresponds to 1, 5, 4, and 13%, respectively.

Table 7.2. Concentration profile of PFCAs in samples. In the event of N = 2 distinct samples (not duplicate of the same sample), the range is reported and for N ≥ 3, the mean ($\bar{x}$) and standard deviation (σ) is reported.

	PFOA	PFNA	PFDA	PFUnA	PFDoA
Ringed Seals Liver, ng/g wet weight* [38]					
1993, N = 2, range	<3.6 – 4.5	1.8 - 2.7	1.1-1.3	2.5 - 3.3	0.49 -0.57
2000, N = 2, range	<3.6	2.1 – 2.3	1.3 – 2.1	2.8 – 5.2	0.47 -1.2
2004, N = 2, range	<3.6 - 6.2	3.6 - 7.3	2.9 - 4.9	5.7 - 12.3	1.8 - 1.8
Polar Bear Liver, ng/g wet weight*[3]					
2002, N = 7, range	2.9 – 13	108-230	35-76	56-78	4.7-8.2
Dolphins plasma, ng/g wet weight* [39]					
Sarasota Bay, N = 2, range	<0.5 - 5.5	6.1 - 23	13.1 - 23.4	19.1 - 38.6	0.3 - 5.2
Indian River Lagoon, N = 2, range	16.6 - 38.1	10 - 10.2	12.6 – 19.3	11 – 18.1	1.7 – 2.1
Charleston, N = 2, range	42.7 – 67.0	67.5 - 89.6	133 – 170	94.4 – 108.5	8.4 – 12.3
Lake Ontario biota, ng/g wet weight					
Zooplankton 2004	1.9	0.5	0.3	0.8	0.3
Zooplankton 2006	0.6	0.2	0.5	0.2	0.2
Mysids	3.9	2.8	1.2	0.8	0.4
Diporeia	67	37	16	18	5.0
Sculpin, N = 5					
$\bar{x}$	88	90	39	49	15
σ	21	19	9.4	13	4.6
Smelt, N = 5					
$\bar{x}$	6.8	20	11	12	3.9
σ	3.0	11	6.7	8.6	2.6
Alewife, N = 4					
$\bar{x}$	1.4	0.9	0.9	1.3	0.9
σ	1.0	0.4	0.2	0.4	0.6
Trout, N = 3[41]					
$\bar{x}$	1.8	1.4	1.8	1.5	0.7
σ	1.8	1.0	1.1	0.6	0.2
Human Sera, N = 15, ng/g wet weight[36]					
$\bar{x}$	4.4	0.8	0.2	0.05 – 0.07	<LOD
Median	4.2	0.7	0.2	Only	
σ	2.0	0.3	0.1	detected in 2 samples	
Char Lake Sediment, ng/g dry wt					
0 – 1 cm depth	2.2	0.6	0.3	0.2	0.1
1 – 1.5 cm depth	1.1	0.2	0.1	0.1	0.1
1.5 – 2 cm depth	1.2	0.1	0.2	0.1	0.1
Lake Ontario Sediment, ng/g dry wt					
Stn 40, 2 – 3 cm depth (2002)	2.7	2.0	3.2	<LOD	<LOD
Stn 1014, 0 – 3 cm depth (1998)[43]	3.0	0.3	0.5	0.5	<LOD
Precipitation, ng/L[8]					
Toronto, ON	1 - 11	0.5 – 9.7	<0.07 – 1.0	<0.07 – 3.7	<0.07 – 5.2
Egbert, ON	0.7 – 3.8	0.4 – 4.1	<0.07 – 1.1	<0.07 – 1.2	<0.07 – 3.3
Surface Water, ng/L					
Char Lake[9]	2.6	0.5	4.2	4.9	<LOD
Amituk Lake[9]	4.1	0.3	1.1	2.5	0.4
Lake Ontario[44]	5.9	2.0	<LOD	<LOD	<LOD

*PFCA concentrations that were less than the method detection limit (MDL) were not quantified. In these cases, <MDL is reported. For ringed seal liver the MDL was 3.6 ng/g ([38]), for dolphin plasma was 0.5 ng/g[39], and sediment was 0.1 ng/g.

7.3.1 PFOA Isomer Profiles

Branched PFOA isomers were detected in humans, Lake Ontario biota, and dolphins. To describe the isomer profile, the area of each branched PFOA isomer peak was divided by the *n*-isomer peak area and multiplied by 100% (Fig. 7.2). Ringed seals and polar bears from the Canadian Arctic did not contain linear or branched PFOA above detection limits.

Abiotic samples, Lake Ontario and Arctic lake sediment and water, and rainwater all contained *n*-PFOA, 5*m*-PFOA and *iso*-PFOA. In rainwater samples, 4*m*-PFOA was also present. Lake Ontario surface waters were the most isomer laden containing 3*m*, 4*m*, 5*m* and *iso*-PFOA. In all samples, the most abundant branched PFOA isomer was *iso*-PFOA, which is also the most prevalent in ECF PFOA (~ 9% by weight). In technical ECF PFOA, *iso*:*n* and 5*m*-PFOA:*n* ratios were 13% and 4% respectively. These ratios were much smaller in samples. For example, *iso*-PFOA:*n*-PFOA in smelt, sculpin, and diporeia was 0.6, 0.6, and 0.9% and 5*m*-PFOA:*n*-PFOA was 0.2, 0.2, and 0.4%.

PFOA isomer profile in Arctic

Branched PFOA isomers were <LOD in Resolute Bay ringed seal livers and Sanikiluaaq polar bear livers. Arctic sediment from the basin of Char Lake on Cornwallis Island in Nunavut, Canada at depths of 0 – 1, 1 – 1.5 and 1.5 – 2.0 cm corresponded to temporal intervals 1997 – 2004, 1990 – 1996, and 1983-1989[9]. The different depth samples did not vary greatly in their 5*m*-PFOA: *n*-PFOA ratio (1 – 2%) but *iso*-PFOA:*n*-PFOA decreased with depth where the most recent layer corresponded to 3% and the lowest layer (1.5 to 2.0 cm) was 1.0%. The top two layers of sediment samples (1990-2004) from Char Lake were more enriched in *iso*-PFOA isomers than sediment from Lake Ontario.

Branched PFOA isomers (5*m*-PFOA and *iso*-PFOA) were observed in surface water from both Char Lake and Amituk Lake at low levels. Char Lake had a slightly greater proportion of 5*m* and *iso*-PFOA (0.3 and 0.6% respectively) than in Amituk Lake (0.2 and 0.5%).

PFOA isomer profile in Lake Ontario

In biological samples containing branched PFOA, multiple branched isomers were observed (Fig. 7.3). In mysis, sculpin, diporeia, one alewife, and two smelt, 5*m*-PFOA and *iso*-

PFOA were detected and in two sculpin, two smelt, and composite diporeia, 3*m*-PFOA was also observed.

Isomer patterns in sediment acquired from two stations in Lake Ontario were also determined. Station 1014 in Lake Ontario was along the southeastern coast, near Rochester, NY, USA and Station 40 in the center of the lake. Both samples contained similar isomer patterns and the proportion of branched isomers was slightly greater than in biota. The ratios 5*m*-PFOA:*n*-PFOA and *iso*-PFOA:*n*-PFOA ranged from 0.5-0.6% and 0.9-1 %, respectively, in sediment which approximates the corresponding ratios in diporeia (0.4% and 0.9%).

Lake Ontario surface waters collected at 3 different stations contained all the major branched isomers in ECF PFOA, 3*m*-PFOA, 4*m*-PFOA, 5*m*-PFOA, and *iso*-PFOA. The proportion of these isomers is the highest reported for any sample in this research including, sediment and biota. However, the branched isomer composition in surface water was less than technical ECF PFOA.

PFOA isomer profile in Dolphins

In dolphin plasma, the PFOA isomer profile contained numerous branched isomers in the majority of specimens from Sarasota, Indian River Lagoon, and Charleston, South Carolina. Dolphins from Charleston and Indian River Lagoon all contained 3*m*-PFOA, *n*-PFOA, 4*m*-PFOA, 5*m*-PFOA and *iso*-PFOA. The branched isomer:*n*-PFOA ratios ranged from 0.05 – 0.1%, 0.05 – 0.1%, 0.03 – 0.2%, and 0.2 – 0.5% for 3*m*-PFOA, 4*m*-PFOA, 5*m*-PFOA, and *iso*-PFOA.

PFOA isomer profile in precipitation

Precipitation samples from two sites in Ontario were also analyzed. The isomer profile in precipitation was comparable in both areas, in which 5*m*-PFOA:*n*-PFOA and *iso*-PFOA:*n*-PFOA ranged from 0.6 – 1 % ($\bar{x}$ = 0.8%, σ = 0.3%) and 1 – 4% ($\bar{x}$ = 2%, σ =0.9%), respectively. Branched isomers were also noted in perfluorohexanoate (PFHxA) and perfluoroheptanoate (PFHpA) profiles. The branched isomer profile consisted of a single isomer for these shorter chain PFCAs. Four of the five samples had a branched PFHpA isomer, the ratio of which to *n*-PFHpA ranged from 0.6 to 1%. Two samples contained a branched PFHxA isomer, normalized to *n*-PFHxA ranged from 0.4 to 0.8%.

PFOA isomer profile in humans

The percentage of branched isomers in human blood PFCA isomer profiles have been reported previously[36]. Here we focus on the individual branched isomers of that study and compare the overall pattern to other samples. In all fifteen samples 5*m*-PFOA and *iso*-PFOA were observed with mean ratios to *n*-PFOA corresponding to 0.2% and 2% ($\sigma = \pm 0.13$ and 0.61, respectively). These values are close to those observed in Lake Ontario biota. Eleven of the human samples also contained 3*m*-PFOA and 4*m*-PFOA with ratios to *n*-PFOA consisting of 0.08 ($\sigma = \pm 0.03$) and 0.3 ($\sigma = \pm 0.07$).

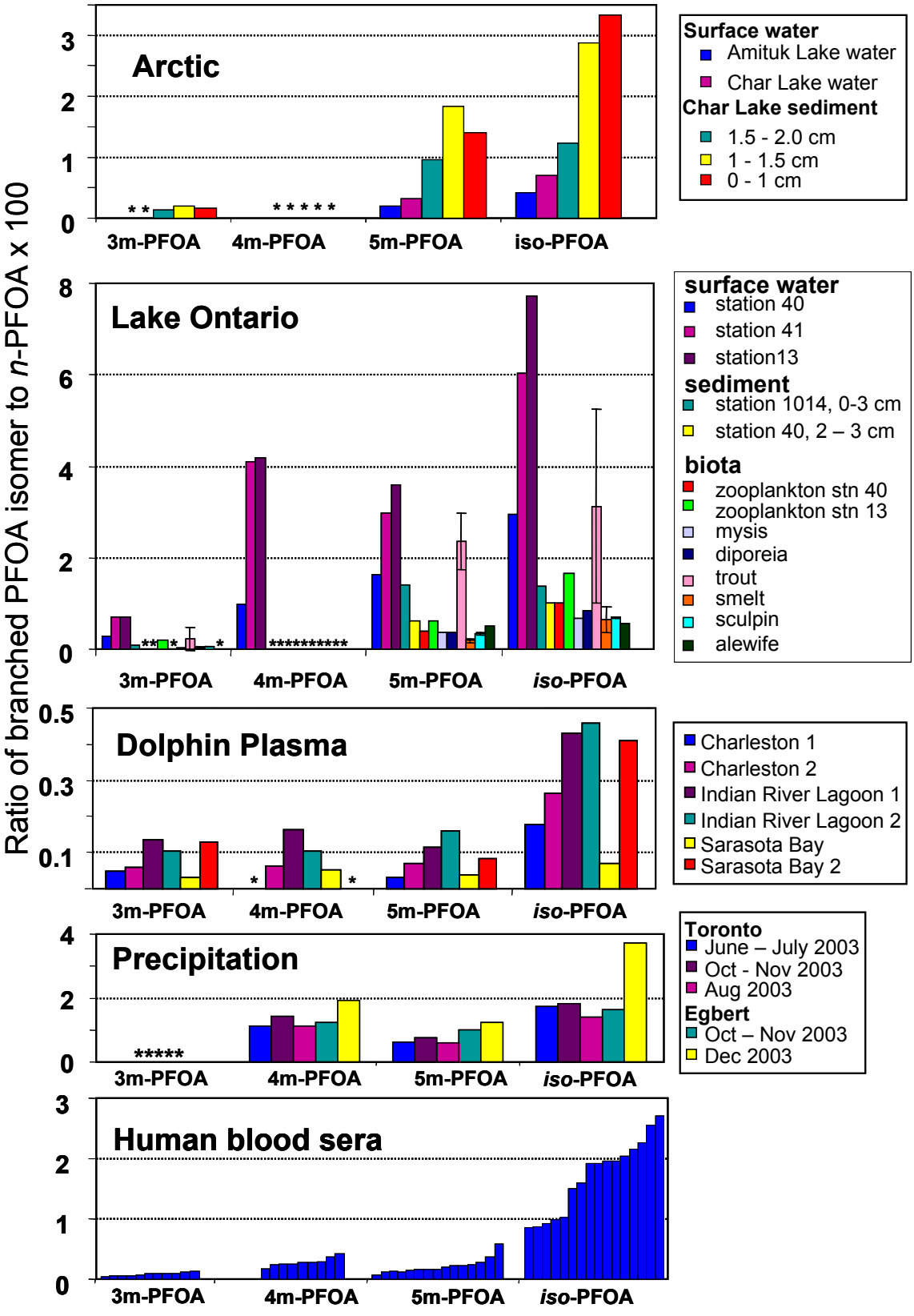

Figure 7.2. PFOA isomer profiles in environmental samples. Mean values plotted for trout, smelt, sculpin and alewife with standard deviation represented by error bars. * = less than LOD

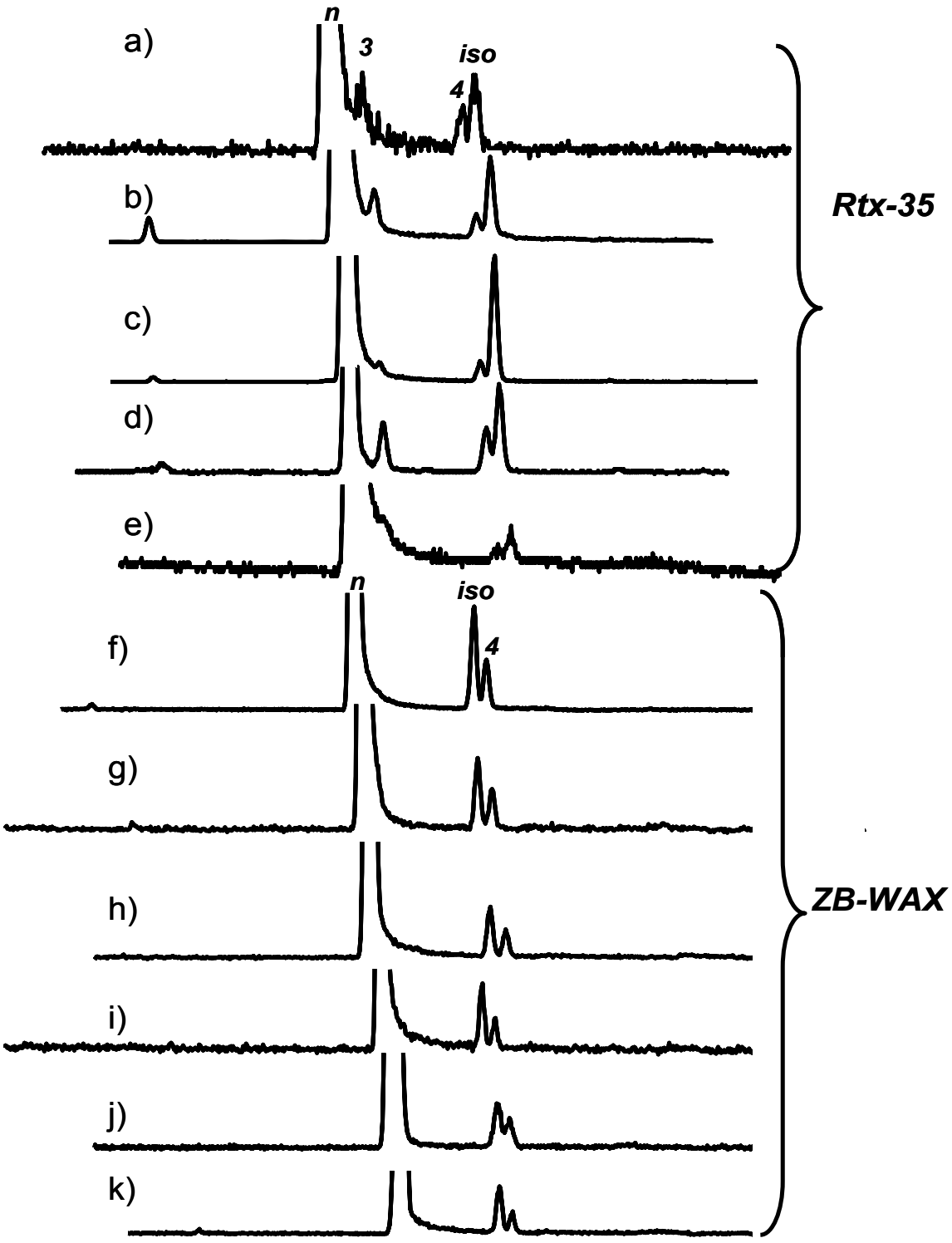

Figure 7.3. GC-MS chromatogram of PFOA isomers in a) Toronto rain, b) Indian River Lagoon dolphin blood, c) human blood, d) Lake Ontario Station 41 surface water, e) Amituk Lake surface water, f) Char Lake sediment, g) trout whole body homogenate, h) mysis, i) zooplankton, j) Lake Ontario sediment and k) smelt homogenate . Chromatograms a) to e) were acquired with Rtx-35 and d) to i) with ZB-WAX.

7.3.2 Isomer Profiles of PFNA, PFDA, PFUnA and PFDoA in Environmental Samples

Branched PFNA isomer profiles in samples fell into one of three categories: no branched isomers, only *iso*-PFNA, or multiple branched PFNA isomers (Fig. 7.4). No branched PFNA was observed in precipitation samples, Lake Ontario sediment and most Lake Ontario biota, including mysis, zooplankton, trout, and alewife.

Branched PFNA in ringed seals from Resolute Bay consisted solely of *iso*-PFNA. Similarly, *iso*-PFNA was observed in only a single polar bear from the Canadian Arctic. The ratio of *iso*:*n*-PFNA in ringed seals was 0.3% (σ , ±0.13) and in the one polar bear liver was 0.4%.

All four branched PFNA isomers and *n*-PFNA were detected in Char Lake sediment. The branched isomers appeared in a similar pattern to branched isomers in ECF PFOA. In these Arctic sediment samples, *n*-PFNA constituted 96-97% and *iso*-PFNA made up 2 – 3% in the PFNA isomer profile. PFNA-1:*n*-PFNA increased with depth from 0.06, 0.2, and 0.3% corresponding to 0 – 1, 1- 1.5 and 1.5 – 2.0 cm slices. The same slices demonstrated a similar increase in PFNA-3: *n*-PFNA corresponding to 0.6, 0.8, and 1% and likewise for PFNA-4:*n*-PFNA, 0.2, 0.4, and 0.4%. With increasing depth *iso*-PFNA:*n*-PFNA was 2, 3, and 1%. The lack of agreement of *iso*-PFNA with the other branched PFNA isomers upon depth analysis may be preliminary evidence that *iso*-PFNA is from a different source or that its profile is not easily discerned due to multiple sources. Unlike sediment, *iso*-PFNA was the only branched PFNA isomer observed in surface waters from Char and Amituk Lakes revealed *iso*-PFNA with *iso*:*n*-PFNA ranging from 0.8 to 1%.

In the PFNA isomer profile, only *iso*-PFNA, was observed in smelt, sculpin, and diporeia. There was little variance in *iso*:*n*-PFNA (±σ) between Lake Ontario biota as evidenced by 0.41% (±0.21), 0.56% (±0.23), and 0.57% in smelt, sculpin, diporeia and seals.

