Estimated Dietary Exposure to Perfluorinated Compounds in Canada

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Abstract

Perfluorinated carboxylates (PFCAs), sulfonates (PFSs) and perfluoroalkylsulfonamides (PFOSAs) have been detected in whole blood and serum of non-occupationally exposed humans, yet sources of exposure have not been fully elucidated. The objectives of this study were to estimate dietary exposure to perfluorooctane sulfonate (PFOS), PFCAs (C_7-C_{11}) and fluorotelomer unsaturated carboxylates (FTUCAs) for the general Canadian and Inuit populations prior to the phase-out of perfluorooctyl-sulfonyl production by 3M and voluntary reductions in PFOA emissions under the PFOA stewardship program. PFCs were measured in 65 archived composite food samples prepared for the 1998 Canadian Total Diet Study (TDS) and 68 archived traditional foods from Nunavut using a newly developed methanol extraction combined with a solid phase extraction clean up. Dietary exposure was estimated using food intake data available from studies carried out between 1997 and 1998 in southern Canada and Nunavut.

PFCs were detected in eight composite food samples from the Canadian TDS and in 61 traditional food samples. Elevated concentrations of PFCs were found in caribou liver (6.2±5.5 ng/g), ringed seal liver (7.7, 10.2 ng/g), polar bear meat (7.0 ng/g), beluga meat (7.0, 5.8 ng/g), luncheon meats (5.02 ng/g), cookies (2.7 ng/g), processed cheese (2.1 ng/g) and peppers (1.8 ng/g). Low levels of total PFCs (<1.5 ng/g) were measured in 41 traditional foods including: meat (caribou, ptarmigan, snow goose, bearded seal, walrus, black duck), berries, and fish (lake trout, arctic char). PFCs were not detected in beverages, unprocessed meats, breads, cereals and fruits from the TDS composite samples analyzed.

The ranges of estimated daily exposure to PFCs were between 2 and 59 ng-person^{-1} and 210 to 610 ng-person^{-1} for average Canadians and Inuit in Nunavut respectively. There were no statistically significant differences in mean PFC exposure levels for different age and gender groups in the general Canadian population. Inuit men in the 41 to 60 year old age group had statistically

significantly higher estimated daily exposure to PFCs ($p<0.05$) than younger men and women from the same age group. This higher exposure was associated with the consumption of beluga muktuk, caribou liver and bearded seal intestine.

Traditional foods contributed a higher percentage to PFC exposure than market foods in all age and gender groups for the Inuit population. In general, caribou meat, arctic char meat and cookies contributed most to dietary exposure for Inuit, with caribou flesh contributing 43 to 75 percent to daily PFC dietary exposure. Dietary exposure for the general Canadian population was associated with the consumption of cakes and cookies, processed cheese, and regular cheese.

Levels of dietary exposure to PFCs estimated in these studies do not pose any significant health risk to either population based on current toxicological information.

Abrégé

Les carboxylates perfluorinés (PFCAs), les sulfonates (PFSs) et les perfluoro-alkyl-sulfonamides (PFOSAs) ont été détectés dans le sang entier et le sérum des personnes non exposées professionnellement, cependant les sources d'exposition à ses substances n'est pas entièrement élucidé. Les objectifs de cette étude étaient d'évaluer l'exposition diététique au sulfonate de perfluorooctane, carboxylates perfluorinés (C7-C11) et aux fluorotelomer carboxylates insaturés aux populations canadienne en générale et aux Inuits en particulier avant la suppression progressive de la production de perfluorooctyl-sulfonyl par 3M et la réduction volontaire des émissions de PFOA dans le cadre du programme de préservation PFOA. Des composés perfluorinés ont été mesurés dans 65 échantillons de composés alimentaires archivés, préparés en 1998 pour la TDS (Canadian Diet Study) et 68 échantillons de nourritures traditionnelles archivées du Nunavut, utilisant une méthode d'extraction au méthanol récemment développée, combinée avec une méthode d'extraction en phase solide à balayage. L'exposition diététique a été évaluée utilisant les données relatives à la consommation alimentaire fournies par des études effectuées entre 1997 et 1998 dans le sud du Canada et au Nunavut.

Les PFCs ont été détectés dans huit échantillons de composés alimentaires du TDS canadien et dans 61 échantillons de composés alimentaires traditionnels. Des concentrations élevées de PFCs ont été trouvées dans le foie du caribou (6.2±5.5 ng/g), le foie annelé du phoque (7.7, 10.2 ng/g), la viande d'ours blanc (7.0 ng/g), la viande de beluga (7.0, 5.8 ng/g), les pâtés de viandes (5.02 ng/g), les biscuits (2.66 ng/g), le fromage fondu (2.12 ng/g) et les poivres (1.79 ng/g). Des concentrations réduites de PFCs total (<1.5 ng/g) ont été mesurés dans 41 composés alimentaires traditionnelles comprenant: viande (caribou, lagopède alpin, oie des neiges, phoque barbu, morse, canard noir), baies et poissons (truite de lac, omble de l'arctique). Les PFCs n'ont pas été détectés dans les boissons, viandes non préparées, pains, céréales et fruits des échantillons analysés de TDS. L'estimation journalière de l'exposition quotidienne au PFCs était entre 2 à 59 ng

par individus pour les canadiens moyens et entre 210 à 610 ng par individus pour les Inuits du Nunavut. Aucune différence statistiquement signifiante n'a été trouvé dans les niveaux d'exposition moyens de PFC par rapport aux différents groupes d'âge et de sexe dans la population canadienne générale. L'exposition quotidienne au PFCs ($p<0.05$) des hommes Inuits entre 41 à 60 ans était statistiquement plus élevée que chez les plus jeunes hommes et les femmes de la même catégorie d'âge. Cette exposition plus élevée a été associée à la consommation du beluga muktuk, du foie de caribou et de l'intestin du phoque barbu.

Les nourritures traditionnelles ont contribué à un pourcentage plus élevé à la consommation de PFC que des nourritures commercialisées dans tous les groupes d'âge et sexe chez la population d'Inuit. Généralement les viandes de caribou, d'omble de l'arctique et les biscuits ont contribué pour la plupart à l'exposition diététique des Inuits, avec la chair de caribou contribuant entre 43 à 75% de l'exposition diététique quotidienne au PFC. L'exposition diététique à la population canadienne générale a été associée à la consommation des gâteaux, biscuits, fromage fondu et fromage régulier.

Les niveaux d'exposition diététique aux PFCs estimée dans ces études ne posent aucun risque sanitaire significatif à l'une ou l'autre populations au regard des informations toxicologiques courantes

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Contribution of Authors

This is a manuscript-based thesis and includes two manuscripts to be submitted for publication (Chapter 2 and Chapter 3). Chapter 2 is authored by myself and co-authored by John Moisey, Robert W. Dabeka and my supervisors Drs. Laurie Chan and Sheryl Tittlemier. Chapter 3 is authored by myself and co-authored by Brent Tague and my supervisors Drs. Murray Humphries, Sheryl Tittlemier and Laurie Chan. The contributions of each of the authors of the manuscripts are stated below:

The project was initiated by L. Chan (McGill University) and S. Tittlemier (Health Canada). R. Dabeka organized the 1998 Total Diet Study. Method development and laboratory work were carried out by S. Ostertag (McGill University). The analysis of samples by HPLC-MS/MS was carried out by John Moisey and Brett Tague (Health Canada). All data and statistical analyzes were carried out by S. Ostertag. The work of S. Ostertag was supervised by S. Tittlemier, L. Chan and M. Humphries and funded by grants provided to L. Chan and S. Tittlemier. The manuscripts were written by S. Ostertag and edited by the co-authors.

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List of Acronyms

Group	Acronym	Name
Perfluorinated carboxylates (PFCAs)	PFHpA	Perfluoroheptanoate
	PFOA	Perfluorooctanoate
	PFNA	Perfluorononanoate
	PFDA	Perfluorodecanoate
	PFUnDA	Perfluoroundecanoate
	PFDoDA	Perfluorododecanoate
	PFTeDA	Perfluorotetradecanoate
Perfluorinated sulfonates (PFSs)	PFHxS	Perfluorohexane sulfonate
	PFOS	Perfluorooctane sulfonate
Perfluorinated sulfonamides (PFOSAs)	N-MePFOSA	N-methyl perfluorooctane sulfonamide
	N-EtPFOSA	N-ethyl perfluorooctane sulfonamide
Perfluorinated sulfonamidoacetic acid (PFOSAAs)	N-MePFOSAA	N-methyl perfluorinated sulfonamidoacetic acid
	N-EtPFOSAA	N-ethyl perfluorinated sulfonamidoacetic acid
Perfluorinated sulfonamidoethanols (PFOSEs)	N-MePFOSE	N-methyl perfluorooctane sulfonamidoethanol
	N-EtPFOSE	N-ethyl perfluorooctane sulfonamidoethanol
Perfluorinated sulfonamido ethylacrylate (FOSEAs)	N-MeFOSEA	N-methyl perfluorooctane sulfonamido ethylacrylate
Fluorotelomers	FTOH	Fluorotelomer alcohol
	FTCA	Fluorotelomer carboxylic acid
	FTUCA	Fluorotelomer unsaturated carboxylic acid
PFC precursors	POSF	Perfluorooctanesulfonyl fluoride
	APFO	Ammonium perfluorooctanoate
	APFN	Ammonium perfluorononanoate
Surfactant	PAPS	Polyfluoroalkyl phosphate surfactants

Chapter 1: Introduction and Literature Review

Introduction

Perfluorinated compounds (PFCs) have been produced since 1947 due to their unique surfactant properties. Recently it was realized that the production and use of PFCs use had resulted in the contamination of humans, oceans, surface water, air, fish and wildlife with perfluorinated carboxylates (PFCAs), sulfonates (PFSs), telomer alcohols (FTOHs) and perfluoroalkylsulfonamides (PFOSAs). Human exposure to perfluorinated sulfonates (perfluorooctane sulfonate and perfluorohexane sulfonate), PFCAs and PFOSAs has been observed in non-occupationally exposed men and women in North America (Calafat et al., 2006a; Olsen et al., 2005d), South America (Calafat et al., 2006b; Kannan et al., 2004), Europe (Kannan et al., 2004) and Asia (Harada et al., 2007; Kannan et al., 2004; Taniyasu et al., 2003). Following the detection of perfluorinated sulfonates and PFCAs in humans and the environment, PFOS-related production was phased out in 2001 (Renner, 2001) and the U.S. EPA initiated the PFOA stewardship program to encourage the voluntary reduction of PFCA emissions and impurities (U.S.EPA, (accessed June 2007)).

Exposure of humans to PFCs occurs globally, however in general concentrations of PFOS and PFOA are higher in North America (Kannan et al., 2004; Kuklenyik et al., 2005; Olsen et al., 2003b) than Europe (Fromme et al., 2007; Kannan et al., 2004). Recent dietary exposure estimates suggest that the consumption of store-bought and restaurant foods results in daily exposure that ranges from 4 ng/kg body weight/day (Tittlemier et al., 2007) to 100 ng/kg body weight/day (Mortimer et al., 2006). Elevated exposure of PFCs has been associated with the consumption of fish in Polish individuals (Falandysz et al., 2006). Dietary exposure to PFCs may be elevated in people consuming wildlife species as a result of bioaccumulation and bioconcentration in food webs (Martin et al., 2004a; Tomy et al., 2004a). Canadian Inuit are vulnerable to increased exposure to environmental contaminants due to their consumption of various wildlife species, which include high-trophic level animals that accumulate mercury and

polychlorinated biphenols (PCBs). PFOS and PFCAs have been detected in species that are frequently hunted and consumed by Inuit (Kannan et al., 2001b; Martin et al., 2004a; Smithwick et al., 2005a; Tomy et al., 2004a), which suggests that consumers of traditional foods are exposed to these compounds.

Dietary exposure to PFCs may also result from the use of food-packaging coatings such as polyfluoroalkyl phosphate surfactans (PAPs) and N-ethylperfluorooctanesulfonamide (N-EtPFOSA) (Begley et al., 2005; Tittlemier et al., 2006). Previous studies have shown that N-EtPFOSA and PAPs can be metabolized to PFOS and PFOA. N-EtPFOSA has been detected in the liver of beluga and narwhal in the Arctic (Tomy et al., 2004a). Animal feeding trials have demonstrated that PAPs can be metabolized to PFOA in rats (D'Eon J and Mabury, 2007) and N-EtPFOSA underwent biotransformation to PFOS and PFOSA in rainbow trout liver microsomes (Tomy et al., 2004b).

The effects of chronic exposure to PFOS and PFOA have not been studied extensively in humans to date. A recent study suggests that PFOA and PFOS exposure *in utero* at levels found in the general North American population may affect development of fetuses (Apelberg et al., 2007a). However, adverse health effects or statistically significant changes in serum biochemistry and haematological variables were not observed in a population exposed to elevated levels of PFOA (Emmett et al., 2006). Elevated PFOS and PFOA exposure in fluorochemical workers have been associated with increased cholesterol, triglycerides and thyroid hormone (T3) levels (Olsen et al., 2003a; Olsen et al., 1999). Animal feeding trials have indicated that PFOA exposure causes liver toxicity, carcinogenicity, increased liver weight and developmental effects (Abbott et al., 2007; Biegel et al., 2001; Butenhoff et al., 2002; Butenhoff et al., 2004; Thomford, 2002; White et al., 2007). Developmental effects of PFOA exposure *in utero* include reduced survival and incomplete lung-development (White et al., 2007). PFOS exposure in cynomolgous monkeys led to reduced

body weight, greater liver weight, lower serum total cholesterol and changes in levels of thyroid simulating hormone (TSH) and total T3 (Seacat et al., 2002).