Human blood sera and dolphin plasma contained multiple branched PFNA isomers. At most, 4 branched PFNA isomers were determined and labeled according to elution order, PFNA-1, *n*-PFNA (PFNA-2), PFNA-3, PFNA-4 and *iso*-PFNA (PFNA-5). Of the four branched PFNA

isomers, only PFNA-4 and *iso*-PFNA were observed in human blood. Collectively these constituted at most 2% of the PFNA isomer pattern. In these samples, PFNA-4:*n*-PFNA and *iso*-PFNA:*n*-PFNA (± σ) corresponded to 0.50% (0.17) and 1.16% (0.30). Most of the dolphin samples (Indian River Lagoon (N=2), Sarasota (N=1), and Charleston (N=1)) contained both PFNA-4 and *iso*-PFNA. The ratio of these to *n*-PFNA ranged from 0.10 to 0.52% for *iso*-PFNA and 0.02 to 0.06% for PFNA-4. One dolphin sample from Charleston was void of any branched PFNA and one from Sarasota only contained *n*-PFNA and *iso*-PFNA. All branched PFNA isomers were <LOD in rainwater samples.

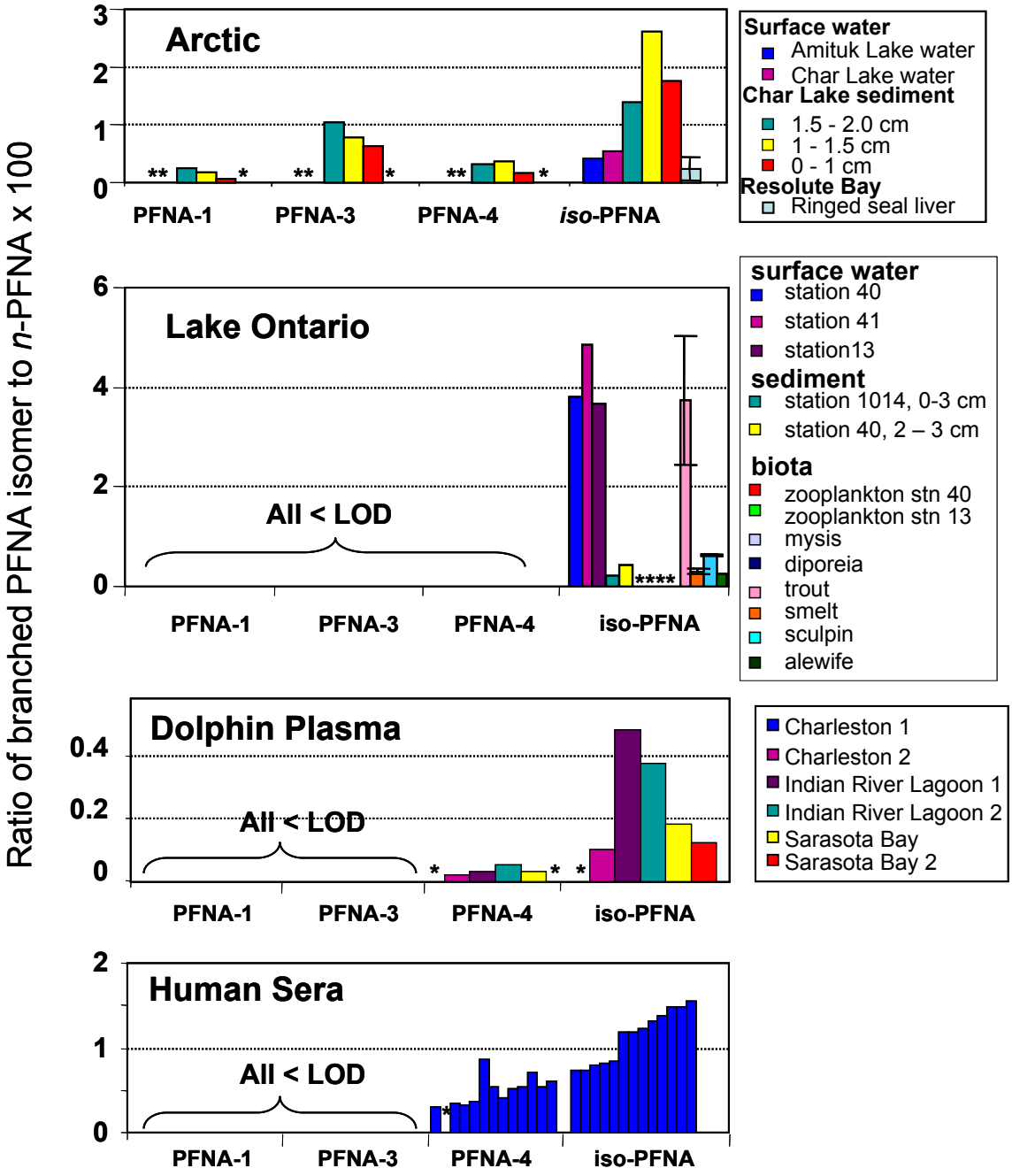

Figure 7.4. PFNA isomer profile in environmental samples. Mean values plotted for trout, smelt, sculpin and alewife with standard deviation represented by error bars. * = less than LOD

Branched isomers of longer chain PFCAs were not observed >LOD in surface waters, both in the Arctic and Lake Ontario as well as Lake Ontario sediment. In the remaining samples, a single branched isomer was observed for the longer chain PFCAs (Fig. 7.5). Based on the difference in retention time of the branched isomer and *n*-isomer, we hypothesized that this isomer corresponded to the isopropyl isomer. In Lake Ontario biota, at all levels of the foodweb, the greatest % of branched PFCA was consistently noted in PFUnA, followed by PFDoA. This pattern was also observed in dolphins from Florida and South Carolina. In addition, *iso*-PFDA was present in these samples but constituted a much smaller percentage of the total PFDA (maximum 0.74%). Branched PFDA and PFDoA was absent in human blood but one human blood sample contained *iso*-PFUnA (*iso:n*-PFUnA 2%)

Samples from the Arctic demonstrated a different pattern in % branched PFCA whereby the greatest percentage was observed for PFDoA, ranging from 4 to 8% branched PFDoA. Branched isomers of PFDA (1 – 4%) and PFUnA (1 – 2%) were also apparent in sediment, seals, and polar bears of this region and in each sample. Unlike samples from the temperate zones, the contribution of branched even-numbered carbon PFCAs was greater than the odd-chain lengths. Included in Fig. 7.5 was PFCA isomer data for polar bears from the Canadian Arctic in Sankiluaq (56.5 N 79.2 W), near southeastern Hudson Bay, from a previously published study[35]. Although this area is more south and east of Cornwallis Island, from which ringed seals and lake sediment were acquired, a similar even-odd pattern was observed whereby branched PFDoA and branched PFDA exceeded other chain lengths. The longer chain isomer profile in precipitation from Egbert and Toronto followed this trend more than Lake Ontario biota whereby two of the precipitation samples contained *iso*-PFDA (*iso:n*-PFDA 2 – 4%)and one had *iso*:PFDoA (*iso*:*n*-PFDoA 1%).

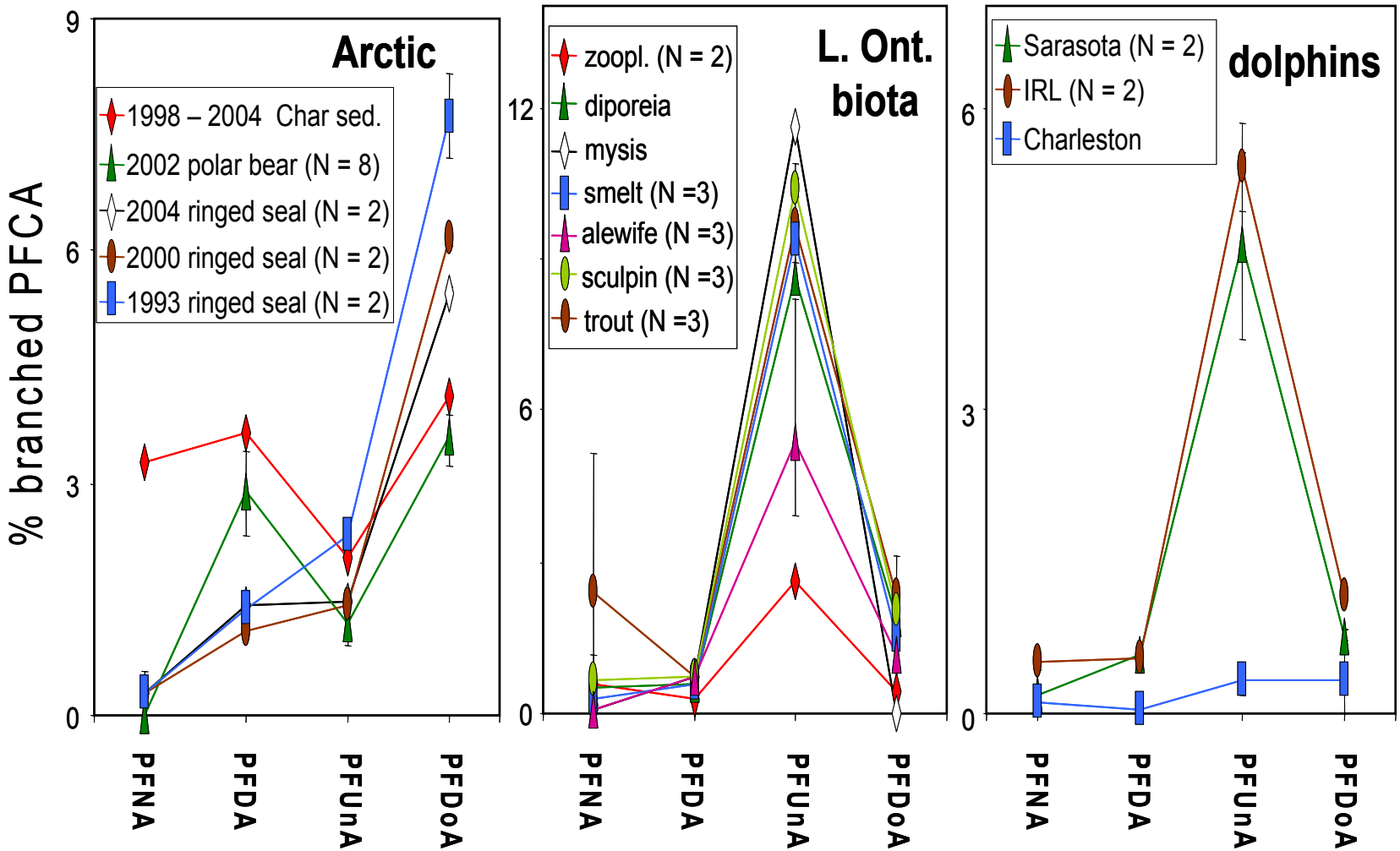

Figure 7.5. PFNA, PFDA, PFUnA and PFDoA isomer profiles in Arctic sediment and biota (ringed seals from Resolute Bay and polar bears from southeastern Hudson Bay[35], Lake Ontario biota and dolphins from Indian River Lagoon (IRL) and Sarasota in FL and Charleston, SC). Each data point is mean and error bars are standard deviation. % branched isomer refers to % isopropyl isomer except in the case of PFNA in Char Lake sediment and dolphins in which case there were multiple branched isomers. Longer chain PFCAs were not detected in Lake Ontario sediment and are thus omitted from this figure.

7.3.3 Environmental Significance

Of the PFCA isomer profiles determined in environmental samples, only PFOA and PFNA contained multiple branched isomers. The branched PFOA isomers observed in these biotic and abiotic samples were consistent with an ECF signature as evidenced by their identification in an authentic ECF PFOA standard. Intentional industrial synthesis of *n*-PFNA is only known to be conducted by telomerization[10], however, PFNA is a by-product in ECF perfluorooctyl-based synthesis. A recent presentation reported PFNA impurities to comprise 0.2% by weight of industrial ECF PFOA and that 65% of the PFNA impurity consisted of branched isomers. Thus, detection of multiple branched PFNA isomers is also indicative of an ECF signature.

The most abundant branched isomer of PFNA and PFOA in samples was the isopropyl isomer. This was expected given that *iso*-PFOA is the most abundant branched isomer in ECF PFOA and by analogy, *iso*-PFNA is likely the dominant branched isomer in the ECF PFNA by-product. In ECF PFOA, *iso*-PFOA:*n*-PFOA was 13% but in environmental samples this ratio did not exceed 6% and was frequently below 1%. The same ratio for PFNA in samples was at most 3%. Thus, although the presence of multiple branched isomers, as noted in PFOA and PFNA in some samples, was consistent with the ECF isomer signature, the elevated composition of *n*-isomer was probably not exclusively by ECF.

Environmental Significance of Biological PFOA and PFNA Isomer Signature

Observation of PFCAs in precipitation[5,8] was representative of atmospherically-derived PFCAs. Estimates of the pK_a of PFOA ranges from 2.8 to -0.5[45]. Thus, at environmental pH, PFOA and longer chain PFCAs would primarily exist in the anion form and non-volatile. Therefore, it is unlikely that PFOA and other PFCAs in rainwater are from volatilization. Branched PFOA isomers in rainwater resembled the ECF signature which could be indicative of atmospheric oxidation of perfluorooctylsulfonamide isomers. The atmospheric oxidation pathway is also expected to produce shorter chain analogs and indeed, branched isomers in PFHxA and PFHpA were noted in these precipitation samples. Isomer discrimination is assumed to be negligible in precipitation. Thus the isomer profile in precipitation can be compared directly to technical grade ECF PFOA. The presence of only 3-7% branched PFOA

isomers in precipitation suggested that at most, 70% of *n*-PFOA in precipitation was coming from a linear source such as 8:2 FTOH (assuming ECF perfluorooctylsulfonamides are 80% *n*-isomer). In the mechanism proposed by Ellis et al.[14], *n*-PFNA, *n*-PFOA and shorter chain length PFCAs would be produced by 8:2 FTOH oxidation. The absence of branched PFNA isomers in rainwater was consistent with an 8:2 FTOH input. The lack of branched PFNA in rainwater compared to its presence in human blood was further suggestive that humans are exposed to directly manufactured ECF PFNA (as an impurity in ECF PFOA). The lack of branched PFNA in precipitation is unlikely due to challenged LOD since the concentration of PFNA in rainwater was on the same order of magnitude to human blood.

The Canadian Arctic biota monitored in this study did not contain branched PFOA isomers >LOD, possibly due to a low bioaccumulation potential. In addition, we hypothesize that directly emitted ECF PFOA is only a minor source of PFCAs in the Arctic environment which may account for ECF PFOA isomers in Lake Ontario biota but not Arctic biota. Both Amituk and Char Lake surface waters contained low levels of branched PFOA isomers. Amituk Lake is considered an isolated lake, thus, a major source of its PFCA content is presumably atmospherically derived[9]. Thus the presence of branched ECF PFOA isomers in Amituk Lake surface waters is indicative of atmospheric transport and oxidation of ECF PFSAm[9]. Since Char Lake is also situated on Cornwallis Island and has similar concentrations as Amituk Lake, the data suggests that a similar input exists for Char Lake. However, Stock et al. detected perfluorodecanesulfonate in Char Lake sediment which is not known to have an atmospheric input[9] and is a component of aqueous film forming foams (AFFFs). This may be related to the branched PFNA isomer profile observed in Char Lake sediment. Multiple branched PFNA isomers were detected in Char Lake, consistent with an ECF source. Linear and branched PFNA isomers are components of ECF perfluorooctanoate[37], used in AFFF formulations and may represent local contamination through the use of AFFF. Char Lake is in close proximity to the Resolute airport and could have received minor inputs of perfluorinated acids in AFFF from incoming dust. In our analyses of Arctic biota, branched PFNA was rarely observed in polar bears[35] and only *iso*-PFNA was detected in ringed seals, suggesting that the ECF branched PFNA contamination of Char Lake sediment was locally constrained. If indeed ECF branched PFNA isomers are the result of AFFF use near Char Lake, then branched PFOA isomers may also be attributed to the same source. Unfortunately sediment from Amituk Lake was not available for comparison.

In Lake Ontario biota, Martin et al. reported sculpin and diporeia as having the greatest concentration of PFCAs[40]. This was attributed to diet because sculpin feed primarily on diporeia[46] which may account for the similarity in isomer profile. Surface sediment in Lake Ontario matched the PFOA isomer profile in diporeia (Fig. 7.2). The presence of branched PFOA in diporeia suggested that this organism may have greater exposure compared to pelagic fish or that the mechanism of accumulation was more efficient than in fish. Diporeia is a burrowing benthic invertebrate and could have been exposed to sediment-bound PFCAs and freely dissolved PFCAs in the sediment pore water[47] through diet and also habitat. Like fish, uptake/elimination of pollutants from water via respiratory membranes is possible but in addition, diporeia also possess the carapace across which uptake and elimination can occur[48]. Sediment-bound PFCAs may be bioavailable as demonstrated in a recent experiment in which bioaccumulation of PFCAs was observed in an aquatic oligochaete, *Lumbriculus variegates*, which are usually partially embedded in sediment in shallow waters [49,50]. In that study, the half life of *n*-PFOA in the oligochaete was 17 days, much longer than 3-5 day half-life measured in rainbow trout. An alternative explanation for elevated levels of linear and branched PFOA in diporeia is that they are generated PFOA *in situ* via biotransformation of PFOA precursors (such as polymers) in the detritus that diporeia subsist on. Experiments designed to determine whether biologically-mediated reactions yield PFOA from fluoropolymers are currently underway, however, biotransformation of certain perfluorooctyl-based fluoromonomers (polymer intermediates), and surfactants have been shown to yield PFOA [51,52]. Water samples from Lake Ontario were considerably more enriched in branched PFOA isomers than biota or sediment. Comparing water from station 40, 41 and 13, station 40 had the least proportion of branched isomers. Station 41 water samples had a larger proportion of branched PFOA than station 40 even though both stations are very close to each other. The difference may be influenced by the date of collection. Station 41 water was collected in 2001 whereas station 40 water samples were obtained in 2002. Water from station 13 had the greatest proportion of branched PFOA and may indicate proximity to source since this station is closer to the periphery of Lake Ontario. In sediment, biota and surface water, *n*-PFNA and *iso*-PFNA were not accompanied by other branched PFNA isomers. This was surprising given that ECF PFOA isomers were observed in all levels of the Lake Ontario ecosystem.

Recently Houde et al. determined PFOS isomers in the same Lake Ontario food web samples[53]. Unlike PFOA which likely has both telomer and ECF inputs, the sole source of

PFOS is presumably ECF-based. Therefore, PFOS isomer distribution in the environment is informative with respect to isomer discrimination processes (physical properties, transport, transformation, biological elimination, etc.). As determined by liquid chromatography, technical ECF PFOS consisted of 70% *n*-isomer and 20% monomethyl-substituted PFOS[53]. However, surface water from Lake Ontario was comprised of 50 to 60% *n*-PFOS and 43 to 50% monomethyl branched PFOS isomers[53]. Fish and invertebrate consisted of 89 to 100% *n*-PFOS[53]. The authors of that study postulated that isomer contamination profile in biota reflected the contamination pattern in sediment, suggesting preferential sorption of *n*-PFOS to sediment and greater water solubility of the branched isomers.

Results of the current research are consistent with the interpretation by Houde et al. whereby the PFOA isomer profile in sediment was similar to biota and water samples were more isomer laden. Despite having a greater proportion of branched PFOA than biota, the isomer profile in Lake Ontario surface water contained more *n*-PFOA than in technical ECF PFOA. This may imply additional contribution of a linear source such as telomerization.

In humans, a source of PFOA branched isomers may be ECF PFOA, which has been synthesized in large quantities since the late 1950s as an emulsifier for use in fluoropolymer synthesis[10]. ECF perfluorooctylsulfonamides, although ubiquitous in the atmosphere with indoor air concentrations vastly exceeding those in outdoor air[54], are not expected to be a major source of ECF PFOA isomers because perfluorooctylsulfonamides are metabolized to PFOS and not PFOA[27,55]. Also, *n*-FTOHs are likely candidates as *n*-PFOA and to a lesser extent, *n*-PFNA sources, based on their elevated concentrations in indoor air[16,56], inhalation or ingestion of residuals in surfactant and polymers for human use[57,58], and metabolism to primarily *n*-PFOA and the minor alpha oxidation product, *n*-PFNA[34]. The ability to discern the degree of telomer versus ECF exposure based on measurement of % *n*-isomer in the isomer profile is could be impeded by biological discrimination of structural isomers as shown in recent rodent pharmacokinetic experiments in which the major branched isomer components of ECF PFOA were eliminated faster than *n*-PFOA[59-61].

Dolphin blood plasma revealed PFOA and PFNA isomer profiles similar to humans in which multiple branched isomers of each were observed. In humans, branched isomers of PFOA and PFNA comprised a greater proportion of the isomer profile compared to any of the other

biological samples in this study. This abundance of branched isomers may be due to prolonged residence of PFOA in humans. The half-life of PFOA has been estimated as 4 years in humans[62], much longer than that measured in any other organism including mice, rats, fish and monkeys[63]. PFOA does not bioaccumulate in rainbow trout[64], which may account for its low concentration in Lake Ontario lake trout[40] and in Arctic seals which feed on fish and invertebrates[38]. PFOA appeared to bioaccumulate in dolphins from Charleston in a study by Houde et al. in which *n*-PFCAs were measured in water, sediment and fish of that area[65]. In that study, effluent from wastewater treatment plants in Charleston contained high concentrations of PFCAs and were postulated to be a significant source[65]. This may account for the similarity in PFOA and PFNA isomer profiles in humans and dolphins. It is interesting that although dolphins contained a lower proportion of branched isomers compared to humans, dolphin plasma concentrations of *n*-PFOA and *n*-PFNA were 5.5-67.0 ng/ml and 10 – 89.6 ng/ml[39], much higher than that of humans, 2.4 – 4.4 ng/g and 0.5 – 1.1 ng/g[36], respectively. Isomer analysis of food sources and environmental water of dolphins would contribute to the understanding of source exposure for dolphins. The presence of multiple branched PFNA isomers is consistent with the ECF isomer signature and may be indicative of exposure to ECF PFNA impurity in ECF PFOA.

Environmental Implication of Isomers of PFDA, PFUnA, PFDoA

Branched isomers, assumed to be isopropyl version, of PFDA, PFUnA, and PFDoA were detected in a number of samples (Fig. 7.5). The pattern of isomer distribution appeared dependent on geography. In dolphins, Lake Ontario biota, and one human blood sample, the PFCA with the greatest proportion of branched isomer was PFUnA. These results corroborate findings reported by Furdui et al. in which Great Lakes trout contained isopropyl PFUnA and PFTrA[66]. In ringed seals, Arctic sediment, and previously analyzed polar bears from the Canadian Arctic[35], the PFCA with the greatest proportion of isopropyl isomer was PFDoA. In abiotic samples from temperate regions, detection of isopropyl isomers of longer PFCAs was infrequent. Lake Ontario sediment did not contain any *iso*-PFUnA or *iso*-PFDoA. Of the PFCAs from PFNA to PFDoA in precipitation, two samples contained *iso*-PFDA and one contained *iso*-PFDoA but *iso*-PFUnA was <LOD.

Detection of *n*-isomers of PFDA, PFUnA and PFDoA in rainwater[8] was indicative of atmospheric oxidation of 10:2 and 12:2 FTOHs. Of the 5 rainwater samples, 2 contained a small amount of a single branched PFDA isomer (3% of the PFDA isomer profile) and of these 2 samples, one contained a branched PFDoA isomer (1% of the PFDoA isomer profile). Some plausible explanations for the presence of longer chain PFCA branched isomers in rain are further discussed further below.

ECF perfluoroalkyl compounds are known to be composed of linear and mixed branched isomers. In the case of human blood, *iso*-PFNA was accompanied by a second branched isomer and was hence, indicative of an ECF input. In contrast, the observation of a single branched isomer, *iso*-PFDA, *iso*-PFUnA, and *iso*-PFDoA was inconsistent with an ECF signature. Telomerization, a radical addition reaction, is known to conserve the geometry of its starting materials. It has been widely assumed that telomer chemistry only produces linear perfluoroalkyl chains. However, numerous patents and other peer-reviewed scientific literature describe the use of perfluoroisopropyl iodide ($(CF_3)_2CFI$) as a telogen in telomerization[67-69].

Entry of *iso*-perfluoroundecyl and *iso*-perfluorononyl substructures into search engines revealed that both C-11 and C-9 *iso*-perfluoroalkyl iodides are commercially available and have an extensive record in the patent literature. Perfluoroalkyl iodides are unstable and considered intermediates for further syntheses. Preparation of *iso*-perfluoroalkyl telomer iodides and telomer olefins with 5, 7, 9, and 11 perfluorinated carbons was described by Wroblewska et al.[70]. According to patent literature, these compounds may be further reacted to form isopropyl fluoro-acrylate monomers[71-74] for polymer synthesis used as anti-soiling in textile treatments, batteries, optical recording discs, etc. and in one case, perfluorinated acids in fire-fighting formula [75]. Furthermore, *iso*-9:2 FTOH has been employed in analytical chemistry applications[76,77] and surface properties of *iso*-PFCAs ranging from 5 to 15 carbons were investigated by the Naval Research Laboratory[78]. Even more patents describe the synthesis of *iso*-perfluoroalkyl geometry in epoxypropane-containing compounds (also known as “oxiranes”) such as (3-(perfluoro-9-methyldecyl)-1,2-epoxypropane) for surface treatments. The environmental chemistry of these compounds has not yet been reported. Regardless of their purpose, production may be limited considering the *iso*-PFCAs were no more than 12% of the isomer signature per PFCA. Temporal analysis of Great Lakes trout revealed an increasing

proportion of *iso*-PFUnA and *iso*-PFTrA from 1979 to 1988 and decreasing from 1993 to 2004[66], suggesting a decline in use or emissions.

An isopropyl fluorotelomer odd-chain length precursor may be a plausible candidate for the observed pattern of long chain *iso*-PFCAs. This is considered with the following hypothetical precursor, an isopropyl 11:2 FTOH ($(CF_3)_2CF(CF_2)_8CH_2CH_2OH$, 11:2 *iso*-FTOH). In humans, 11:2 *iso*-FTOH, if present as a residual, would be ingested or inhaled and metabolized to *iso*-PFUnA (major) and *iso*-PFDoA (minor). Given that most fluorotelomer products consist of a mixture of chain lengths based on differential rates of polymerization, minor impurities expected in telomer 11:2 *iso*-perfluoroalkyl formulations would be 9:2 and 13:2 *iso*-perfluoroalkyl compounds. Metabolism of 9:2 *iso*-FTOH and 13:2 *iso*-FTOH would yield major products *iso*-PFNA and *iso*-PFTrA and minor products *iso*-PFDA and *iso*-PFTA. In the current research, only one human blood sample contained *iso*-PFUnA but many Lake Ontario biota and dolphins contained long chain *iso*-PFCAs, centered around *iso*-PFUnA. This might suggest that a specific fluorotelomer formulation, surfactants or polymers, based on *iso*-PFUnA could undergo biotransformation in waste treatment plants and then undergo transport to other waterways.

Samples from the Arctic including sediment, polar bears and seals, were dominated by *iso*-PFDoA compared to *iso*-PFUnA in temperate regions. This was reminiscent of the even-odd pattern previously observed in Arctic organisms whereby the odd chain length PFCA is more concentrated than the preceding even numbered PFCA[3]. For the branched isomers, the opposite trend was apparent in which *iso*-PFDoA exceeded *iso*-PFUnA and *iso*-PFDA was greater than *iso*-PFNA. This suggested atmospheric oxidation of *iso*-perfluoroundecyl-based precursor. The hypothetical precursor, *iso*-11:2-FTOH, would likely yield *iso*-PFUnA and *iso*-PFDoA and of the two, *iso*-PFDoA would have a greater bioaccumulation potential[64] and as such, reach higher concentrations than *iso*-PFUnA with increasing trophic level. These results are partially supported by the detection of *iso*-PFDA and *iso*-PFDoA in some of the rainwater samples of this study, which are assumed to be atmospherically-derived. Further work is obviously needed on this hypothetical compound.

7.4 Conclusions

Two industrial synthetic pathways, ECF and telomerization which differ in isomer composition, have likely contributed to the global distribution of PFOA. However, based on isomer analysis of PFOA in environmental samples, source assignment is not straight-forward. This is mainly due to biological isomer discrimination which may lead to enrichment in *n*-PFOA in biota. Research presented in this paper demonstrates that analysis of isomers in longer chain PFCAs such as PFNA to PFDoA may provide additional information to allow source elucidation. In addition, PFCA isomer analysis in abiotic samples, which are not affected by isomer discrimination, is also imperative. Exposure to industrially produced ECF PFOA appears to result in branched isomers of both PFOA and PFNA. This was observed in mid-latitude organisms such as dolphins, Lake Ontario biota, and humans. In remote areas where atmospheric inputs possibly dominate, isomer profiles revealed branched PFOA and typically only *n*-PFNA (>99%) due to atmospheric reactions of ECF perfluorooctylsulfonamide isomers and *n*-8:2 FTOH. This research also indicated the environmental presence of long-chain perfluoroalkyl compounds with terminal isopropyl geometry.

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CHAPTER EIGHT

Summary, Conclusions, and Future Work

8.1 Summary and Conclusions

The broad objective of this thesis was to investigate the utility of PFA isomer analysis as a tool to elucidate industrial sources in the environment. The industrial origin of PFAs is environmentally relevant, given the continued production of fluorotelomer chemicals, toxicity of fluorotelomer intermediates[1,2], and potential for long range atmospheric transport leading to widespread dissemination[3]. As part of the research in this thesis, environmental monitoring revealed the presence of ECF signature isomers and predominance of *n*-isomer. In subsequent chapters, the hypothesis that physical properties and biological handling influence the environmental PFA isomer profile was tested. The findings of these research endeavors are summarized below.

In Chapter 1, physical properties of PFA precursors specifically, PFSAm and a homologous series of FTOHs, were assessed. Experimental slow-stirring log K_{OW} values ranged from 1.97 to 5.58 for this suite of compounds and were correlated with computer modeled molar volumes. A +0.6 to 0.7 increase in log K_{OW} with each additional CF_2 in FTOHs, however, this trend deteriorated with 10:2 FTOH. The measured log K_{OW} corresponding to 10:2 FTOH was 2.91 and much lower than the predicted value of 6.2-6.5. This result suggested that the hydrophobicity and liphophobicity of 10:2 FTOH imparted by the perfluoroalkyl chain length resulted in diminished partitioning into octanol and water. Another surprising result was that structural isomers of N-EtFOSE did not exhibit any differences in K_{OW}. Based on this finding, it was postulated that equivalent K_{OW} of N-EtFOSE isomers may be reflective of competition between activity coefficients in octanol and water. Water solubilities of the *n*-isomer of N-EtFOSE were determined at fixed temperatures from 15 to 30 ºC using the generator column technique and ranged from 77 to 123 μg/l. Chromatograms of N-EtFOSE from the generator column revealed enhanced concentration of branched isomers in the saturated water extract compared to the technical mixture. Molecular volume was estimated for each isomer class. Branched isomers had lower molecular volumes than the n-isomer which may contribute to enhanced water solubility. Vapour pressure may also be related to molecular volume and thus it is expected that branched isomers could be more volatile. Vapour pressure is likely a major factor in determining transport of FTOHs and PFSAm compared to water solubility.

In Chapter 3, a method for analyzing PFCA isomers was introduced. Given the resolving power of GC and the absence of LC-related PFOA contamination, a derivatization reaction was employed for GC-MS analysis. This method was adapted from that of Scott et al. previously used for haloacetic acids analysis[4]. Using this method, seven isomers of an authentic ECF PFOA standard were separated. The method was employed to characterize PFCA isomers in polar bears from the Canadian Arctic and eastern Greenland. The PFOA isomer pattern in Greenland polar bear samples showed a variety of branched isomers while only *n*-PFOA isomer was observed in Canadian samples. The presence of branched isomers suggests some contribution from ECF sources. However, in comparison to the amount of branched isomers in the ECF PFOA standard, such minor percentages of branched PFCAs suggested additional input from an exclusively linear isomer source. Samples from both locations had primarily (>99%) linear isomers of PFNA and PFTrA. Branched isomers of PFDA, PFUnA, and PFDoA were also noted. Unlike the PFOA isomer signature, only a single branched isomer peak on the chromatograms was observed for these longer chain PFCAs. Based on the range of chain lengths, a fluorotelomer precursor with isopropyl geometry was proposed as a candidate for these PFCA isomers. Differences in contamination profile between Greenland and Canada may be indicative of air flow whereby contaminants in Greenland reflect contribution from sources in North America and Europe.

The method outlined above was also employed to analyze human blood samples in Chapter 4. Routes of PFCA exposure for the general human population were hypothesized to be diverse given direct (industrially produced) and indirect (production from precursor fluorinated organics) sources. PFOA was the dominant PFCA (mean 4.4 ng/g). Blood serum isomer profiles consisted of predominantly (mean 98%) the *n*-isomer for each PFCA (C8-C11). There were similarities in branched isomer patterns of an ECF PFOA standard to both PFOA and PFNA in blood whereby multiple branched isomers were detected. Direct exposure to ECF PFOA, which has a legacy of production for use in fluoropolymer industry, was postulated to be a source of the observed branched isomer pattern. PFNA isomers are a minor component of ECF PFOA and considered a byproduct of the perfluorination reaction[5]. Predominance of *n*-PFCA isomers and the [even PFCA] > [odd PFCA] concentration trend in blood is suggestive of additional input from a strictly linear perfluoroalkyl source. One potential explanation for the pattern is metabolism of *x*:2 FTOH to PFCAs in which beta oxidation is more prevalent compared to alpha oxidation[2]. As such, the even carbon numbered PFCA would be the major product of *x*:2

FTOH metabolism and the odd carbon numbered PFCA with one additional carbon would be a minor metabolite (i.e. [PFOA]>[PFNA])[2]. Polar bear livers (and other Arctic organisms) demonstrated an opposite trend whereby [odd PFCA]> [even PFCA] (i.e. [PFNA]>[PFOA], [PFUnA]>[PFDA], etc)[6,7]. The contrast in PFCA profile between Arctic organisms and humans suggested a differing mechanism. In remote areas such as the Arctic, atmospheric oxidation of FTOHs is expected to prevail and based on the mechanism elucidated by Ellis et al., similar yields of even and odd-numbered PFCAs occurs[8]. However, the longer odd-numbered PFCA is more bioaccumulative leading to the [odd]>[even] pattern. Another marked difference between PFCA contamination of polar bears versus humans is that PFNA in polar bears was not consistent with an ECF signature. Together these results indicate a major difference in PFOA and PFNA sources whereby humans are exposed to legacy ECF PFOA (and PFNA impurity) whereas in polar bears this is not evident. In polar bears, ECF branched PFOA isomers are instead more likely to arise from atmospheric PFSAm oxidation[9,10].

Using rats as a model for mammals, the biological handling of PFA isomers was investigated in Chapter 5. Male and female rats were administered PFA isomers in a 12-week dietary exposure, followed by a 12-week depuration period. The diet consisted of approximately 500 ng g^{-1} each of ECF PFOA, ECF PFOS, *n*-PFNA, and *iso*-PFNA. Blood sampling during the exposure phase revealed preferential accumulation of the *n*-isomers of PFOA, PFNA and PFOS compared to the majority of branched isomers. During depuration, females excreted all isomers faster than males, but both sexes eliminated branched isomers from blood more rapidly than the corresponding *n*-isomer. In males, elimination half-lives for PFOA isomers ranged from 3 to 21 days, for PFNA isomers from 30 to 48 days, and for PFOS isomers from 20 to 103 days. Two relatively minor isomers of ECF PFOA and one branched PFOS isomer had longer elimination half-lives than the corresponding *n*-isomer. However, there were overlapping confidence intervals for the elimination rate constants for *n*-PFOS and the major branched isomers of ECF PFOS. This was supported by observations in human blood in which the isomer profile was consistent with the composition of the technical ECF product[11,12]. These results indicated that caution should be applied when extrapolating pharmacokinetics of PFA isomers in rats to other organisms, based on dosage level and species. The mechanism for biological discrimination amongst PFA isomers is not yet known and might be due to differences in binding efficiency to transporters (such as organic anion transporters or serum proteins) involved in mediating uptake, excretion or re-absorption.

A similar type of pharmacokinetic experiment was then applied to fish in Chapter 6. Previous research in fish has suggested diet to be a major source of PFAs[13,14]. Thus, rainbow trout were fed ECF PFOA isomers (6.9 μg/kg/d), *n*-PFNA (1.2 μg/kg/d), and isopropyl PFNA (0.96 μg/kg/d) for 4 weeks. Elimination kinetics of PFCA isomers were assessed by blood monitoring when fish were switched to a clean diet. At steady state, the accumulation ratio ($PFCA\ isomer_{tissue} \div PFCA\ isomer_{feed}$) was lower for most branched isomers compared to the *n*-isomer. This trend was consistent with elimination kinetics of ECF PFOA and PFNA isomers whereby faster clearance of the branched isomers occurred. Blood elimination half lives were faster in fish than male rats and ranged from 2 to 6 days for PFOA isomers and 10 to 16 days for PFNA isomers. The experiment was redone at a dose approximately 6x lower for PFOA and similar kinetics were observed, yielding steady state liver PFOA concentrations of 5-8 ng/g, generally higher than what has been observed in fish from the natural environment[14,15].

In Chapter 7, linear and branched PFCA isomer patterns were determined in a diverse set of abiotic and biotic samples, ranging from remote to urban locations. In human blood, Arctic lake sediment and surface water, Lake Ontario sediment and surface water, Ontario rainwater, and Lake Ontario biota, the PFOA isomer profile contained branched isomers congruent with an ECF input, however, *n*-PFOA predominated (>90%) more than in the technical ECF product. PFNA isomer pattern was variable ranging from no branched isomers to only *iso*-PFNA to multiple branched isomers. Precipitation, Lake Ontario sediment and the majority of Lake Ontario biota did not contain branched PFNA above detection limits. In both ringed seals and surface waters from isolated lakes of the Canadian Arctic, low levels of *iso*-PFNA were observed. Human blood, Arctic sediment, and dolphin plasma all contained multiple branched PFNA isomers similar to an ECF signature. Consistent with earlier findings in polar bears, a single branched isomer of PFCAs from PFDA to PFDoA was observed in ringed seals, dolphins, and Lake Ontario biota but were less prevalent in human blood, precipitation and sediment. These results supported the hypothesis that long range transport of linear volatile precursors, subsequent atmospheric degradation and deposition is a major source of PFCAs in the Arctic environment. The presence of branched PFOA implies additional contribution from ECF, likely PFSAm. Consistent with previous observations in humans, abiotic and biotic samples in temperate regions indicated ECF PFOA exposure. This was corroborated by the presence of multiple PFNA isomers resembling an ECF signature which were attributed to PFNA impurity in

ECF PFOA. PFOA production by telomerization (post 2002) represented an additional potential direct source of *n*-PFOA. Precipitation is an important sample to probe atmospheric inputs. Unlike PFNA in humans, in precipitation PFNA was not consistent with an ECF signature. This further reinforces the suggestion that atmospheric reactions of linear fluorotelomers are a source of *n*-PFCAs. By extension, this mechanism is applicable to remote regions. Detection of an isopropyl isomer of PFDA, PFUnA, and PFDoA may be preliminary evidence of a fluorinated organic precursor with a terminal isopropyl geometry. However, beyond presentation in patent literature, evidence of perfluoroalkyl isopropyl production has not been reported by major manufacturers.

In conclusion, this thesis examined the environmental fate and disposition of important group of persistent pollutants, PFAs, from a relevant but novel perspective of industrial isomer signature. Physical and biological properties of PFA precursor and PFA isomers were investigated as well as monitoring of PFA isomers in the abiotic and biotic environment. Abiotic properties demonstrated differences in physical properties of PFSAm isomers whereby branched isomers were more water soluble than the *n*-isomer. Unexpectedly, K_{OW} did not indicate any appreciable differences among isomers. From these results it was suggested that the ratio of hydrophobicity and lipophobicity affiliated with the perfluoroalkyl tail is independent of perfluorocarbon arrangement. Water solubility of the isomers may be related to molar volume, from which relative vapour pressures can be predicted whereby the smallest molar volumes would translate to the greatest vapour pressure. Vapour pressure is likely to influence transport of PFSAm isomers and as such be related to PFA isomer patterns. Biologically, major branched isomers of ECF PFOA and PFOS (internal methyl branch and isopropyl) were eliminated faster than the *n*-isomer in rats and fish. This was surprising due to the conservation of isomer profile in ECF PFOS and PFOS in human blood. This research highlighted the need to understand the underlying mechanism mediating pharmacokinetics of PFA isomers which may constrain extrapolation from animal-based models to humans. Research presented in this thesis illustrated that PFA (PFOS and PFCA) isomers are present in the abiotic and biotic environment, in temperate regions and reaching to remote Arctic locations. As expected branched PFOA isomers consistent with ECF production have a presence in the environment. In temperate regions, industrially produced ECF PFOA was expected to be a major source of branched isomers, given its legacy and volume of production. However, the PFOA isomer pattern in sediment of Lake Ontario was mostly linear and in addition, concentration of *n*-PFOA in the Great Lakes was

comparable to that in isolated Arctic lakes[16]. These findings suggested that a linear fluorotelomer atmospheric source may also contribute to the Great Lakes isomer pattern and predominance of *n*-PFOA in Lake Ontario water supports this theory. Possibly corroborating this was the detection of mostly linear PFOA in Ontario rainwater. In the Arctic, PFOA isomers consistent with an ECF signature were also detected. It is suggested that the origin of these isomers is ECF PFSAm which likely undergo long range atmospheric transport and undergo atmospheric reactions to produce the isomers. The major difference in ECF signature between remote and temperate regions is the presence of ECF PFNA isomers in temperate regions and their absence in the Arctic. ECF PFNA is an impurity in ECF PFOA, constituting approximately 0.2% by weight. ECF perfluorononyl analog isomers are not expected to be a significant component of PFSAm, although this has not been confirmed. Like PFSAm, PFOS is also an industrial product of ECF perfluorooctylsulfonyl fluoride and analysis of ECF PFOS reveals that PFNA is a minor component, consisting of only 0.002% by weight[5]. Thus longer chain impurities in ECF PFSAm alone do not account for the distribution of PFNA, PFDA, PFUnA, etc. in the Arctic environment. It has been suggested that oceanic transport of PFOA from temperate regions could lead to significant contamination of remote areas[17-19]; however, the isomer analysis in this thesis does not corroborate this theory. If ECF PFOA was a significant source of PFOA in the Arctic, then presumably PFNA would also consist of ECF-type isomers. Polar bears are arctic apex predators, which predominantly feed on ringed seals and are at the top of the Arctic marine food chain and as such have the some of the highest reported levels of PFNA (108-230 ng/g)[6] reported in any environmental samples, rivaled only by dolphins from Charleston and Delaware Bay(11 – 547 ng/g PFNA)[20]. In this research, ECF PFNA isomers were apparent in dolphins but not in polar bears.