Rationale

Exposure of humans to PFCs has been established worldwide, yet sources of exposure have not been fully elucidated. Exposure estimates for Canadians suggest that more than half of total daily adult exposure results from the consumption of contaminated food (Tittlemier et al., 2007). However, there is no national contaminant exposure program in Canada and a comprehensive analysis of dietary exposure to PFCs has not been conducted to date. Previous studies of PFCs in Canadian foods have been limited to the analysis of fast foods, meats and eggs and the dietary exposure estimate was based on a dietary intake survey conducted by Nutrition Canada and Food and Drug Directorate in 1970-1972 (Directorate, 1981). Recent data on dietary intake based on the response of the general Canadian population to 24-hour dietary recalls indicated that changes in diet have occurred since 1972 (Gray-Donald et al., 2000).

Insufficient information is available to estimate dietary exposure to PFCs for Inuit in Nunavut. Levels of perfluorinated compounds in appropriate species, tissues and preparations of arctic wildlife commonly consumed by Inuit people are not available. However, Inuit dietary exposure to PFCs may exceed that of the general Canadian population, given that PFCs were detected at low concentrations in only a few store-bought foods (Tittlemier et al., 2007), yet their presence has been observed in the liver and blood of species frequently consumed by Inuit (Martin et al., 2004a; Tomy et al., 2004a).

Objectives

1. Develop a method to measure PFOS, PFCAs (C_7-C_{11}) and FTUCAs (6:2, 8:2 and 10:2) in diverse food matrices.
2. Measure the concentrations of PFOS, PFCAs (C_7-C_{11}) and FTUCAs (6:2, 8:2 and 10:2) in archived market and traditional foods.
3. Estimate dietary exposure to PFOS, PFCAs (C_7-C_{11}) and FTUCAs (6:2, 8:2 and 10:2) for the general Canadian population and Inuit from Nunavut for the late 1990s.
4. Assess the health risk associated with dietary exposure estimates for the general Canadian population and Inuit.

Literature Review

1. Perfluorinated Compounds

1.1. Description of Perfluorinated Compounds

Perfluorinated compounds (PFCs) have been synthesized since 1947 (3M, 1999) via electrochemical fluorination (ECF), fluorotelomer iodide oxidation, fluorotelomer olefin oxidation and fluorotelomer iodide carboxylation (Prevedouros et al., 2006). PFCs are diverse chemicals characterized by repeating fluorocarbon chains and a sulfonyl or carboxyl head group (Kissa, 2001). Perfluorooctanesulfonate and perfluoroctanoate are similar to fatty acids with highly hydrophobic and lipophobic perfluorinated carbon tails, and strongly polar (hydrophilic) sulfonyl or carboxyl head groups, respectively (Lehmler, 2005). The predominant PFCs produced include perfluorinated carboxylates (PFCAs), perfluorinated sulfonates (PFSs), perfluorinated sulfonamides and sulfonamidoethanols (PFOSAs and PFOSEs) and perfluorotelomer alcohols and carboxylic acids (FTOHs and FTCAs).

Perfluorooctanesulfonyl fluoride (POSF) is produced by electrochemical fluorination of octanesulfonyl fluoride, then POSF is reacted with various hydrocarbons to produce N-methyl or N-ethylperfluorooctanesulfonamide (PFOSA) and N-methyl or N-ethylperfluorooctanesulfonamidoethanol (PFOSE) (Lehmler, 2005). PFOSE and PFOSA are important intermediates for 3M's products (Lehmler, 2005). 3M Corporation began to phase-out the production of POSF in 2000 (Renner, 2001).

PFCAs and telomer alcohols are produced as intermediates or final products through a telomerization process (Schultz et al., 2003). Fluorinated alkyl substances based on telomerization are produced by a number of companies including DuPont, Asahi Glass, Atofina, Clariant, and Daikin (Schultz et al., 2003). Fluorinated telomeric alcohols and ammonium perfluorooctanoate

(APFO) are precursor compounds in the production of fluorinated polymers and are present as residuals in final products (Dinglasan-Panlilio and Mabury, 2006).

1.2. Production and Use

PFCs have been produced due to their unique surfactant properties. PFCs are used as surface treatments, paper and packaging protectors and performance chemicals (3M, 1999). The ammonium salts of perfluorooctanoate (APFO) and perfluorononanoate (APFN) are used primarily in the manufacturing process of fluoropolymers such as polytetrafluoroethylene (PTFE) and polyvinylidine fluoride (Prevedouros et al., 2006). PFCAs are used in breathable waterproof fabrics, biomaterials, insulators for electric wires, planar etching of fused silica, foam fire extinguishers, floating agents and other applications (Kissa, 2001). PFOA is also formed in trace amounts during the manufacturing of some telomer-based products (Kissa, 2001). Fluorotelomer-based production has approximately doubled between 2000 and 2004, and production volumes in 2004 were approximately 11-14 x 10^6 kg yr^{-1} (Prevedouros et al., 2006).

Global APFO manufacturing emissions were approximately 45 tonnes in 1999 and are expected to decrease to 7 tonnes in 2006 (Prevedouros et al., 2006). Total global emissions of PFCAs was estimated to be between 3200 and 7300 tonnes (Prevedouros et al., 2006). In 2000, nearly 3 million kg PFOS were produced in the United States (Lehmler, 2005); however, 3M voluntarily phased out the production of perfluorooctylsulfonyl-based compounds in 2001 (Renner, 2001).

1.3. Analytical Methods

Limits in methods and analytical instruments have made the analysis of perfluorinated compounds in biological matrices challenging. In 2001, a method was developed that permitted the analysis of low levels of fluorinated organic compounds in human blood, sera and other biological matrices (Hansen et al., 2001). The precise identification of many perfluorinated compounds is now

possible through the use of high-pressure liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

1.4. Regulation

The United States Environmental Protection Agency (USEPA) issued a significant new use rule (SNUR) under section 5(a)(2) of the Toxic Substances Control Act (TSCA) for 75 perfluoroalkyl sulfonates in 2003 (Agency, 2002). The USEPA initiated the 2010/15 PFOA Stewardship Program in January 2006 to promote the reduction of PFOA emissions and content in products (Agency, (accessed June 2007)). Eight companies in the fluorochemical industry have committed to reduce emissions of PFOA by 95 percent by 2010 and eliminate emissions and content in products by 2015 (Agency, (accessed June 2007)). Environment Canada issued a temporary ban on four fluorotelomer polymers found in stain repellents in 2004 (Renner, 2004).

1.5. Environmental Sources

Emissions of PFCAs during manufacturing and industrial and consumer uses have released an estimated 3200-6900 tonnes of PFCAs to the environment (Prevedouros et al., 2006). Residual impurities in POSF-based products and fluorotelomer-based products have resulted in the release of 30-350 tonnes of PFCAs to the environment (Prevedouros et al., 2006). A reduction of greater than ninety percent in APFO environmental releases has been reported in recent years (Pelley, 2004) and ammonium perfluorononanoate (APFN) releases are expected to decline by 67 percent (Pelley, 2004).