8.2 Future Research

This thesis presented novel research on the isomer aspect of perfluoroalkyl-containing pollutants including physical properties, biological properties and environmental monitoring. As this is an emerging field of research, new directions for further investigations are a consequence of findings of this thesis. In particular, further avenues of research pertaining to perfluoroalkyl isomers include PFOS isomer monitoring, environmental chemistry and properties of PFSAm isomers, monitoring airborne PFSAm isomers, exploring factors governing isomer pharmacokinetics, biomonitoring of PFA isomers in terrestrial food chains, temporal human blood samples and water samples, and expanding the roster of fluorochemical interest to unknown precursors.

8.2.1 PFOS Isomers

One of the difficulties in assigning source based on PFOA isomer analysis was the complexity arising from potential contributions of *n*-PFOA from both telomer and ECF sources. Like PFOA, environmental PFOS has potential inputs from indirect and direct sources; however, PFSAm precursors and industrial PFOS were largely manufactured by ECF, reducing any confusion regarding industrial source. Thus, environmental analysis of PFOS isomers may provide insight on the extent of processes which may alter isomer signature between source and sink. To date PFOS isomers have been investigated in human blood[11,12] and an unpublished study on Lake Ontario food web samples[21]. Further research regarding environmental PFOS isomers such as samples from the Arctic as well as samples from temperate regions including abiotic (precipitation, sediment core depth samples, water) and biota would likely fill a major research gap. Though these compounds have largely been phased out, there is some evidence that production continues by other manufacturers[22]. In addition, studying PFOS isomer patterns in the environment can be informative regarding the magnitude of influence of processes leading to isomer discrimination. However, PFOS isomer patterns would likely have limited utility in contrasting between indirect and direct sources.

8.2.2 Environmental Chemistry and Properties of PFSAm and PFSAm-Related Materials

PFSAm are comprised of characteristic ECF isomers, however, biotransformation[23,24] and atmospheric oxidation[9,10] studies have focused only on the *n*-isomer. In this thesis, the assumption was made that these reactions would conserve the isomer pattern in PFOS and PFCA products, however, this has not been confirmed. In particular atmospheric oxidation likely yields perfluorinated ketone which may undergo further reactions. An isomer-specific product study of PFOS and PFCAs obtained from smog chamber studies of PFSAm isomers would be informative. Furthermore, PFSAm compounds are considered residuals in phosphate ester surfactants and polymers. Although the environmental chemistry of these residuals has been investigated, the stability of the polymers and surfactants has not been explored. The fate of these manufactured items is presumably landfills and perhaps sewage treatment plants where biodegradation processes are relevant. Biotransformation of PFSAm polymers and surfactants including attention to component isomers should be studied as these may be particularly pertaining to aquatic organisms. Begley et al. demonstrated the ability for fluorotelomer phosphate surfactants to migrate from food packaging[25] and D'eon et al indicated rodent metabolism of such chemicals generated PFCAs[26], highlighting potential indirect exposure to PFCAs in humans through treated food-contact paper. N-EtFOSE phosphate esters were also used in similar applications and could have been a source of PFOS isomers in humans. 3M has asserted that PFSAm-related compounds are presumably a major source of PFOS exposure because PFOS itself was only a minor industrial product[27].

As described above, reaction mechanisms may influence isomer patterns of PFAs. However, physical properties may also have an impact on transport of PFSAm isomers and as a consequence, isomer patterns of PFAs. In findings presented in Chapter 2, water solubility of branched isomers was greater than the *n*-isomer and may have been related to molar volume. Molar volume also relates to vapour pressure and could suggest branched isomers are also more volatile than the *n*-isomer. Vapour pressure measurements of PFSAm isomers should be conducted but without available purified isomers, some obstacles exist. A challenge of applying GC retention method is the separation of isomers on a boiling point column and secondly, interactions with the stationary phase[28]. Employing the gas saturation method is more promising although classical methodology would have to be adapted to eliminate the incorporation of glass wool to which PFSAm adsorb. Furthermore, monitoring of PFSAm

isomer profiles in air samples collected from urban, rural, and remote regions may be informative regarding physical properties and reactivity of isomers.

Research presented in Chapter 2 revealed similar octanol-water partitioning of N-EtFOSE isomers and a diminished partitioning into either phase for 10:2 FTOH. K_{OW} of other ECF PFSAm isomers such as N-EtFOSA and N-MeFOSE should be investigated to see whether this trend holds. It was suggested that the low K_{OW} of 10:2 FTOH was due to competing activity coefficients in water and octanol. Interrogation of the interface between octanol and water should be conducted to determine whether 10:2 FTOH was indeed accumulating at the interface forming a tertiary fluorinated phase. Scattering spectroscopy or turbidity measurements may be a useful tool for such research. Also, longer chain lengths of FTOHs should be tested to decipher whether the trend is a function of fluorocarbon chain length.

8.2.3 Biological factors mediating PFA isomer kinetics

In Chapters 5 and 6, biodiscrimination of PFA isomers was demonstrated in rats and fish, whereby preferential excretion of the major branched isomer occurred. This was surprising given the consistency in PFOS isomers in humans compared to technical grade ECF products. The mechanism mediating this discrimination is not presently known. Currently blood transport of PFAs is thought to occur by binding to blood proteins and enterohepatic circulation is also known to take place[29-31]. Furthermore re-absorption of PFAs from the renal filtrate via OATPs has been postulated[32,33]. The behaviour of isomers in each of these processes has not been researched. Isomer-specific competitive binding assays with serum albumin and OATPs may illuminate the mechanisms involved in isomer discrimination.

8.2.4 Sample selection for PFA monitoring

The source of PFAs in the Arctic is of considerable debate. While atmospheric transport and oxidation of precursors is one theory[3,8], another is the oceanic transport of industrially synthesized PFOA and PFOS from temperate regions[18,19]. Based on the latter hypothesis, the presence of these PFAs in polar bears and ringed seals in Arctic regions would be expected via consumption of lower components of the marine food chain. Analysis of PFAs in terrestrial food chains has not been conducted with the exception of wood mice residing near a fluorochemical

manufacturing plant[34] and captive panda bears in zoos[35]. Both Arctic and temperate food chain organisms should be studied for PFAs. In the Arctic, the terrestrial food chain consists of lichen, caribou and wolf with additional inputs from air and snowpack meltwater[36]. In temperate regions, a terrestrial food chains include organisms such as soil dwelling invertebrates, small rodents, and fox[37,38].

In Chapter 3, PFCA isomers in polar bears revealed patterns associated with geographical location whereby Canadian Arctic was markedly different from Eastern Greenland. Smithwick et al. also observed distinctions in PFA contamination in polar bears with geographic location in a circumpolar study[39]. Samples from Alaska may reveal a different pattern given this region is subject to inputs from Asian air masses. Thus, isomer analysis of Alaskan biota may indicate usage and production patterns in Asia. Similarly, northern versus southern hemispheres could also yield contrasting results given atmospheric circulation patterns. FTOH and PFSAm air concentrations decrease from mid-latitudes southward (i.e. Germany to southern tip of Africa)[40]. Karrman et al. revealed PFA concentrations in humans from rural and urban areas of Australia to be similar to analogous subjects in Europe and North America but this is most likely representative of modern life and not long range transport of precursors[41]. Thus a suitable approach would be sampling in non-industrialized areas of the southern hemisphere.

One of the most important areas of research on PFCA isomers is in water samples. Research presented in this thesis and elsewhere[42] has demonstrated selective excretion of branched PFAs; however, PFAs are environmentally persistent and as such, waterways could be a major sink for branched PFA isomers. In Chapter 7, food web samples and surface waters from Lake Ontario were analyzed for PFCA isomers. Correlations were observed between diet, water-borne environment, and body burden. Additionally, the PFOA isomer profile in Arctic lakes appeared predominantly linear (>99%) compared to that of Lake Ontario. Isomer analysis of ocean water samples would be valuable. Recently Yamashita et al. suggested PFOS and PFOA in ocean waters could be used as tracers for oceanic transport[43]. Analysis of ECF isomers in this type of sample could be useful in examining the dominance of oceanic transport especially since the majority of ECF PFOA production occurred in the eastern US and Europe with only little production in Asia.

Recently, a decline in PFOS blood concentrations were observed from the general human population which corroborated the half-life estimated using PFOS monitoring in blood of fluorochemical retirees[44,45]. In Chapter 5, potential problems with extrapolating laboratory-based pharmacokinetics to realistic environmental exposure were discussed. Analysis of PFA isomers in human blood before and after ECF phase-out may permit calculation of half-lives corresponding to isomers in humans. Results from such a study would illuminate whether pharmacokinetics at low environmental exposures mirror rodent models in which branched isomers had significantly shorter half-lives. However, it would be important to determine whether human exposure to ECF sources has indeed declined post cessation of production. In Chapter 4, potential indirect and direct sources of PFOA was discussed. Fluorotelomer compounds were suggested as a major source of exposure. Although fluorotelomer intermediates have been observed in biological samples[7] and rainwater[15,46,47] and PFSAm metabolites are present in human blood[48,49], fluorotelomer intermediate metabolites have not been confirmed in human blood. Investigation of FTCA and FTUCA in humans of the general population should be carried out to assess the relative contributions of sources but may be challenging given the intermittent residence of these fluorotelomer intermediates due their labile nature.

8.2.5 Elucidation of Unknown Precusors

In Chapters 3 and 7, analysis of environmental samples unexpectedly revealed the presence of isopropyl isomers of PFDA, PFUnA, and PFDoA in a wide variety of samples collected across North America. Industrial production of long chain isopropyl perfluoroalkyl-containing compounds has not yet been reported. Based on patent literature, a number of candidates were proposed in Chapter 7 including isopropyl FTOHs, FTOs, and oxiranes. Many manufacturers are not transparent about the specific fluorochemicals in production which is unfortunate from an environmental interest. As such, it is important to improve awareness regarding unknown emerging or legacy perfluoroalkyl-containing compounds which may contribute to overall perfluorinated pollution.

Currently, monitoring of FTOHs, FTOs and PFSAm in air is conducted using single-ion-monitoring corresponding to these chemicals[50-52]. The results presented in Chapters 3 and 7 suggest monitoring should be expanded to include ions corresponding to odd-chain length FTOs

and FTOHs. Furthermore, fluorochemical residuals components in surfactants and polymers should be fully characterized, which would likely involve a suite of analytical methods including ^{19}F-NMR and MS. In the research by Dinglasan-Panlilio et al., biodegradation of a series of fluorinated monomers was investigated[53]. In that study two ester linked monomers underwent biodegradation releasing FTOHs and subsequent production of PFCAs[53]. However, an oxetane monomer did not follow this mechanism but did degrade[53]. Deciphering intermediates and products of this degradation may yield pertinent information regarding unknown perfluoroalkyl-compounds. Discovery of previously unknown perfluoroalkyl-compounds would likely introduce the classical avenues of environmental chemistry research – monitoring, physical properties, toxicity, biotic and abiotic reactions.

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APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER TWO:

Water Solubility and Octanol-Water Partition Coefficient of Perfluorooctylsulfonamides and Fluorotelomer Alcohols

Table A-S1. Isomer composition in technical N-EtFOSE, based on chromatographic peak area. Mean and standard deviation based on five analyses of ECF N-EtFOSE

Isomer	% isomer in technical N-EtFOSE (mean ± standard deviation)
1	1.33 ± 0.08
2	0.72 ± 0.04
3 (*n*-isomer)	79.47 ± 0.36
4	3.46 ± 0.12
5	0.38 ± 0.03
6 (isopropyl)	9.25 ± 0.23
7	4.83 ± 0.10
8	0.33 ± 0.07
9	0.23 ± 0.06

Table A-S2. Molar volumes estimated by Hyperchem and modified McGowan method

Compound	**Hyperchem molar volume (cm^3/mol)**	**Modified McGowan molar volume (cm^3/mol)**
4:2 FTOH	317	135
6:2 FTOH	398	178
8:2 FTOH	479	222
10:2 FTOH	561	265
PFOSA	483	226
N-EtFOSA	548	254
N-EtFOSE	605	288

Table A-S3. Percent isomer composition of N-EtFOSE in standard compared to aqueous fractions from generator column (mean standard deviation) in C^{sat}_{w} experiments, 15 to 30 °C using area counts.

N-EtFOSE Isomer	ECF Standard	15 °C	20 °C	25 °C	30 °C
1	1.3 ± 0.08	4.3 ± 0.81	4.5 ± 0.80	4.2 ± 0.72	4.0 ± 0.55
2	0.7 ± 0.04	3.4 ± 0.78	3.6 ± 0.70	3.1 ± 0.64	3.1 ± 0.44
3 (*n*-isomer)	79.5 ± 0.36	47.5 ± 8.49	45.9 ± 8.30	47.8 ± 5.34	50.0 ± 4.88
4	3.5 ± 0.12	11.8 ± 2.17	12.4 ± 2.15	12.1 ± 1.76	11.4 ± 1.25
5	0.4 ± 0.03	2.9 ± 0.75	3.3 ± 0.83	2.7 ± 0.67	2.8 ± 0.46
6 (isopropyl, inferred)	9.3 ± 0.23	13.7 ± 1.19	13.4 ± 1.15	14.0 ± 1.09	13.3 ± 0.65
7	4.8 ± 0.10	12.9 ± 2.09	13.1 ± 1.99	12.9 ± 1.56	12.3 ± 1.12
8	0.3 ± 0.07	2.4 ± 0.60	2.6 ± 0.53	2.3 ± 0.45	2.2 ± 0.31
9	0.2 ± 0.06	1.1 ± 0.28	1.2 ± 0.24	1.1 ± 0.20	1.0 ± 0.14

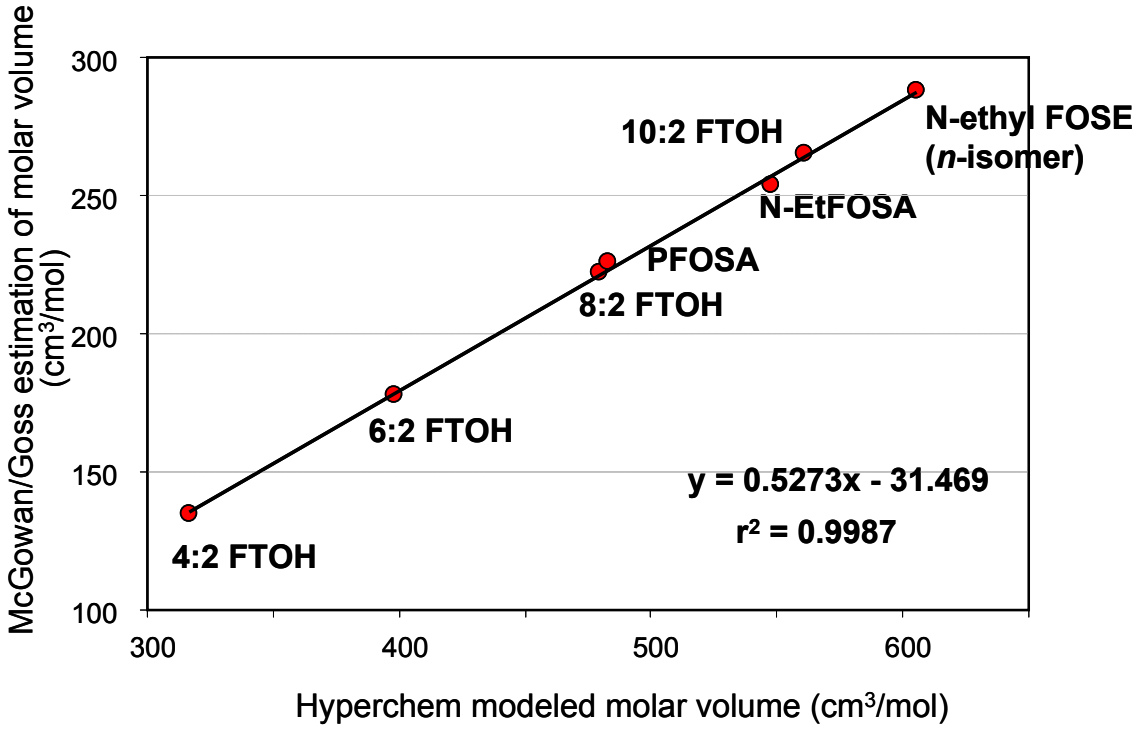

Figure A-S1. Molar volumes estimated using the McGowan equation[1] with the fluorine substituent value recommended by Goss et al.[2] versus those modeled by Hyperchem software.

The results demonstrate that Hyperchem captures the trend in molar volumes but systematically over-predicts the molar volume by a factor of 2.

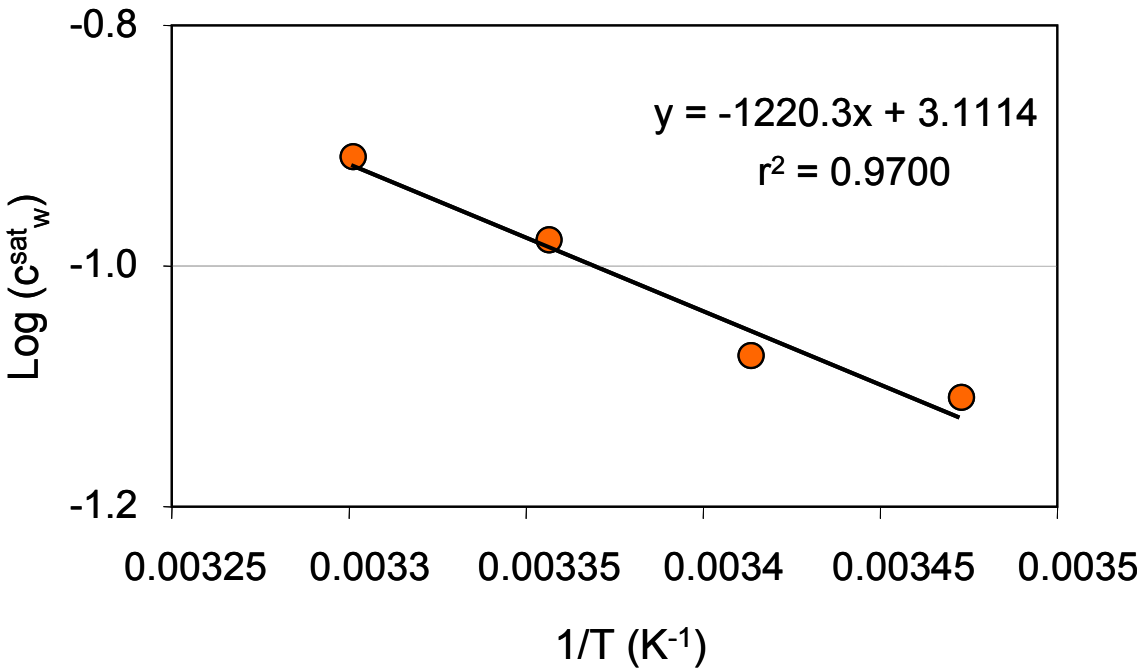

Figure A-S2. Linear regression of log C^{sat}_{w} ($g \cdot m^{-3}$) versus inverse temperature. The slope of the linear regression is equal to $\Delta_{ws}H_i$ /(2.303*R) where $\Delta_{ws}H_i$ is the enthalpy of solution for linear N-EtFOSE and R is the universal gas constant (8.31 $J \cdot mol^{-1} \cdot K^{-1}$). Thus $\Delta_{ws}H_i$ is 23.37 kJ/mol.

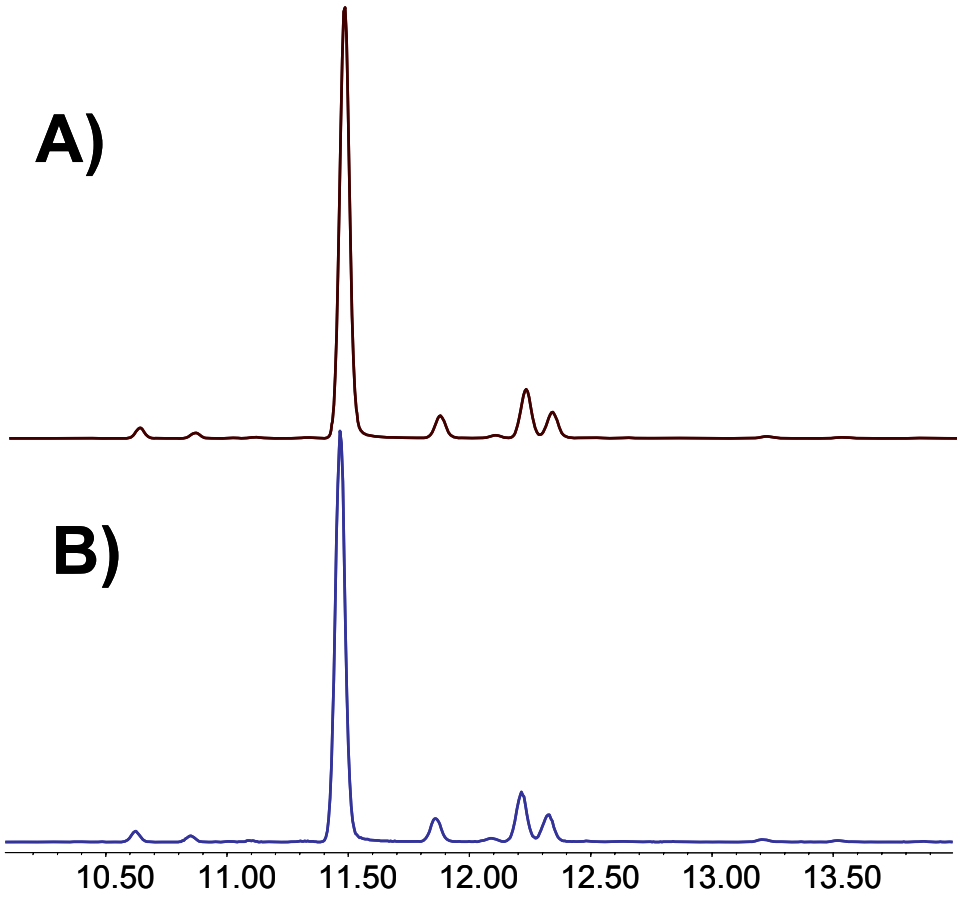

Figure A-S3. N-EtFOSE isomer profile in **A)** octanol phase and **B)** aqueous phase of K_{OW} system at equilibrium. The same pattern was observed in both chromatograms suggesting similar K_{OW} values for each isomer.

CALCULATION OF ACTIVITY COEFFICIENT IN OCTANOL (γ_O)

	C^{sat}_w (mg/L) [3]
4:2 FTOH	974
6:2 FTOH	18.8
8:2 FTOH	0.194
10:2 FTOH*	0.00304

* estimated by linear regression of ln C^{sat}_w vs fluorotelomer chain length for 4:2 through 8:2 FTOH data, y = -2.1303x + 15.507, r^2 = 0.9982

Sample calculation for 4:2 FTOH

Molar C^{sat}_w = 0.974 g/L ÷ 264 g/mol = 0.00369 mol/L, using CSATW given in reference [3] (see above table).

Activity coefficient in water γ_w**:**

γ_w= 1÷(0.00369 mol/L * 0.018L/mol) = 15063

$\gamma_o = \gamma_w \div K_{OW}$ = 15063 ÷ $10^{1.97}$ = 161 , where K_{OW} is the measured value in Table 2.5.

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APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER FOUR:

Isomer Distribution of Perfluorocarboxylates in Human Blood

– Potential Correlation to Source

Extraction, Derivatization, GC-(NCI) MS Analysis and QA/QC of PFCAs in Human Serum

Extraction of PFCAs from Human Blood Serum

All PFCA standard stock solutions and other reagent solutions were made with 18MΩ water unless otherwise stated. Extraction was conducted by combining 4 ml human blood serum sample, 10 ng of $^{13}C_4$-PFOA internal standard, 4 ml 0.25M sodium carbonate (ACP, Montreal, PQ, Canada, >99%), 2 ml 0.5M tetrabutylammonium hydrogen sulfate (Sigma Aldrich, Oakville, ON, Canada, >99%) (adjusted to pH10 using sodium hydroxide, >95%, Fisher Scientific, Ottawa, ON, Canada), and 10 ml methyl-*tert*-butyl ether (MTBE) (VWR International, Mississauga, ON, Canada, >99.98%). These contents were shaken in a polypropylene centrifuge tube for 10 min. After centrifuging for 5 minutes, the MTBE layer was transferred to a 2nd clean tube and remaining sample was shaken for an additional 10 min with 10 ml of MTBE. Following centrifugation for 10 min, the MTBE layer was removed and combined with the 1st MTBE layer. The combined MTBE phase was(*31*) taken to just dryness with N_2 gas (Pre-purified grade, BOC Gases, Mississauga, ON, Canada).

PFCA Derivatization in Blood Extracts

Immediately after drying, the extract was acidified to pH 1.0 using concentrated HCl (Fisher Scientific, Ottawa, ON, Canada, 36.5%). To this 0.1 g of sodium chloride (ACP, >99%) was added along with 20 ml of ethyl acetate (VWR International, >99.99%). Dicyclohexylcarbodimide (DCC) (1 ml of 1 M solution in ethyl acetate) was introduced prior to addition of the derivatizing agent 2,4-difluoroaniline (2,4-DFA) (1 ml of 1 M solution in ethyl acetate). Both DCC and 2,4-DFA were from Sigma-Aldrich with >99% purity. The sample was vortexed for 30 sec. to complete the reaction. A series of washes of the organic phase were conducted in the following order 5 ml 10% HCl, 5 ml aqueous saturated sodium bicarbonate, 5 ml aqueous saturated sodium chloride (both salts from ACP, >99%). Washes were shaken with the sample, centrifuged for 5 min., and then aqueous layer was drained off from the organic layer. After final wash, sodium sulfate (ACP, >99%) was added as a drying agent and the organic layer was concentrated to 2 ml. Removal of polar constituents was performed by running the concentrated derivatized extract through a silica (0.75 g) gel column with elution of

the derivatives via 15 ml toluene (1.5% 2-propanol, 1.5% *n*-hexanol). The eluant was concentrated to 0.5 ml using a slow stream of N_2 gas. Toluene (99.5%), 2-propanol (>99%), and 1-hexanol (>99%) were acquired from Caledon Laboratories Ltd. (Georgetown, ON, Canada), Vexchem (Aurora, ON, Canada), and Sigma Aldrich, respectively.

GC-(NCI)MS Parameters for Determination of derivatized PFCAs

The injector was held at 190˚C for the duration of each run. Initial oven temperature was kept at 80˚C for 1.5 min, ramped at 0.7˚C/min to 110˚C and held for 5 min. A 0.7˚C/min ramp was applied to reach 140˚C. The final step of the oven program was a 30˚C/min ramp to 250˚C and held for 8.00 minutes. Helium was used as the carrier gas with 1.1 ml/min flow rate. At the detector, the transfer line was held at 250˚C, ion source and quadrupole both at 150˚C.

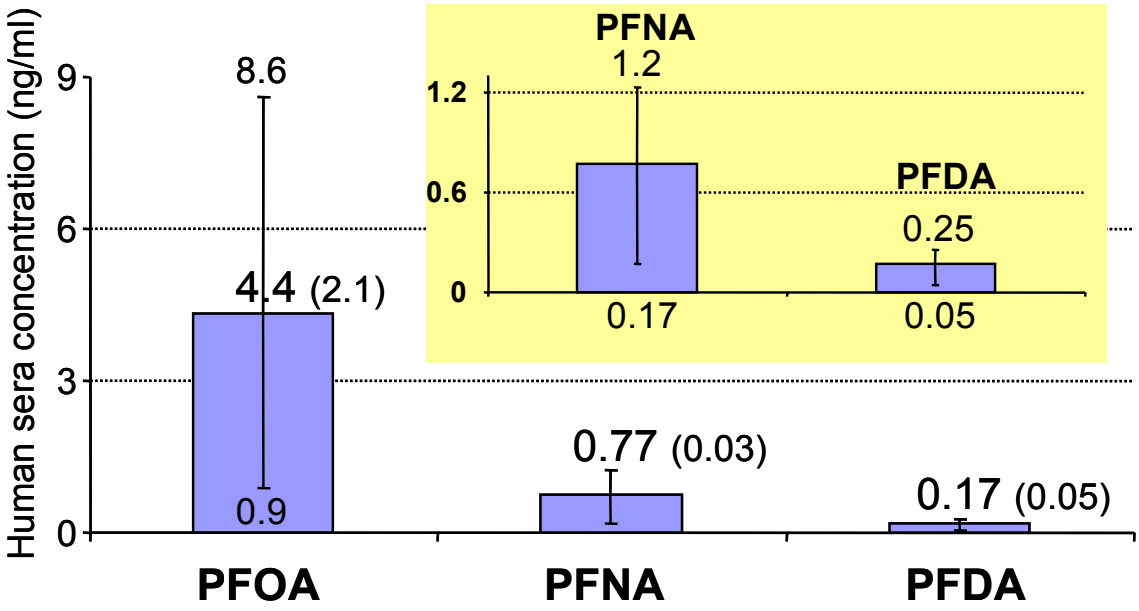

Figure B-S1. Linear PFCA concentrations in 16 pooled human blood serum samples. Mean represented by columns, standard deviation is in brackets and maximum and minimum indicated by bar limits. Linear PFUnA was detected in 2 samples at 0.048 and 0.067 ng/ml. PFDoA and PFTrA not observed >LOD in any blood sample.

Table B-S1. External calibration curve slope and correlation coefficients for linear PFNA and Isopropyl PFNA using 0.5, 2.0, 20.0, and 40.0 ng standards.

	Slope in linear regression of x(PFCA mass in ng) and y (PFCA peak area)	Linearity of calibration curve R^2
Linear PFNA	y = 0.0998x	0.9995
Branched Isopropyl PFNA	y = 0.0984x	0.9996

Table B-S2. Recovery of 7 isomers of 10 ng ECF PFOA from 4 ml Aldrich human serum matrix (N=3).

Isomer	**Average Recovery (%)**	**Standard dev.**
Linear	98.0	3.5
1	91.3	12.9
2	72.1	8.9
3	89.2	4.3
4	73.4	10.2
5	102.3	5.5
6	97.1	5.4
7	82.2	7.1
8	84.0	7.7

Table B-S3. Recovery of 0.76 ng/ml PFNA, 0.20 ng/ml PFDA, and 0.06 ng/ml PFUnA in Aldrich human serum matrix (N=3).

Isomer	**Average Recovery (%)**	**%RSD**
Linear PFNA	115	13
Linear PFDA	107	15
Linear PFUnA	97	11

GC-(NCI)MS Chromatograms of PFCA isomers in human blood sera

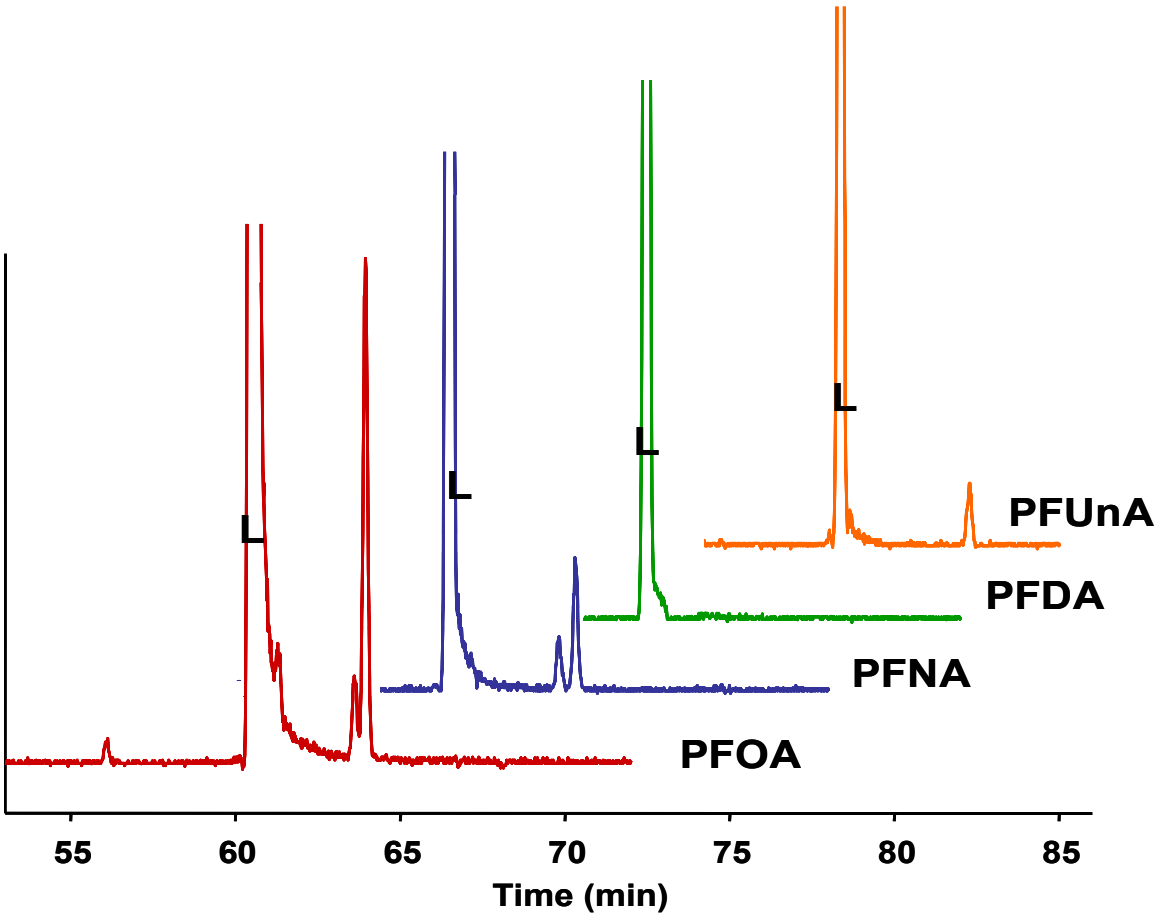

Figure B-S2. PFCA Isomer profile in pooled human blood serum sample. Peaks labeled with "L" correspond to linear isomer and smaller peaks are ascribed to branched isomers. PFCA levels in this sample were found to be 3.4 ng/ml PFOA, 0.5 ng/ml PFNA, 0.2 ng/ml PFDA, and 0.07 ng/ml PFUnA. The isomer profile consisted of 1.4% branched PFOA, 1.9% branched PFNA, and 2.3 % branched PFUnA.

Identification of isopropyl branch PFCA isomer.

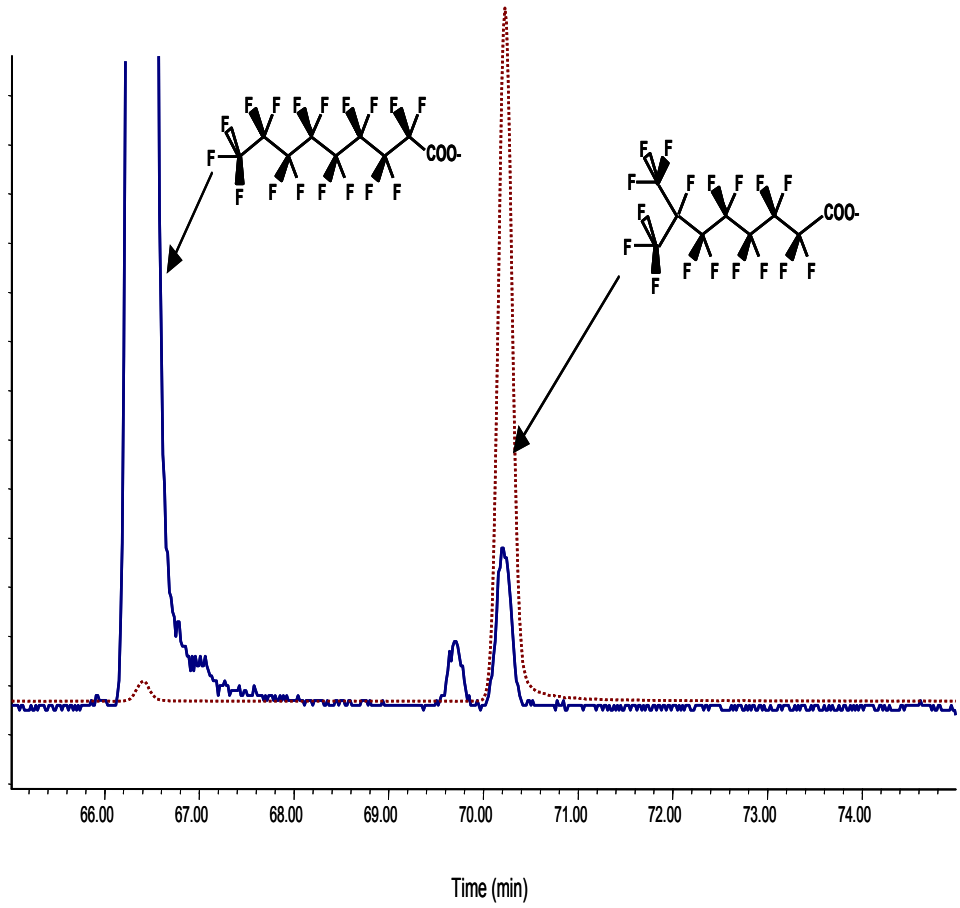

Figure B-S3. PFNA isomer signature in blood sample (blue solid line) superimposed on isopropyl PFNA branch standard chromatogram (red dashed line). Magnified view of PFNA branched isomers in blood is presented.

APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER FIVE

Disposition of Perfluorinated Acid Isomers in Rats; Part II Subchronic Dose

Table C-S1. Body mass, feed consumption (FC) rate, total consumed chemical, and liver somatic index (LSI) for male rats.

	Male Rats					
	Controls		Treated			
	Control 1 sacrificed at week 5	Control 2	1 sacrificed at week 5	2	3	4
Initial mass (g)	393	427	414	374	392	377
Mass at end of uptake, wk 12 (g)	573 at wk 5	691	560 at wk 5	654	598	489
Mass at end of depuration, wk 25 (g)	-	830	-	790	696	687
Mean FC rate (± std dev) during 12 wk uptake (g feed/d)	27 (4)	28 (5)	26 (5)	27 (5)	25 (3.5)	27 (3)
Daily Dose (µg PFA/d)						
n- PFOS	-	-	11	11	11	11
n- PFOA	-	-	11	11	10	11
n-PFNA	-	-	14	14	13	14
iso-PFNA	-	-	13	13	12	13
Cumulative Dose (mg PFA in 12 wk)			At 5 wk			
n- PFOS	-	-	0.387	0.964	0.893	0.964
n- PFOA	-	-	0.368	0.916	0.848	0.916
n-PFNA	-	-	0.488	1.216	1.126	1.216
iso-PFNA	-	-	0.447	1.114	1.031	1.114
Dose expressed per mean body weight during uptake phase						
Daily dose (mg/kg/d)						
n- PFOS	-	-	0.023	0.022	0.021	0.027
n- PFOA	-	-	0.022	0.021	0.020	0.025
n-PFNA	-	-	0.029	0.028	0.027	0.033
iso-PFNA	-	-	0.026	0.026	0.025	0.031
Cumulative dose (mg/kg)						
n- PFOS	-	-	0.79	1.88	1.80	2.23
n- PFOA	-	-	0.75	1.78	1.71	2.12
n-PFNA	-	-	1.00	2.37	2.27	2.81
iso-PFNA	-	-	0.92	2.17	2.08	2.57
LSI at time of sacrifice	3.93	3.15	3.20	2.84	3.04	3.02

Table C-S2. Body mass, feed consumption (FC) rate, total consumed chemical, and liver somatic index (LSI) for female rats.