Environmental sources of PFOA and PFOS include wastewater treatment plants (Boulanger et al., 2005a; Boulanger et al., 2005b; Hansen et al., 2002; Higgins et al., 2005; Saito et al., 2004), releases during application of N-Methyl perfluorooctane sulfonamido ethylacrylate (N-MeFOSEA) (3M, 1999), releases from fluorochemicals in landfills following degradation of fluoropolymers (Ellis

et al., 2003) and local contamination from the use of aqueous fire-fighting foams (Moody and Field, 1999; Moody et al., 2003).

Atmospheric transport has been suggested to result in a flux of PFCAs (0.1-10 t/year) to the Arctic (Ellis et al., 2003). Between 2 and 12 tonnes of PFOA are estimated to reach the Arctic annually via oceanic transport (Prevedouros et al., 2006). PFOS concentrations measured in high Arctic icecaps suggests that 18 to 48 kg PFOS were transported north of 65 degrees latitude in 2005 (Young et al., 2007).

1.6. Routes of Exposure

Exposure estimates for Canadians suggest that more than half of total daily adult exposure results from the consumption of contaminated food (Tittlemier et al., 2007). Other potential sources of exposure for humans include drinking water (Harada et al., 2003), dust (Kubwabo et al., 2005), solution-treated carpeting and treated apparel (Washburn et al., 2005).

Food may be a source of PFCAs, PFOSAs and PFSs for humans (D'Eon J and Mabury, 2007; Mortimer et al., 2006; Sinclair et al., 2007; Tittlemier et al., 2006; Tittlemier et al., 2007). Polyfluoroalkyl phosphate surfactans (PAPs) and N-ethylperfluorooctanesulfonamide (N-EtPFOSA) used in food packaging have been shown to migrate into foods (Begley et al., 2005; Tittlemier et al., 2006). Animal feeding trials have demonstrated that PAPs can be metabolized to PFOA in rats (D'Eon J and Mabury, 2007) and N-EtPFOSA can be metabolized to PFOSA and PFOS (Tomy et al., 2004b). These studies suggest that foods that are in contact with fluorinated food-packaging coatings may be a source of PFOS and PFCAs for consumers.

1.7. Concentrations and Trends

1.7.1. Concentrations

1.7.1.1. Wildlife

PFCs have been detected in wildlife species (plasma, blood and liver samples) from urban, rural and remote locations (Bossi et al., 2005; Falandysz et al., 2007; Giesy et al., 2001; Kannan et al., 2002; Kannan et al., 2001; Martin et al., 2004a; Martin et al., 2004b; Smithwick et al., 2005; Tomy et al., 2004a). Reported concentrations of PFOS in wildlife range from less than detection limits in polar skua (*Catharacta maccormicki)* from Antarctica to 3680 ng/g in mink (*Mustela vison)* from Midwestern United States (Giesy et al., 2001). PFOS concentrations were found to be greatest in proximity to populated and industrialized regions compared to remote marine regions (Giesy et al., 2001) and bioconcentration of PFOS was observed in an Arctic marine ecosystem (Tomy et al., 2004a). The concentrations of PFOS exceeded those of PFOA in Arctic marine mammals (Martin et al., 2004a). PFCAs were analyzed in Arctic wildlife species and the greatest concentrations were measured in mammals feeding higher in the food chain (Martin et al., 2004a).

1.7.1.2. Humans

Studies have been carried out worldwide to determine current and past levels of perfluorinated compounds in occupationally and non-occupationally exposed humans (Guruge et al., 2005; Hansen et al., 2001; Inoue et al., 2004; Kannan et al., 2004; Kubwabo et al., 2004; Kuklenyik et al., 2005; Kuklenyik et al., 2004; Olsen et al., 2003a; Olsen et al., 1999; Olsen et al., 2004; Olsen et al., 2003b; Olsen et al., 2003c; Olsen et al., 2005b; Olsen et al., 2003d; So et al., 2006; Taniyasu et al., 2003; Yeung et al., 2006). The study of PFCs in human tissue (milk, blood, cord blood, serum, plasma and liver) has revealed that human exposure to PFCs is a global phenomenon. PFOS has consistently been found at greater concentrations than PFOA and other PFCAs in human serum and plasma samples. The greatest concentrations of PFCs have been detected in fluorochemical workers with mean serum PFOS concentration of 2.19 mg/L in 1997 in Antwerp, Belgium and Decatur, Alabama (Olsen et al., 1999). Mean serum levels of PFOS in the general population range from 0.98 μg/L on an organic tea farm in Sri Lanka (Guruge et al., 2005) to 55.4 μg/L in Gdansk,

Poland (Kannan et al., 2004). Levels of PFOS and PFOA in human serum are generally greater in North America (Kannan et al., 2004; Kuklenyik et al., 2005; Olsen et al., 2004; Olsen et al., 2003b; Olsen et al., 2003c) than in Europe (Fromme et al., 2007; Kannan et al., 2004).

Japanese men had approximately twice the serum PFOS concentrations than women (Harada et al., 2004). Greater concentrations of PFOS were detected in premenoposal women than postmenoposal women in Japan (Harada et al., 2005) and increasing PFOS and PFOA concentrations were observed in older females (Fromme et al., 2007).

1.7.2. Temporal Trends

1.7.2.1. Humans

Few studies have assessed temporal trends of PFC exposure in humans. A four-fold increase in PFOA concentrations was observed in serum samples from Kyoto, Japan between 1983 and 1999 (Harada et al., 2007). The concentrations of PFOS, PFOA, perfluorooctane sulfonamidoacetate (PFOSAA) and PFHxS increased from 1974 to 1989, but remained stable from 1989 to 2001 in participants representative of the US population (Olsen et al., 2005b). A pilot study of PFOS and PFOA concentrations in blood donors from the American Red Cross suggests that concentrations of these two compounds have declined from 2000 to 2005 (Olsen et al., 2007).

1.7.2.2. Environment

Limited studies have been published regarding temporal trends of PFCs in the environment. Decreasing trends in PFOS concentrations in the Arctic have been observed recently in ice samples from high-arctic ice caps and ringed seal (*Phoca hispida*) liver from Arviat and Resolute Bay, Nunavut (Butt et al., 2007; Young et al., 2007). Concentrations of PFCAs (C_9-C_{15}) continue to increase in both populations of seals (Butt et al., 2007). PFOS concentrations in archived guillemot eggs from Sweden increased from 25 ng/g in 1968 to 614 ng/g in 2003,

with a sharp peak in 1997 (Holmstrom et al., 2005). PFOS and PFCA (C_9-C_{11}) concentrations increased in polar bear liver samples from Alaska and northern Baffin Island, with doubling times ranging from 3.6 +/- 0.9 years for PFNA and 13.1 +/- 4.0 years for PFOS from 1972 to 2002 (Smithwick et al., 2006).