	Female Rats					
	Controls		Treated			
	Control 1 sacrificed at week 5	Control 2	1 sacrificed at week 5	2	3	4
Initial mass (g)	262	261	281	308	287	281
Mass at end of uptake, wk 12 (g)	317 At 5 wk	337	324 At 5 wk	417	385	377
Mass at end of depuration, wk 21 (g)	-	373	-	490	437	450
Mean FC rate (± std dev) during 12 wk uptake (g feed/d)	18 (2)	17 (3)	20 (4)	20 (3)	20 (3)	21 (2)
Daily Dose (µg PFA/d)						
n- PFOS	-	-	8.5	8.5	8.5	8.9
n- PFOA	-	-	8.1	8.1	8.1	8.5
n-PFNA	-	-	10.7	10.7	10.7	11.3
iso-PFNA	-	-	9.8	9.8	9.8	10.3
Cumulative Dose (mg PFA in 12 wk)			At 5 wk			
n- PFOS	-	-	0.298	0.714	0.714	0.748
n- PFOA	-	-	0.283	0.680	0.680	0.714
n-PFNA	-	-	0.375	0.899	0.899	0.949
iso-PFNA	-	-	0.344	0.823	0.823	0.865
Dose expressed per average body weight during uptake phase						
Daily dose (mg/kg/d)						
n- PFOS	-	-	0.028	0.023	0.025	0.027
n- PFOA	-	-	0.027	0.022	0.024	0.026
n-PFNA	-	-	0.035	0.030	0.032	0.034
iso-PFNA	-	-	0.032	0.027	0.029	0.031
Cumulative dose (mg/kg)						
n- PFOS	-	-	0.98	1.97	2.13	2.27
n- PFOA	-	-	0.93	1.88	2.03	2.17
n-PFNA	-	-	1.24	2.48	2.68	2.89
iso-PFNA	-	-	1.14	2.27	2.45	2.63
LSI at time of sacrifice	3.36	3.08	3.82	2.65	2.38	3.33

Table C-S3. Male rats - blood concentration of *n*-PFOA, *n*-PFNA, *iso*-PFNA, and *n*-PFOS. For *n*-PFOA, *n*-PFNA, and *iso*-PFNA each value is the mean of 4 rats until day 38 and then each subsequent value is the mean of 3 rats. Numbers in brackets represent standard error of the mean (SEM). PFOS numbers refer to only one data point.

exposure day	ng/g *n*-PFOA	ng/g *n*-PFNA	ng/g *iso*-PFNA	ng/g *n*-PFOS
1	39.99 (3.35)	24.48 (3.64)	42.30 (1.83)	14.80
2	125.53 (46.82)	26.62 (1.64)	46.68 (6.77)	
3	112.71 (17.98)	67.40 (3.25)	113.17 (18.91)	
4	276.23 (93.98)	102.98 (13.84)	168.45 (26.17)	61.73
5	170.86 (62.67)	92.56 (9.97)	156.06 (17.50)	
8	439.80 (11.45)	188.73 (7.20)	291.93 (5.99)	76.14
11	322.02 (115.44)	209.16 (35.52)	316.84 (53.95)	86.52
17	478.19 (97.80)	364.04 (109.64)	559.26 (188.18)	82.64
22	937.61 (128.61)	539.96 (40.10)	681.30 (43.61)	272.75
26	774.09 (212.25)	784.03 (89.48)	899.38 (119.47)	409.75
32	793.30 (248.73)	1058.8 (136.7)	1159.5 (229.2)	614.18
38	975.04 (150.46)	1039.7 (78.4)	1139.8 (103.9)	558.97
50	1050.0 (185.7)	1386.5 (174.0)	1322.4 (276.1)	731.56
57	800.59 (219.08)	1328.6 (103.2)	1513.5 (227.8)	904.27
72	855.74 (325.77)	1551.6 (43.1)	1294.6 (127.0)	1012.47
84	638.93 (123.15)	1509.67 (84.25)	1350.33 (72.07)	976.18

Table C-S4. Female rats - blood concentration of *n*-PFOA, *n*-PFNA, *iso*-PFNA, and *n*-PFOS. For *n*-PFOA, *n*-PFNA, and *iso*-PFNA each value is the mean of 4 rats until day 38 and then each subsequent value is the mean of 3 rats. Numbers in brackets represent standard error of the mean (SEM). PFOS numbers refer to only one data point.

exposure day	ng/g n-PFOA	ng/g iso-PFNA	ng/g n-PFNA	ng/g n-PFOS
1	24.47 (3.96)	46.66 (2.11)	43.17 (24.11)	37.29
3	29.55 (8.95	60.89 (16.82)	49.71 (16.21)	85.55
5	20.43 (3.54)	61.45 (15.28)	116.99 (22.44)	91.78
8	45.38 (11.67)	56.32 (4.33)	154.93 (11.19)	189.77
11	53.23 (19.75)	65.72 (14.90)	184.61 (30.51)	193.16
17	63.61 (23.90)	44.94 (8.24)	206.51 (24.27)	308.59
22	105.35 (13.52)	41.97 (14.09)	149.79 (10.32)	424.63
26	15.19 (2.24)	50.61 (10.14)	163.73 (22.82)	506.62
32	48.85 (30.80)	65.26 (16.41)	228.10 (16.96)	729.03
38	23.06 (4.93)	68.50 (10.80)	223.05 (25.61)	816.67
50	46.82 (19.29)	36.59 (11.81)	150.58 (25.37)	1264.91
57	78.55 (33.27)	38.29 (13.41)	174.05 (21.04)	1308.73
72	28.38 (6.48)	56.08 (22.79)	185.36 (33.17)	1490.31
84	8.87 (7.08)	28.09 (18.98)	116.22 (55.21)	1517.21

Table C-S5. Male rat relative accumulation coefficients (C_{ra}) of PFOA isomers and PFNA isomers at Day 35.

	fat	muscle	gonad	spleen	kidney	Lung	Heart	blood	liver
3m-PFOA	0.084	0.23	0.27	0.22	0.29	0.32	0.18	0.46 (0.08)	0.30
***n*-PFOA**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.000 (0)	1.00
4m-PFOA	0.29	0.31	0.39	0.32	0.54	0.33	0.39	0.51 (0.20)	0.65
5m-PFOA	0.21	0.20	0.31	0.15	0.32	0.24	0.23	0.48 (0.20)	0.17
***iso*-PFOA**	0.87	0.96	0.99	0.89	0.95	1.02	1.07	1.10 (0.15)	0.71
PFOA-6	0.05	0.06	0.14	0.28	0.95	0.08	0.09	0.24 (0.05)	0.32
PFOA-7	<	0.04	0.01	0.24	0.20	0.00	0.04	0.13 (0.10)	0.31
PFOA-8	0.09	0.14	0.23	0.23	0.09	0.08	0.14	0.33 (0.08)	0.15
***n*-PFNA**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
***iso*-PFNA**	1.30	0.93	0.82	1.12	0.74	1.23	1.02	1.43 (0.20)	0.67

Table C-S6. Female rat relative accumulation coefficients (C_{ra}) of PFOA isomers and PFNA isomers at Day 35.

	Fat	muscle	gonad	kidney	lung	Heart	blood	liver
3m-PFOA	<LOD	<LOD	<LOD	0.14	0.00	<LOD	0.380 (0.15)	0.20
***n*-PFOA**	1	1	1	1	1	1	1	1
4m-PFOA	0.08	0.09	0.05	0.28	0.13	0.19	0.437 (0.02)	0.14
5m-PFOA	0.11	0.09	0.06	0.206	0.14	0.13	0.256 (0.02)	0.10
***iso*-PFOA**	0.19	0.20	0.06	0.42	0.26	0.22	1.013 (0.06)	0.24
PFOA-6	<LOD	<LOD	<LOD	0.101	0.00	<LOD	0.134 (0.16)	0.51
PFOA-7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PFOA-8	<LOD	<LOD	<LOD	0.12	0.00	<LOD	0.216 (0.15)	0.4
***n*-PFNA**	1	1	1	1	1	1	1	1
***iso*-PFNA**	0.28	0.28	0.26	0.25	0.28	0.29	0.33 (0.02)	0.24

Table C-S7. Day 84 average relative accumulation coefficients (C_{ra}) in blood of male and female rats (N=2); Range is provided in brackets.

	Male Blood day 84	Female blood day 84
3m-PFOA	0.14 (0.06)	0.12 (0.06)
***n*-PFOA**	1.00	1.00
4m-PFOA	0.21 (0.03)	<LOD
5m-PFOA	0.12 (0.004)	0.01 (0.00)
***iso*-PFOA**	0.61 (0.02)	0.01 (0.00)
PFOA-6	0.18 (0.15)	0.21 (0.02)
PFOA-7	0.02 (0.004)	<LOD
PFOA-8	0.25 (0.20)	0.27 (0.27)
***n*-PFNA**	1.00	1.00
***iso*-PFNA**	1.20 (0.01)	0.15 (0.00)

Table C-S8. Relative accumulation coefficients (C_{ra}) of PFOS isomers in male at Day 35

	iso-PFOS	5m-PFOS	4m-PFOS	3m-PFOS	1m-PFOS
muscle	0.75	1.14	0.50	0.47	2.88
fat	0.73	0.85	0.24	0.34	1.95
gonads	4.53	1.00	0.34	0.37	2.08
lungs	0.50	0.73	0.32	0.33	1.25
heart	0.59	0.64	0.26	0.32	1.60
spleen	0.38	0.40	0.19	0.24	0.61
kidney	0.03	0.50	0.12	0.29	0.30
liver	0.98	0.62	1.22	2.06	0.54

Table C-S9. Relative accumulation coefficients (C_u) of PFOS isomers in female at Day 35

	iso-PFOS	5m-PFOS	4m-PFOS	3m-PFOS	1m-PFOS
Muscle	0.87	0.99	0.67	0.62	1.89
Fat	0.65	0.67	0.38	0.40	1.67
Gonad	0.11	0.11	0.06	0.07	0.26
Lung	0.64	0.61	0.40	0.50	1.03
Heart	0.46	0.49	0.32	0.40	0.86
Spleen	0.29	0.24	0.15	0.15	0.25
Kidney	0.07	1.40	1.15	1.11	0.77
Liver	0.76	0.42	0.64	1.17	0.37

Table C-S10. Relative accumulation coefficients (C_{ra}) of PFOS isomers in male and female blood at Day 35 and Day 84

	n-PFOS	iso-PFOS	5m-PFOS	4m-PFOS	3m-PFOS	1m-PFOS	tbutyl-PFOS	PFOS-7	PFOS-8	PFOS-9
day 38 male	1.00	1.27	2.00	0.94	0.71	6.17	0.33	2.74	0.49	3.32
day 84 male	1.00	1.04	1.45	0.85	0.54	4.45	0.17	1.85	0.24	2.76
day 38 female	1.00	0.99	1.55	1.15	1.28	3.60	0.21	1.88	0.16	2.61
day 84 female	1.00	0.99	1.21	0.73	0.71	3.97	0.05	1.49	0.03	2.64

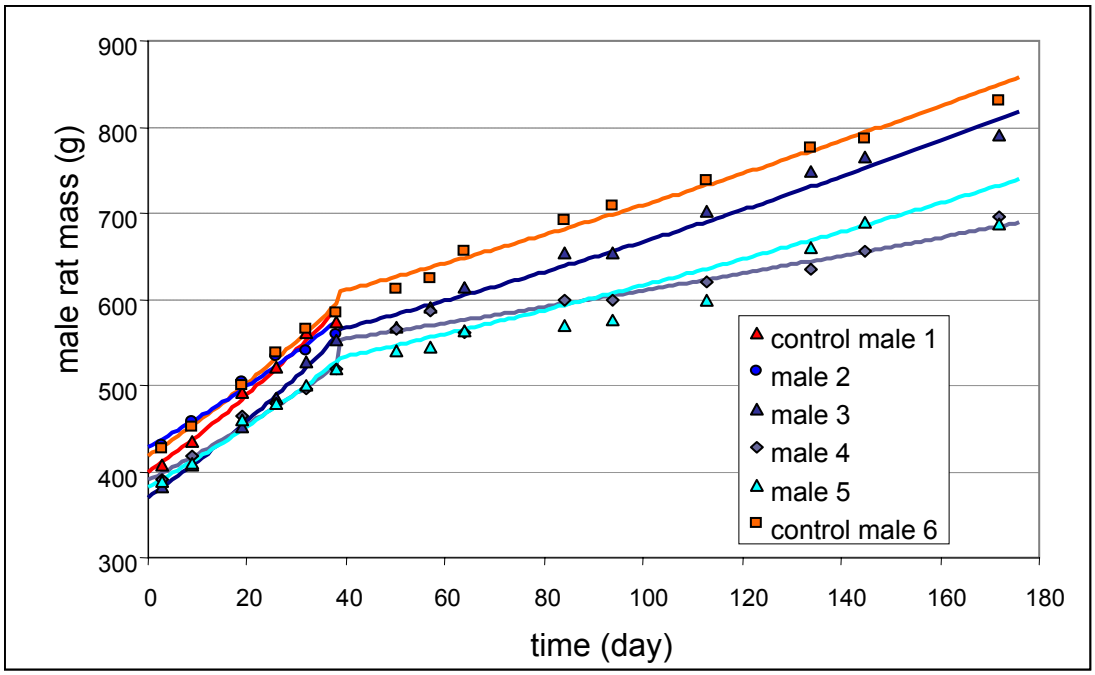

Figure C-S1. Measured male rat mass (symbols) and modeled mass (lines) for control and treated population. Control males are # 1 and # 6.

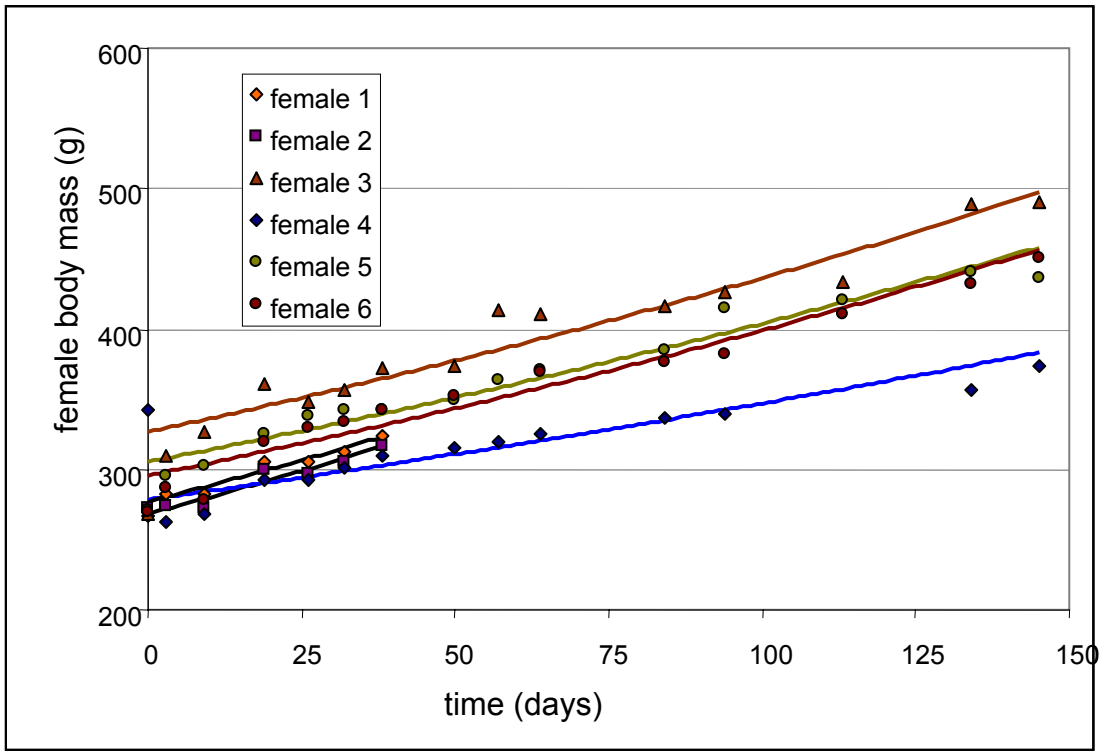

Figure C-S2. Measured female rat mass (symbols) and modeled mass (lines) for control and treated population. Control females are # 2 and # 4.

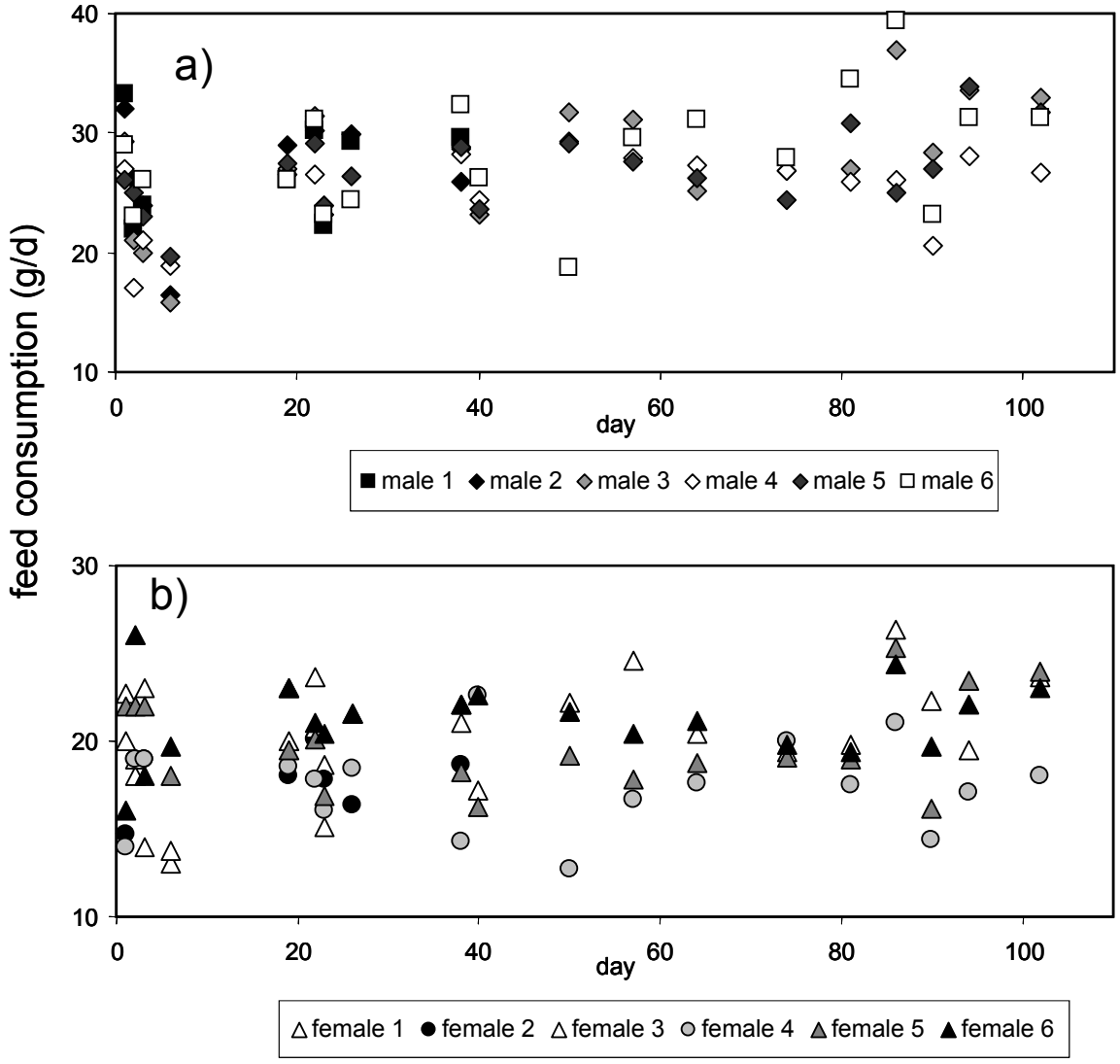

Figure C-S3. Feed consumption rate measured for a) treated males (diamond) and control males (square) and b) treated females (triangle) and control females (circle)

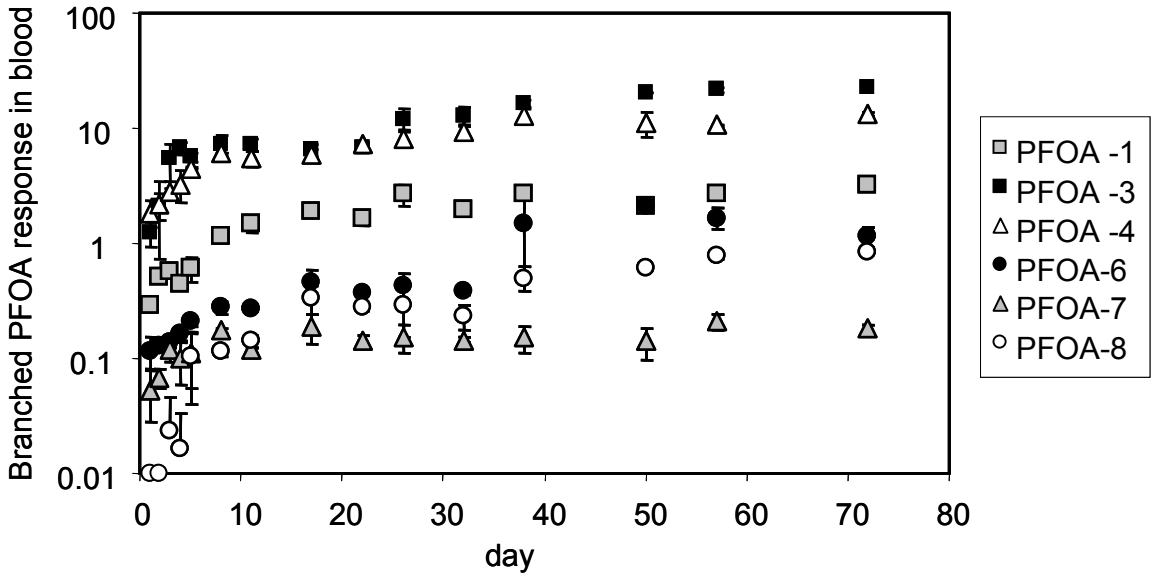

Figure C-S4. Time course for branched PFOA isomers in blood of male rats. Y-scale corresponds to relative response of isomer to internal standard ÷ sample mass. Error bars represent range observed for 3 rats until day 30 and 2 rats beyond. Legend: PFOA 1 = 3m-PFOA, PFOA 3 = 4m-PFOA, and PFOA 4 = 5m-PFOA.