1.8. Toxicokinetics

1.8.1. Tissue Distribution

PFOS is well absorbed orally and distributes in the serum and liver (Johnson et al., 1984; Seacat et al., 2003; Seacat et al., 2002). PFOS is poorly metabolized and excreted, and undergoes extensive enterohepatic circulation (Johnson et al., 1984). PFOS binds highly to beta-lipoproteins (Jones et al., 2003) and albumin and liver-fatty acid binding protein (Luebker et al., 2002).

The ratio of PFOS concentrations in liver to that in serum was calculated to be 1.3:1 for humans based on the analysis of samples collected post mortem and distributed by the International Institute for the Advancement of Medicine (Olsen et al., 2003c). PFCs were analyzed in breast milk from donors in Zhoushan, China with results indicating that low concentrations of longer chain PFCAs ($C \geq 8$), PFOS and PFHxS are present in breast milk (So et al., 2006). The transfer of PFCs from maternal to cord blood was studied and the ratio of PFOS concentrations for maternal to cord blood was calculated to be 0.32 using 15 paired samples collected in Hokkaido, Japan (Inoue et al., 2004a). PFOS, PFHxS, PFOA, perfluorononanoate (PFNA) and perfluoroundecanoate (PFUA) have been detected in seminal fluid (Guruge et al., 2005).

PFOA was found to accumulate in liver> plasma> kidney for male rats and plasma>kidney>liver>ovaries for female rats following dosing with ^{14}C PFOA (9.4 mmol/kg dosed intraperitoneally) (Vanden Heuvel et al, 1991). Subchronic PFOA exposure in Wistar rats resulted in the greatest concentration of PFOA in serum, followed by liver, kidney and lungs (Ylinen and Auriola, 1990). PFOA does not seem to be defluorinated, given that changes in fluoride concentrations were not observed in plasma or urine following dosing and only the parent

compound was present in urine samples, bile extracts and tissue extracts (Vanden Heuvel et al., 1991).

1.8.2. Elimination Rates

The half-life of PFCAs is proportional to chain length in exposed rats and excretion rates of PFOA and PFNA are lower for males than females (Ohmori et al., 2003). Differences in excretion rates of PFCAs associated with chain lengths and gender are likely due to differences in renal clearance (Ohmori et al., 2003). The half-life of PFOA was four hours in rabbits (Johnson, 1995), 20 days in monkeys (Butenhoff et al., 2004) and 3.8 years in humans (Olsen et al., 2005a). Elimination rates for PFOS have been calculated to be 100 days in rats (Johnson et al., 1979), 100 to 200 days in cynomolgous monkeys (Seacat et al., 2002) and five years in humans (Olsen et al., 2005a).

1.8.3. Toxicity

1.8.3.1. Toxicity in Humans

Few studies have addressed the effects of chronic PFOS and PFOA exposure in the general population. Residents of the Little Hocking water district in southeastern Ohio have serum PFOA concentrations that are approximately 70 times greater than those of the general American population (Emmett et al., 2006). However, no changes in serum chemistry or hematologic variables were associated with serum PFOA concentrations in this community (Emmett et al., 2006). A recent study suggests that adverse developmental effects may arise due to exposure to PFOA and PFOS *in utero* at levels found in the general North American population (Apelberg et al., 2007b). Lesser weight and head circumference were statistically significantly associated with cord blood PFOA and PFOS concentrations for newborns (Apelberg et al., 2007b).

Fluorochemical workers have serum PFOA and PFOS concentrations that exceed levels in the general population by up to two orders of magnitude; elevated PFOA concentrations have been associated with prostate cancer (Gilliland and Mandel, 1993), greater cholesterol, triglycerides and thyroid hormone (T3) levels (Olsen et

al., 2003a). Elevated PFOS exposure in fluorochemical workers was positively associated with serum cholesterol and total bilirubin, but not with clinical hepatic enzyme tests (Olsen et al., 1999).

1.8.3.2. PFCA Toxicity

1.8.3.2.1. Acute and Subchronic Toxicity

Acute oral toxicity for PFOA expressed as the LD50 has been determined to be greater than 500 mg/kg for male and female rats (Glaza, 1997) and a lowest observable adverse effect level (LOAEL) was determined to be 100 mg/kg based on peroxisome proliferation, hepatomegaly and weight loss in rats (Berthiaume and Wallace, 2002). Subchronic exposure to PFOA has been studied in cynomolgous monkeys and rats. In cynomolgous monkeys a LOAEL (liver toxicity and possible mortality) and lowest observable effect level (increased liver weight) were determined to be 3 mg/kg/day (Berthiaume and Wallace, 2002; Thomford, 2002). A no observable effect level was determined to be 0.05 mg/kg/day based on increased liver weight and hepatocellular hypertrophy observed at dosing levels of 0.5 mg/kg/day in a rat feeding trial (Perkins, 1992).

1.8.3.2.2. Developmental Toxicity

Developmental toxicity has been associated with PFOA exposure in rat and mice studies (Abbott et al., 2007; Butenhoff et al., 2004; Gortner, 1981; Lau et al., 2004; Lau et al., 2006; Lau et al., 2003; Thibodeaux et al., 2003; White et al., 2007; Wolf et al., 2007). Maternal toxicity (lower weight gain) in Sprague-Dawley rats was observed following PFOA dosing of 150 mg/kg/day and an NOAEL for developmental toxicity was determined to be 150 mg/kg/day. Greater liver weight was observed in 129S1/Svlmj WT mice pups exposed to 0.1 mg/kg/day and reduced pup survival was observed with exposure to 0.6 mg/kg/day (White et al., 2007). The study by White et al. (2007) found that maternal weight gain or loss was less sensitive to PFOA exposure than lactational development of maternal mammary glands. Normal lactational development of maternal mammary glands was altered at a dose of 5 mg/kg/day and dosing for six

days of gestation led to a reduction in neonatal growth and the prevention of female pup mammary epithelial proliferation (White et al., 2007).

1.8.3.2.3. Carcinogenicity and Immunotoxicity

PFOA exposure has been linked to tumours in rats during animal feeding trials. Agonism of the peroxisome proliferation receptor (PPAR-α) has been hypothesized as the mechanism that leads to hepatocellular tumors in rats (Biegel et al. 2001). PFOA exposure resulted in Leydig cell and hepatocellular tumours in rats (Biegel et al., 2001). PFOA exposure has been shown to affect the immune system. Significantly lower thymus and spleen weights and a reduction in the number of thymocytes and splenocytes were observed following PFOA dosing of mice (Yang et al., 2000).

1.8.3.2.5. Mechanism of Action

The effects of PFCAs on various cell lines provide meaningful information regarding their mechanisms of action on mammals. Inhibition of gap junction intercellular communication for WB-F344 rat liver epithelial cell line was associated with PFOA and PFNA exposure (Upham et al., 1998). The binding of endogenous fatty acids to liver fatty acid binding protein was inhibited by PFOA exposure in rats (Luebker et al., 2002) and a dose-dependent activation of PPAR-α was observed in isolated human plasmid cells by Tacaks and Abbot (Takacs and Abbott, 2007). The inappropriate activation of PPAR could lead to mortality or abnormal development (Takacs and Abbott, 2007). The cytotoxicty of PFCAs was demonstrated in human colon carcinoma cells (HCT116) and toxicity was proportional to alkyl residue length and incubation period (Kleszczynski et al., 2007).

1.8.3.3 Toxicity of PFOS

1.8.3.3.1. Acute and Subchronic Toxicity

The toxicity of PFOS has been studied in animal feeding trials involving cynomolgous monkeys and rats. Exposure of male Sprague-Dawley rats to 100 mg/kg PFOS led to peroxisome proliferation, weight loss and increased lauroyl

CoA oxidase enzyme activity (Berthiaume and Wallace, 2002). The effects of PFOS exposure were investigated following a 26-week intragastric dosing study (control, 0.03, 0.15, 0.75 mg/kg/day) of cynomolgus monkeys (Seacat et al., 2002). Gender differences were observed for effects of PFOS exposure; blood cholesterol and estradiol concentrations were statistically significantly lower in males from the highest dose group (0.75 mg/kg/day) than females. Higher TSH and lower T3 were observed for the highest dose group and peroxisome proliferation (palmitoyl CoA oxidase activity) was greatest in females from the highest dose group (below level of biological significance). Lipid-droplet accumulation, centrilobular vacuolation, hypertrophy, mild bile stasis and increased glycogen status were observed in certain individuals of the highest dose group. NOAEL was determined to be 0.15 mg/kg/day in cynomolgus monkeys (Seacat et al., 2002).

1.8.3.3.2. Developmental Toxicity

PFOS exposure was associated with developmental effects following dosing of Sprague-Dawley rats throughout gestation at the following rates: 4 days at 25 mg/kg/day (during different gestational periods) or 0, 25 or 50 mg/kg/day on gestational days (GD) 19 and 20 (Grasty et al., 2003). Dam weight was reduced in all 4-day dosing treatment groups and pup survival was lower in all treated groups compared to controls (Grasty et al., 2003). Dosing later in gestation was associated with a reduction in pup survival and mortality was highest during the first 24 hours after birth. Pups dosed during GD 17-20 experienced nearly one hundred percent mortality within 24 hours of birth and the pups from the earliest three treatment groups had reduced weights. Weight-gain was suppressed in dams following 2-day dosing on GD 19-20 and live litter size and pup weight were lower in both treated groups. The post-natal lungs of dosed animals were similar to the prenatal lungs of control animals

1.8.3.3.3. Mechanism of Action

The effect of PFOS on gap-junction intercellular communication (GJIC) was studied in WB-F344 rat liver epithelial cell line culture and dolphin kidney cell

line culture (Hu et al., 2002). The NOEL for GJIC inhibition was determined to be 3.1 μg/gl for rat and dolphin liver (Hu et al., 2002). PFOS exposure inhibited DAUDA-L-FABP binding *in vitro*, which could cause the displacement of endogenous fatty acids and lead to the activation of PPAR (Luebker et al., 2002).

Chapter 2: Estimation of Dietary Exposure to Perfluorooctanesulfonate, Perfluorinated Carboxylates and Fluorotelomer Unsaturated Carboxylates in the Canadian Population

Manuscript Title:

Estimation of Dietary Exposure to Perfluorooctanesulfonate, Perfluorinated Carboxylates and Fluorotelomer Unsaturated Carboxylates in the Canadian Population

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Abstract

Dietary exposure to perfluorooctane sulfonate and perfluorinated carboxylates (C_7-C_{11}) was estimated for the Canadian population prior to the phase-out of perfluorooctyl-sulfonyl production by 3M and the voluntary reduction in PFOA emissions and product content. Perfluorinated compounds were measured in 65 archived composite food samples prepared for the 1998 Canadian Total Diet Study using a newly developed methanol extraction combined with a solid phase extraction clean up. PFCs were detected in eight samples including processed meats, pre-prepared foods and peppers with a range of concentrations from 0.48 to 5.01 ng/g wet weight. Dietary exposure was estimated for a sample of 326 people using dietary data collected in 1998. Mean exposure to PFCs was estimated for Canadian men and women between the ages of 13 and 65 and ranged from 2 to 59 ng/person/day. There were no statistically significant differences in mean PFC exposure levels for different age and gender groups. Total PFCAs were found to contribute the greatest quantity to PFC exposure followed by PFOS; FTUCAs contributed less than five percent to dietary exposure. Dietary exposure to PFCs was associated with the consumption cakes and cookies, processed cheese, and regular cheese. These levels pose minimal health risks based on current toxicological information.

Introduction

The production and use of perfluorinated compounds (PFCs) over the last fifty years has led to the worldwide contamination of humans, oceans, surface water, air, fish and wildlife with perfluorinated carboxylates (PFCAs), sulfonates, telomer alcohols and perfluoroalkylsulfonamides (PFOSAs). PFCs have been detected in non-occupationally exposed men and women worldwide (Calafat et al., 2006b; Kubwabo et al., 2004; Olsen et al., 2005c; Kannan et al., 2004; Inoue et al., 2004a; Taniyasu et al., 2003). Although human exposure to PFCs has been established for urban and rural populations in both industrialized and non-industrialized countries, the sources of exposure have not been fully elucidated.

The effects of chronic exposure to PFCs are not well understood. Adverse health effects or statistically significant changes in serum biochemistry and haematological variables were not observed in individuals with elevated serum PFOA levels from southeastern Ohio (Emmett et al., 2006). However, reduced weight and smaller head circumference were statistically significantly associated with the concentration of PFOA and PFOS in cord blood of newborns in Baltimore, Maryland (Apelberg et al., 2007b). Elevated exposure to PFCs compared to the general North American population has been observed in fluorochemical workers and has been associated with prostate cancer (Gilliland and Mandel, 1993), higher cholesterol, triglycerides, higher bilirubin (Olsen et al., 1999) and thyroid (T3) levels (Olsen et al., 2003a). Subchronic exposure to PFOA is associated with liver toxicity, increased liver weight and developmental toxicities in laboratory feeding trials (Abbott et al., 2007; Butenhoff et al., 2002; Butenhoff et al., 2004; Thomford, 2002; White et al., 2007). Exposure to PFOS was associated with reduced weight, greater liver weight, reduced serum total cholesterol and changes in thyroid stimulating hormone (TSH) and thyroid hormone (T3) in cynomolgous monkeys (Seacat et al., 2002).