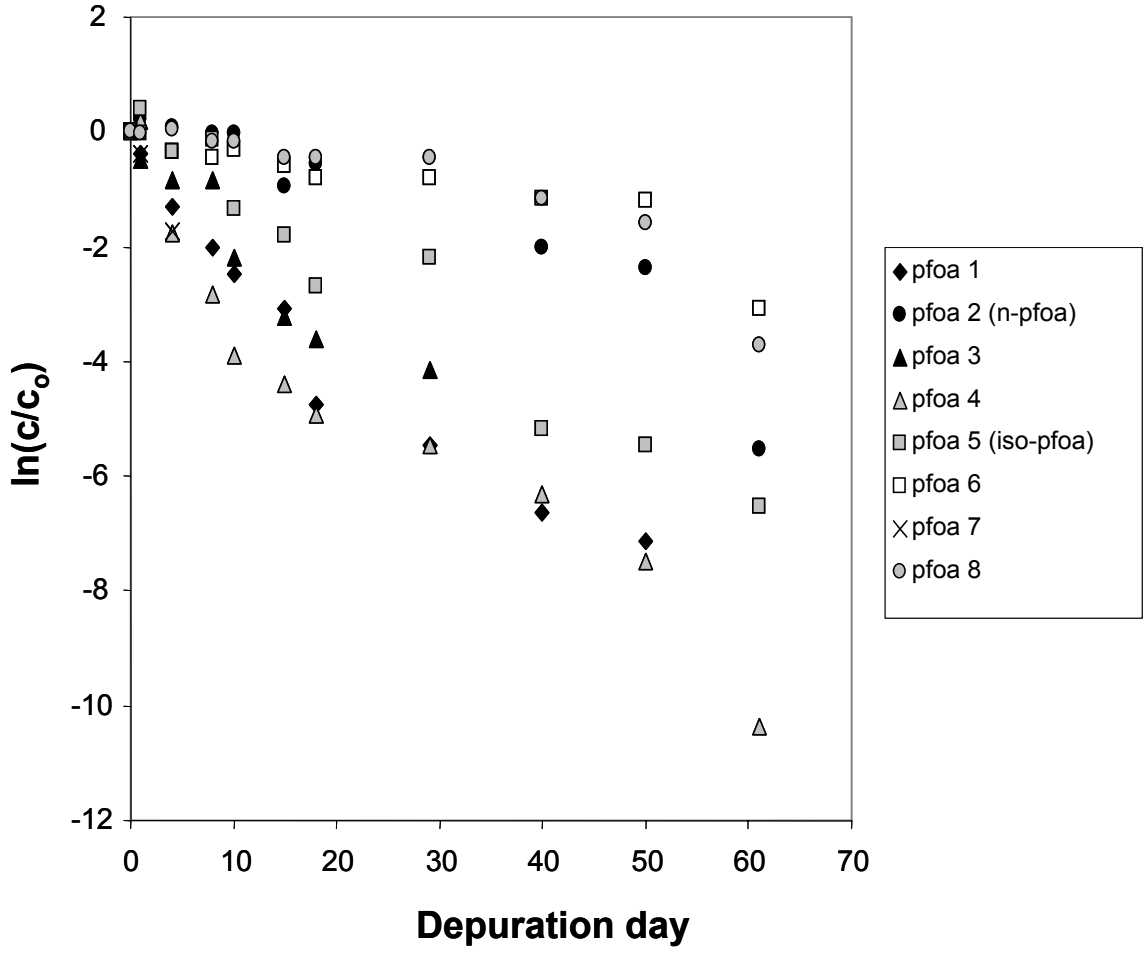

Figure C-S5. Depuration of PFOA isomers in male rat blood. Each point represents average of two male rats. Legend: pfoa 1 = 3m-PFOA, pfoa 2 = n-PFOA, pfoa 3 = 4m-PFOA, pfoa 4 = 5m-PFOA, and pfoa 5 = iso-PFOA.

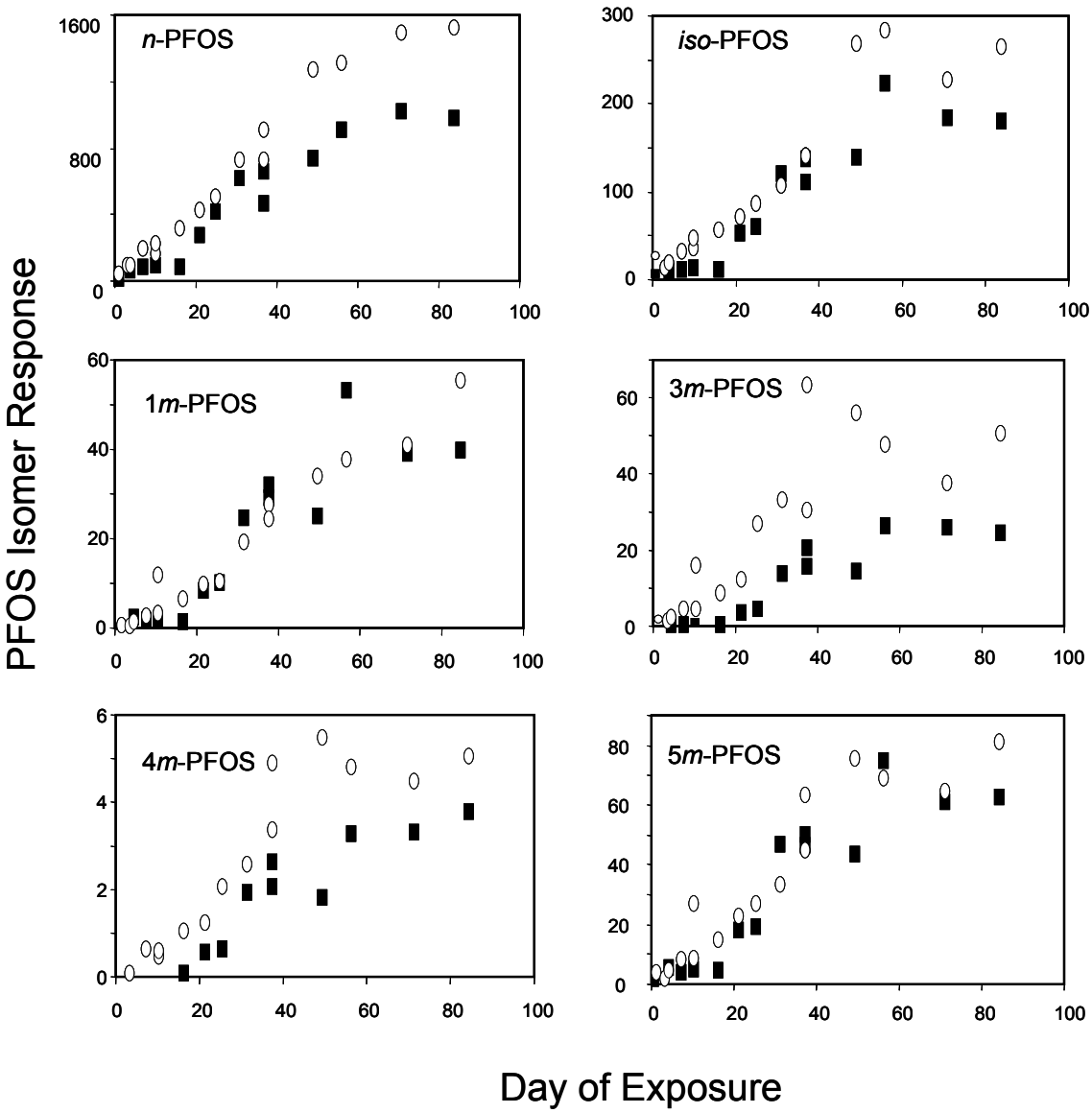

Figure C-S6. Time course of branched PFOS isomers in blood of male rat (■) and female rat (○). Y-scale corresponds to relative response of isomer to internal standard ÷ sample mass except for *n*-PFOS which is in units of ng/g.

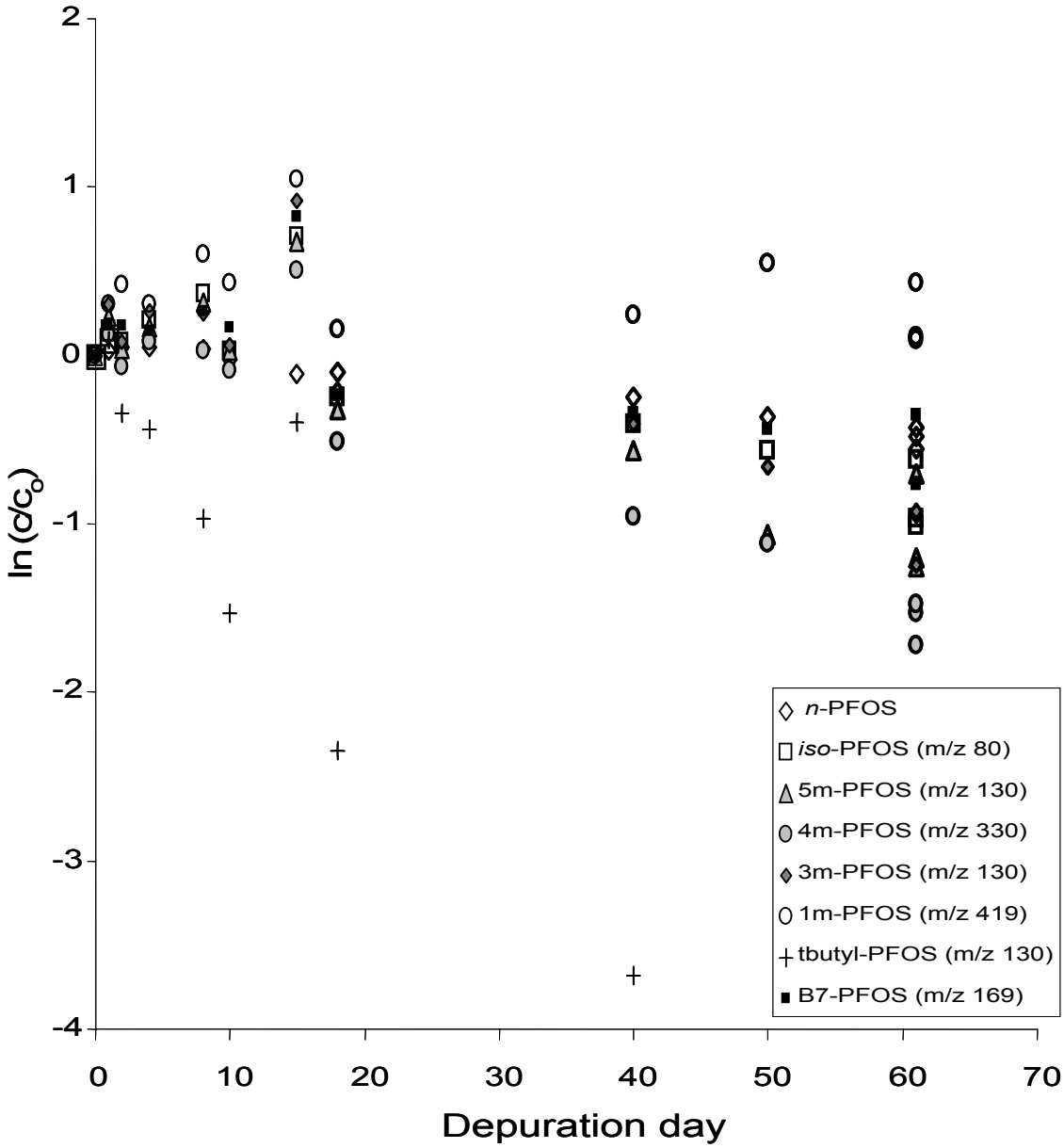

Figure C-7. Depuration of PFOS isomers in female rat blood. Legend indicates PFOS isomer with number in brackets corresponding to daughter ion monitored using tandem mass spectrometry.

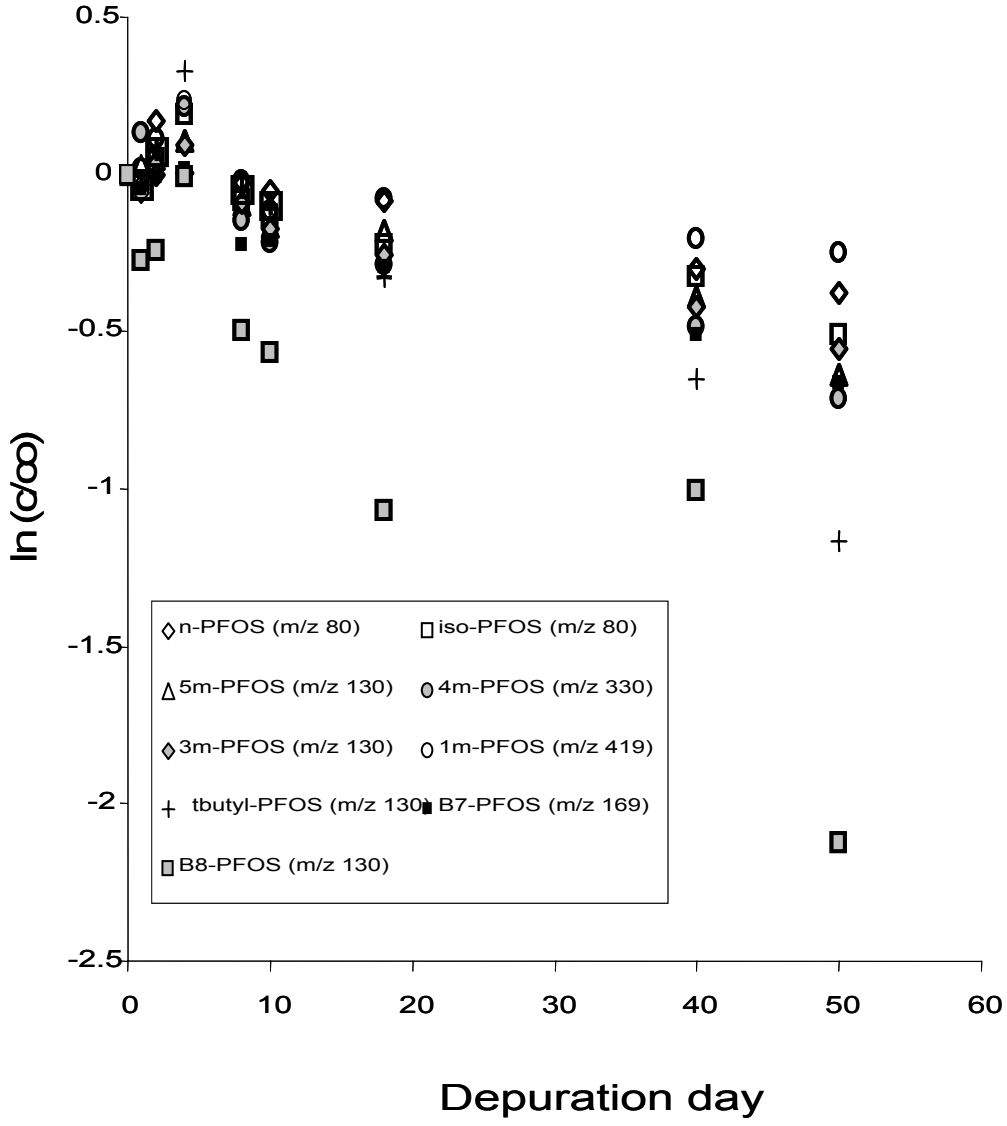

Figure C-8. Depuration of PFOS isomers in male rat blood. Legend indicates PFOS isomer with number in brackets corresponding to daughter ion monitored using tandem mass spectrometry.

APPENDIX D

SUPPORTING INFORMATION FOR CHAPTER SIX

Uptake, Accumulation and Elimination of Perfluorocarboxylate Isomers in Rainbow Trout

Table D-S1. Exposure phase data in blood (ng/g not growth corr), mean N=3

day	*n*-PFOA	*n*-PFNA	*iso*-PFNA
0	1.86	0.73	0.03
1	7.32	2.86	0.69
1.3	8.78	2.56	0.80
2	9.66	2.84	0.74
3	34.14	10.90	5.59
5	44.09	18.21	9.55
9	93.58	40.69	19.76
17	104.36	41.74	19.92
36	79.42	54.51	27.27

Table D-S2. Exposure phase SEM (standard error of mean) ng/g in blood (not growth corr), N=3

Day	*n*-PFOA	*n*-PFNA	*iso*-PFNA
0	0	0	0
1	1.29	0.40	0.35
1.3	3.64	1.08	0.19
2	6.89	2.10	0.59
3	1.84	1.15	1.75
5	10.76	3.16	1.39
9	3.09	5.21	4.93
17	22.51	10.33	6.19
36	10.14	1.69	1.51

Table D-S3. Exposure phase RRF/g blood, mean of N=3; RRF = relative response factor

blood	PFOA-1	PFOA-3	PFOA-4	PFOA-5	PFOA-6	PFOA-8
0	0.000	0.040	0.034	0.095	0.000	0.000
1	0.054	0.254	0.331	0.453	0.025	0.016
1.3	0.095	0.258	0.309	0.953	0.038	0.027
2	0.113	0.402	0.135	1.590	0.054	0.051
3	0.238	0.555	0.442	1.668	0.088	0.091
5	0.354	1.026	1.245	3.406	0.179	0.149
9	0.450	1.068	1.482	4.181	0.184	0.187
17	0.606	1.436	1.751	4.865	0.220	0.232
36	0.530	1.153	1.667	4.504	0.203	0.221

Table D-4 Exposure phase RRF/g blood, SEM of N=3

blood	PFOA-1	PFOA-3	PFOA-4	PFOA-5	PFOA-6	PFOA-8
0	0.000	0.000	0.000	0.000	0.000	0.000
1	0.028	0.042	0.045	0.177	0.013	0.008
1.3	0.023	0.098	0.144	0.159	0.008	0.005
2	0.027	0.109	0.115	0.096	0.005	0.008
3	0.046	0.118	0.224	0.294	0.017	0.010
5	0.045	0.192	0.193	0.424	0.011	0.022
9	0.024	0.089	0.060	0.236	0.030	0.035
17	0.087	0.333	0.435	0.895	0.045	0.023
36	0.055	0.096	0.146	0.300	0.019	0.006

Table D-S5. % PFOA isomer profile at day 36

	PFOA-1	n-PFNA	PFOA-3	PFOA-4	PFOA-5	PFOA-6	PFOA-8
Dose	0.7	80	4.4	3.9	10	0.2	0.2
Blood	0.4	88	1.7	2.6	6.9	0.3	0.3
Kidney	0.4	89	1.7	2.4	6.3	0.3	0.3
Liver	0.3	90	1.5	2.0	5.9	0.3	0.2
Heart	0.4	92	1.5	1.4	3.8	0.2	0.2
spleen	0.2	94	0.8	1.0	3.2	0.2	0.2