A national contaminant exposure program does not exist in Canada, although Health Canada carries out an annual Total Diet Study to monitor contaminant levels in the Canadian food supply. Studies on sources of PFC exposure are limited in Canada and worldwide, however a recent study estimated that the exposure of Canadian adults to PFCs is largely due to the consumption of contaminated foods (Tittlemier et al., 2007). Dietary exposure to PFCs has been estimated to range from 4 ng/kg body weight/day (Tittlemier et al., 2007) to 100 ng/kg body weight/day (Mortimer et al., 2006). However, previous studies of PFCs in Canadian foods have been limited to fast foods and animal products (Tittlemier et al., 2006; Tittlemier et al., 2007) and dietary exposure estimates for Canadians relied on a dietary intake survey conducted from 1970 to 1972 (Directorate, 1981). Recently, dietary intake data were collected in the form of 24-hour recalls to establish current dietary habits of Canadians (Starkey et al.,

2001). The results from this study indicated that changes in diet have occurred since 1970 (Gray-Donald et al., 2000).

In this paper, we present the results from the analysis of PFCs in archived composite samples of store-bought and restaurant foods and estimate daily dietary exposure for the general Canadian population in the late 1990s. Nine fluorinated compounds were analyzed in archived food samples collected in Whitehorse, Yukon Territory, Canada in 1998 for the Total Diet Study conducted by Health Canada. The analysis of archived food samples provides a historical perspective on the presence of PFCAs, PFOS and FTUCAs in market foods and associated dietary exposure. The objective is to verify whether diet is indeed an important source of PFCs for humans. These results will also provide valuable data for reducing dietary exposure to PFCs and monitoring changes in dietary exposure over time.

Materials and Methods

Standards and Reagents. The following perfluorinated and fluorotelomer compounds (purity >95%) were used as standards: perfluoroheptanoic acid (Aldrich, Oakville, ON, Canada), perfluorooctanoic acid (Wellington Laboratories, Guelph, ON, Canada), perfluorononanoic acid (Aldrich), perfluorodecanoic acid (Aldrich), perfluoroundecanoic acid (Aldrich), perfluorododecanoic acid (Aldrich), perfluorotetradecanoic acid (Aldrich), L-perfluorooctane sulfonate (Wellington), 2H-perfluoro-2-dodecenoic acid (Wellington), 2H-perfluoro-2-decenoic acid (Wellington) and 2H-perfluoro-2-octenoic acid (Wellington). The following mass-labelled perfluorinated and fluorotelomer compounds were used as recovery and internal performance standards: 1,2-^{13}C perfluorooctanoic acid (Perkin Elmer, Boston, MA, USA; 98% chemical purity, 99% isotopic purity), perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid (Wellington), 1,2-^{13}C perfluorononanoic acid (3M, 95% chemical purity, 99% isotopic purity), $^{13}C_5$ perfluorononanoic acid (Wellington), 1,2-^{13}C perfluorodecanoic acid (Wellington Laboratories, 98% chemical purity, > 99% isotopic purity), sodium 1,2,3,4-^{13}C perfluorooctane

sulfonate (Wellington, 98% chemical purity, > 99% isotopic purity), L-$^{18}O_2$ PFOS (RTI International, Research Triangle Park, NC, USA), 2H-perfluoro-[1,2-$^{13}C_2$]-2-octenoic acid (Wellington), 2H-perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid (Wellington) and 2H-perfluoro-[1,2-$^{13}C_2$]-2-dodecenoic acid (Wellington).

Given that it was not feasible to prepare matrix-specific calibration curves for the 65 food samples analyzed, matrix effects on ionization were accounted for through the use of instrument performance standards. The instrument performance standards that were used to correct for matrix-effects are given in table 1.

All water used in the method was Milli-Q purified (Millipore, Billerica, MA, USA) and passed through a glass column containing Amberlite XAD-7 resin (Aldrich) to remove any possible perfluorinated contaminants. Methanol (MeOH; Optima, FisherScientific), ammonium hydroxide (Baker analyzed, 29% purity), glacial acetic acid (FisherScientific HPLC grade) and anhydrous sodium acetate were used without extra purification. Weak anion exchange (WAX) cartridges (Oasis®Wax, 6 cc, 150 mg, 30 µm) solid-phase extraction (SPE) cartridges were purchased from Waters (Milford, MA).

Market Food Samples. Composite samples of store-bought and restaurant foods were prepared by Health Canada for the Total Diet Study (TDS) in 1998. Food samples were collected in 1998 from supermarkets and restaurants in Whitehorse, Yukon Territory. Food items were purchased from four grocery stores and restaurants and were prepared as for consumption. Composite food samples were created by combining and homogenizing individual replicate food samples. All samples selected for this study were stored in chemically cleaned polypropylene Nalgene™ containers and lids at –20°C following homogenization. The food samples analyzed correspond to foods consumed by Canadians based on 24-hour recalls carried out in the 1998 Canadian food habits study (Starkey et al., 2001). In total, 65 archived composite samples from the 1998 Health Canada Total Diet

Study were analyzed and the description of the samples analyzed is presented in table 2. Composite samples from various food grouping were analyzed: drinks (n=4), plant-based (n=24), meat and fish (n=10), milk products (n=7), fats and oils (n=2), fast foods and prepared meals (n=16) and sugar (n=2).

Dietary Exposure Estimate and Statistical Analysis. Dietary exposure to PFCs was estimated using food intake data collected in 1998 for the 'Canadian Food Habits' study (Starkey et al., 2001). Food intake data from 24-hour recalls were organized into 96 food groups based on similarities in nutrient content. We matched the food samples analyzed with the food groups based on the contents of each food group to estimate dietary exposure. The concentrations of PFCs in the food groups consumed were multiplied by the quantities of these foods consumed based on 24- hour recalls. Concentrations below the LOD were entered as zero. Daily dietary exposure was estimated for four age categories for both men and women (13-19, 20-40, 41-60, older than 61 years old) based on dietary intake information for 326 individuals. A one-way ANOVA was carried out to identify whether age and gender were statistically significantly associated with PFC exposure. All statistical analyses were performed using SAS v 9.1.3.

Analytical Method. The composite samples were thawed at room temperature and one gram cooked food or two grams raw food (liquid or solid) were taken and placed in methanol-rinsed polypropylene centrifuge tubes. The difference in sample aliquot size was due to the poor yield of supernatant for 2g of cooked (i.e. low moisture) samples following methanol extraction (data not shown). The extraction of PFCAs (C_7-C_{11}), PFOS and FTUCAs was carried out with a methanol extraction modified from the method described by Tittlemier et al. (2005).

Each sample was spiked with 5000 pg recovery standard (50 µL of 100 pg/µL solution made up of $^{13}C_4$PFOA, $^{13}C_2$ PFNA and $^{13}C_4$ PFOS) and 4 mL methanol was added immediately to all samples except liquid samples. Internal recovery

standard was added to liquid samples immediately prior to freeze-drying for six hours (Flexi-Dry MP microprocessor controlled bench top lyophilizer; FTS Systems, Inc., Stone Ridge, NY.). Following freeze-drying, 4 mL methanol were added to the samples and the methanol extraction took place. Tubes were capped, vortexed and placed on an orbital shaker at 200 rpm, 25°C for 4 hours. The tubes were vortexed and centrifuged at 667 *x* g, 10°C for 10 minutes. The supernatant was transferred to pre-cleaned polypropylene centrifuge tubes and two more extractions were carried out under the following conditions: 2 mL methanol were added to the sample, vortexed and placed on the orbital shaker for 10 minutes and centrifuged (same conditions as above). The supernatants were combined, vortexed and dried to 0.5 mL under a gentle stream of nitrogen gas in a water bath at 37°C.