APPENDIX E

SUPPORTING INFORMATION FOR

Distribution of Perfluorinated Carboxylate Isomers in the North American Environment

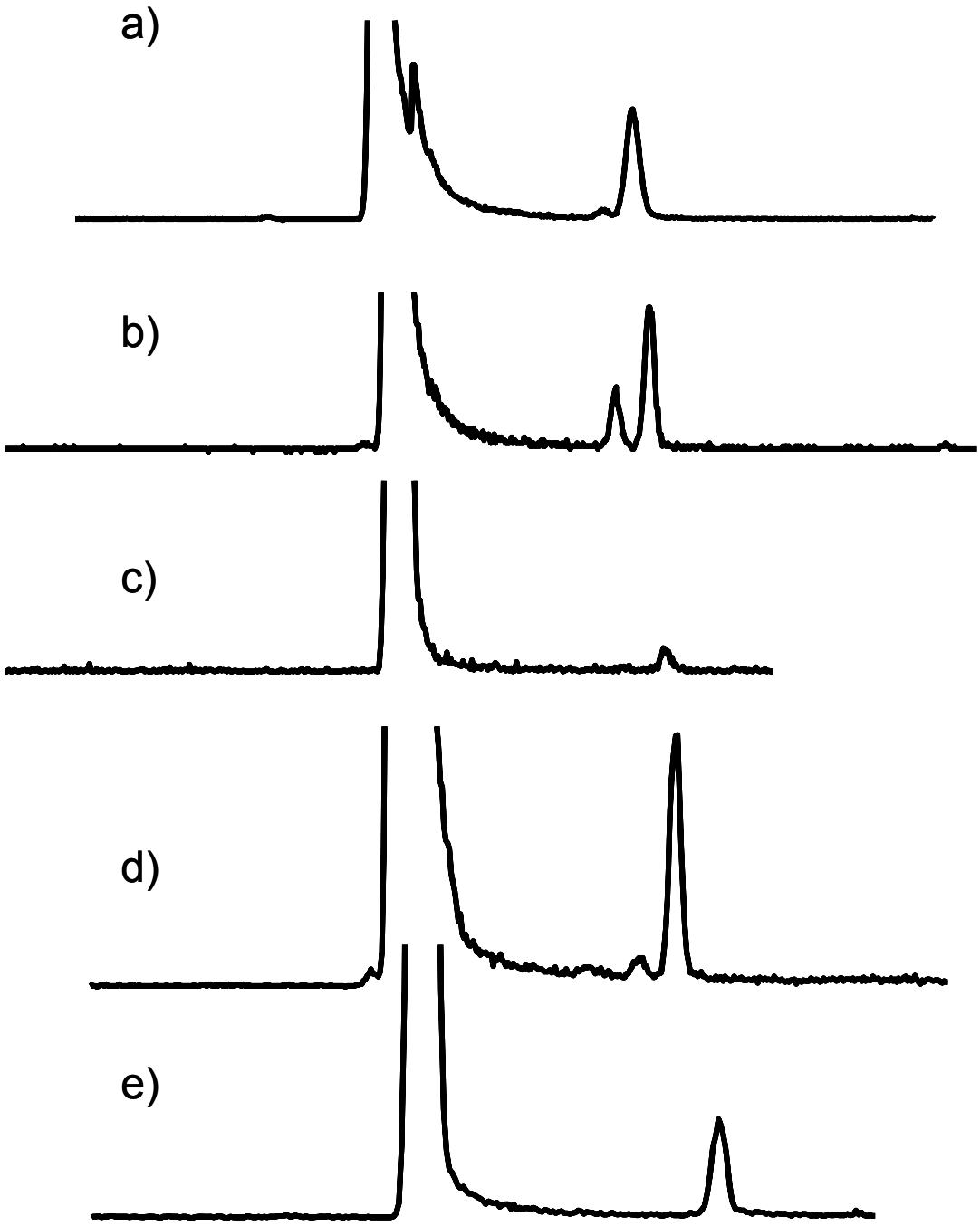

Figure E-S1. GC-MS chromatogram of PFNA isomers in a) Char Lake Sediment, b) human blood, c) ringed seal liver, d) dolphin plasma, and e) trout whole body homogenate using Rtx-35.

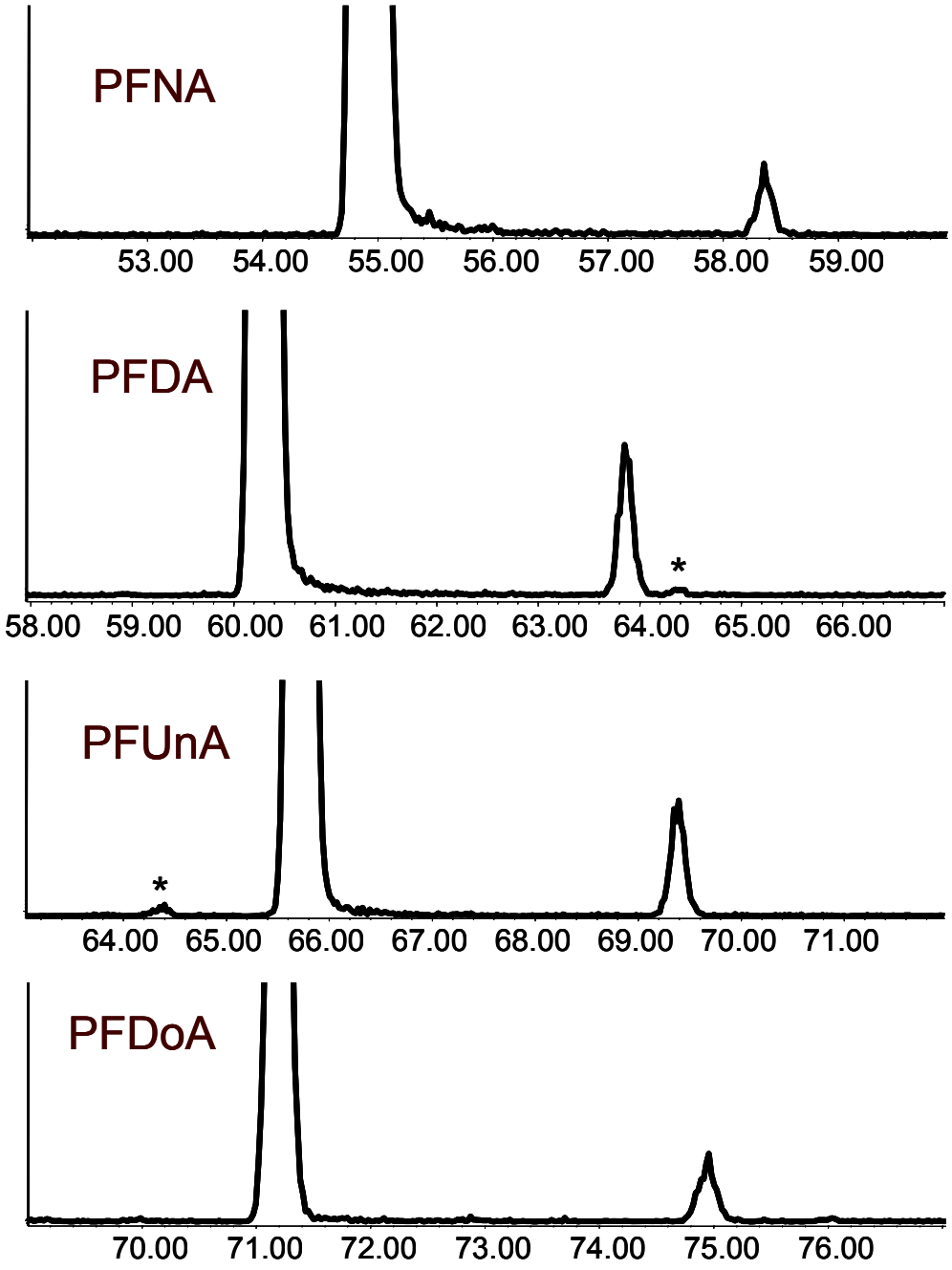

Figure E-S2. GC-MS chromatograms of PFNA, PFDA, PFUnA, and PFDoA isomers in Resolute Bay ringed seal liver from 2004 using Rtx-35. The peak labeled * was not considered an isomer as it appeared in every single ion monitoring (SIM) window.

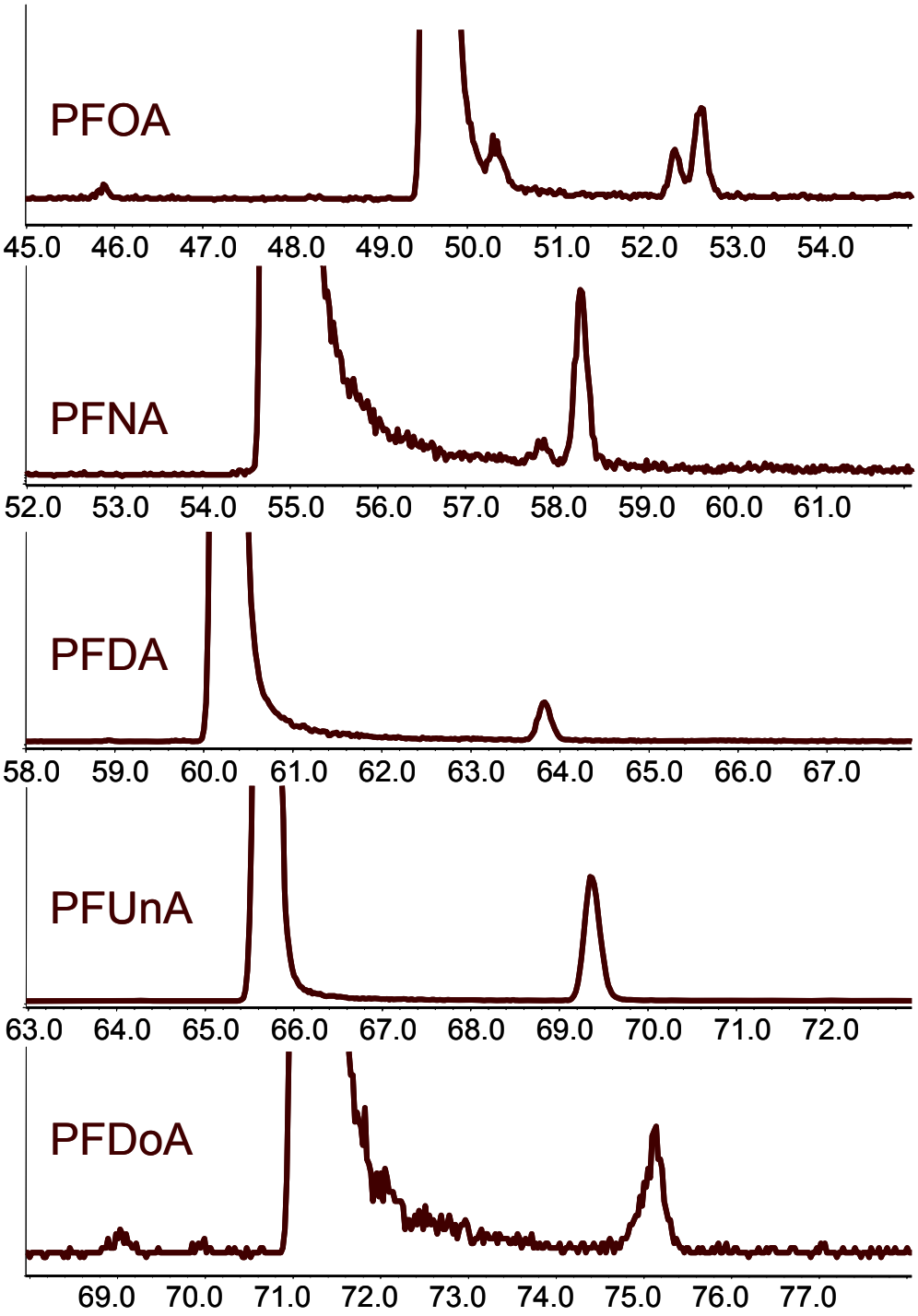

Figure E-S3. GC-MS chromatograms of PFOA, PFNA, PFDA, PFUnA, and PFDoA isomers in dolphin plasma using Rtx-35.

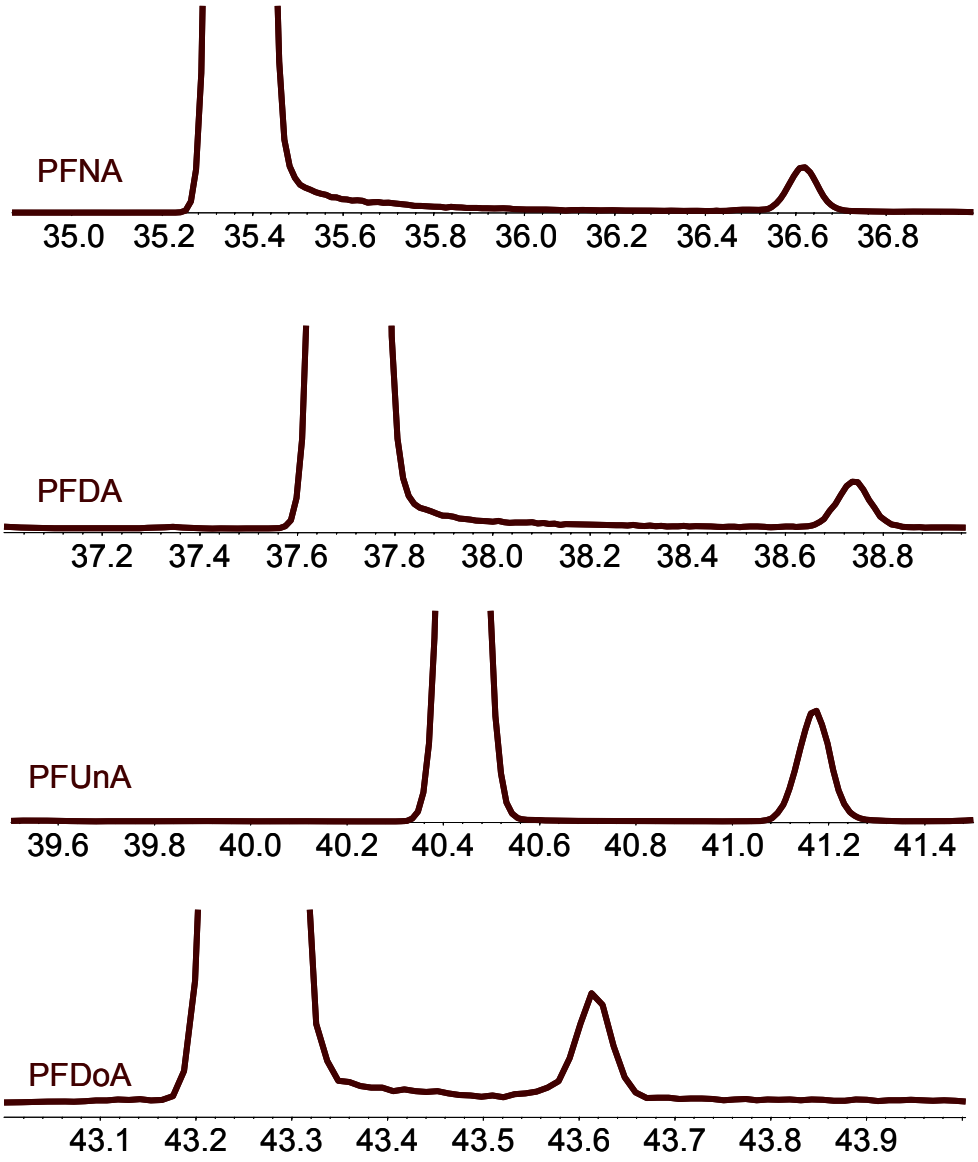

Figure E-S4. GC-MS chromatograms of PFNA, PFDA, PFUnA, and PFDoA isomers in *diporeia* using ZB-WAX.

Table E-S1. Mean ratio of branched PFOA to *n*-PFOA * 100 (standard deviation)

Sample	3m-PFOA	4m-PFOA	5m-PFOA	iso-PFOA
Arctic				
Char Lake surface water	0.09 (0.05)	<LOD	0.3 (0.01)	0.7 (0.05)
Amituk Lake surface water	0.05 (0.01)	<LOD	0.2 (0.03)	0.4 (0.08)
Char Lake sediment				
1.5 – 2.0 cm	0.1	<LOD	1	1
1.0 – 1.5 cm	0.2	<LOD	2	3
0 – 1.0 cm	0.2 (0.03)	<LOD	2 (0.1)	3 (0.1)
Resolute Bay ringed seal liver	---------- <LOD----------			
Sanikiluaaq polar bear liver	---------- <LOD----------			
Lake Ontario				
Sediment				
Station 1014	0.09	<LOD	1.4	1.4
Station 40	<LOD	<LOD	0.6	1.0
Surface water				
Station 40	0.3 (0.01)	1 (0.9)	2 (0.2)	3 (0.2)
Station 41	0.7	4	3	6
Station 13	2 (1)	4 (0.05)	4 (0.4)	8 (0.4)
Biota				
Zooplankton 1	<LOD	<LOD	0.4	1
Zooplankton 2	0.2	<LOD	0.6	1
Mysis	<LOD	<LOD	0.4	0.7
Diporeia	0.03	<LOD	0.4	0.8
Alewife	0.01 (N=1)	<LOD	0.5 (N=1)	0.6 (0.2)
Sculpin	0.1 (0.01)	<LOD	0.3 (0.03)	0.7 (0.02)
Smelt	0.04 (0.01)	<LOD	0.2 (0.04)	0.6 (0.3)
Trout	0.2 (0.2)	<LOD	2 (0. 6)	3 (2)
Precipitation	1 (0.3)	<LOD	0.8 (0.3)	2 (1)
Human Blood	0.08 (0.03)	0.3 (0.1)	0.2 (0.1)	2 (0.6)
Dolphin Blood	0.1 (0.04)	0.1 (0.05)	0.1 (0.04)	0.4 (0.1)

Table E-S2. % *iso*-PFCA composition (standard deviation)

Sample	PFDA	PFUnA	PFDoA
Arctic			
Char Lake surface water	----------<LOD----------		
Amituk Lake surface water	----------<LOD----------		
Char Lake sediment			
1.5 – 2.0 cm	1	<LOD	5
1.0 – 1.5 cm	3	<LOD	<LOD
0 – 1.0 cm	4 (0.2)	3 (2)	4 (0.5)
Resolute Bay ringed seal liver	1 (0.2)	2 (0.5)	7 (1)
Sanikiluaaq polar bear liver	3 (0.5)	1 (0.2)	4 (0.3)
Lake Ontario			
Sediment			
Station 1014	----------<LOD----------		
Station 40	----------<LOD----------		
Surface water			
Station 40	----------<LOD----------		
Station 41	----------<LOD----------		
Station 13	----------<LOD----------		
Biota			
Zooplankton 1	<LOD	3	<LOD
Zooplankton 2	0.6	3	1
Mysis	1	13	<LOD
Diporeia	0.6	9	2
Alewife	0.7 (0.2)	6 (3)	1 (0.4)
Sculpin	0.7 (0.01)	12 (0.2)	2 (0.03)
Smelt	0.6 (0.1)	10 (0.3)	2 (0.6)
Trout	0.7 (0.3)	11 (2)	2 (0.8)
Precipitation	1 (0.3) N = 3	<LOD	
Human Blood	<LOD	0.4 (N=1)	<LOD
Dolphin Blood	0.5 (0.3)	5 (2)	0.8 (0.3)

QA/QC

Water

1 L of HPLC water was rotovapped to 20 ml and derivatized. GC-MS analysis revealed 0.1 ng (N = 3, %RSD 0.5) of *n*-PFOA. No branched isomers or longer chain PFCAs were observed >LOD. 2 ng of ECF PFOA was spiked into 3 L of HPLC grade water and rotovapped to 20 ml. Recovery of *n*-PFOA was 90-93% for N = 3 and the isomer pattern was conserved between replicates and with the technical product. The standard deviation for determination of % n-PFOA was ±0.2%.

Sediment

Sediment from the Char Lake core at 26-27 cm depth was used for spike and recovery measurements and precision. 1 ng of ECF PFOA was spiked into 7-8 g of sediment and extract in the same manner as samples. The % recovery for n-PFOA was 82-84% and the isomer pattern was conserved between duplicate trials and with the technical product. The difference in % *n*-PFOA for N = 2 spiked samples was ±0.3%. The instrument limit of detection corresponded to 0.5 ng ECF PFOA in which 4m-PFOA, 5m-PFOA and iso-PFOA were readily detected. Thus the LOD corresponded to 0.02 ng per isomer.