Clean-up involved a SPE step modified from a method previously described (Taniyasu et al., 2005). Samples were diluted to 50 mL with Milli-Q water immediately prior to SPE. The WAX cartridges were conditioned with 4 mL 0.1% NH_4OH in methanol, 4 mL methanol and 4 mL water, at a rate of 2 drops per second. The samples were loaded and passed through the cartridge at a rate of 1 drop per second. 4 mL of 25 mM sodium acetate buffer (pH 4) were passed through the cartridge prior to elution of analytes to remove biomolecules and lipids and to improve adsorption of target compounds (Taniyasu et al., 2005). Elution was carried out with 4 mL methanol followed by 4 mL 0.1% NH_4OH in methanol at a rate of 1 drop per second. The eluate was dried to 0.5 mL under a gentle stream of nitrogen. The eluate was vortexed, a 250 µL aliquot was taken and internal performance standard (20 µL of a 100 pg/µL solution) and 230 µL water were added. Samples were mixed on a vortex mixer and centrifuged at 2200 *x g* for ten minutes. Approximately 450 µL of sample was then transferred to a polypropylene autosampler vial, capped and stored at 4°C until analysis.

Instrumental Analysis. Samples were analyzed using liquid chromatography negative electrospray tandem mass spectrometry (LC-MS/MS). Samples (10 uL

injection) were chromatographed at ambient temperatures on a 2.1 x 50 mm Genesis C_{18} analytical column (Jones Chromatography Ltd., Hengoed, Mid Glamorgan, UK) and C_{18} guard column (4 mm x 2.0 mm i.d.; Phenomenax, Torrance, CA) installed on an HP 1100 binary pump high performance liquid chromatograph (Agilent, Palo Alto, CA). The mobile phase solutions were 5 mM ammonium formate in Barnstead Diamond water (18 MΩ*cm) (solution A) and a 1:1 (v/v) solution of acetonitrile/methanol (solvent B). The perfluorinated compounds were chromatographically resolved using the following gradient program: 40% B at 0.200 ml /min for one minute, 45% B over 4 min, increasing to 70% B over 8 minutes, 75% B over 2 minutes, and 95% B over 4 minutes and then held at 95% B for 0.1 minutes. The column was then flushed with 40% B for 10.9 minutes. The liquid chromatograph was connected to a VG Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK).

Analysis of PFCs in samples was carried out in the multiple reaction-monitoring mode. Analytes and the transitions monitored are provided in table 1: two transitions were monitored for all native analytes (except PFHpA) and one mass-transition was monitored for the mass-labelled standards (internal and recovery standards). Three chromatographic windows were utilized to separate transitions in the multiple reaction-monitoring program. Operational parameters for the mass spectrometer were as follows: capillary voltage 2.0 kV, source temperature 140°C, and nebulizer and drying gas (N_2) flow rates 20 L/h and 350 L/h, respectively. The collision gas was nitrogen at 2.0 x 10^{-3} mbar and the mass resolution was set at 1.2 mass units at the base for both mass analyzers.

Quantitation. Target analytes were considered to be positively identified if their retention time was within 2.5 percent of the standard retention time, the signal to noise ratio was greater than three and the confirmation mass transition was present (when applicable). Relative response factors were calculated as a ratio of the peak area of the target analyte and that of the corresponding mass-labelled internal performance standard (indicated in table 1). Quantitation was based on

response factors of an external five-point calibration curve made up in methanol and water (1:1 ratio). The curve for each analyte consistently had an r^2 value > 0.98 for all analytes of interest. Concentrations were not recovery-corrected.

The instrument detection limit was estimated for each sample as the concentration for which the corresponding peak had a signal-to-noise ratio of 3. Method detection limits (MDL) were considered as three times the standard deviation of the blanks plus the instrument detection limit (Smithwick et al., 2006) divided by the weight of sample. If the blank was below the instrument detection limit, the MDL was estimated as three times the standard deviation of the lowest concentration standard of the calibration curve (Gomez-Taylor et al., 2003). The limit of quantification was determined as three times the detection limit.

Method Validation. Vegetable soup (n=3), butter (n=3), pizza (n=2), white bread (n=2), sugar (n=2), and arctic char (n=3) were fortified with target analytes (50uL of a 100pg/uL solution) to a concentration of 2.5 ng/g. Fresh beef liver (n=3) was spiked with target analytes (250 uL of a 100 pg/uL solution) to produce a concentration of 12.5 ng/g. Target analytes are given in table 1. The extraction of target analytes and clean-up steps were carried out as described above to determine recoveries for various food matrices.

Quality Assurance and Quality Control. Quality assurance and control steps included methanol laboratory blanks, fortified matrix samples and the addition of mass-labelled recovery internal standards and internal performance standards to each sample. One methanol blank and one homogenized raw ground beef sample fortified with target analytes (50 µL of a 100 pg/µL standard solution) were included in each batch of samples. Blank-subtraction of the samples was carried out to account for the presence of analytes in methanol blanks.

Results

Method Recoveries. Recoveries were consistently greater than 75 percent for most analytes and matrices. Recoveries of PFUA, PFDoDA and PFTeDA were less consistent and mean recoveries were less than 50 percent for most matrices. The three FTUCAs had lower recoveries for the freeze-dried soup samples, than the other foods analyzed. Method recoveries from nine PFC-fortified foods are given in table 3.

Quality Control and Quality Assurance. 10:2 FTUCA, PFDA and PFUA were not detected in the blanks. Concentrations of PFCs detected in methanol blanks are given in table 4. Levels in blanks often exceeded ten percent of the concentration detected in the samples therefore sample concentrations were blank-subtracted. Recovery of PFCs in the fortified beef reference material (n=6) was: 63±6 (PFHpA), 76±12 (PFOA), 81±25 (PFNA), 40±43 (PFDA), 6±11 (PFUA), 54±13 (PFOS), 53±29 (6:2 FTUCA), 57±36 (8:2 FTUCA) and 53±11 (10:2 FTUCA). Average recoveries (± standard deviation) for the three internal recovery standards in samples were 84 ± 20% (n=73), 77 ± 29% (n=73) and 81 ± 23 (n=73) for $^{13}C_4$PFOA, $^{13}C_2$ PFNA and $^{13}C_4$ PFOS respectively

Concentrations in market foods. PFCs were detected in 8 of the 65 composite samples analyzed. The concentrations of individual PFCs detected in the 8 food samples are provided in table 5. In general, PFCs were detected in foods that had undergone processing or were pre-packaged (e.g. luncheon meats, cookies, and pizza). Very few unprocessed foods contained detectable levels of PFCs, although the pepper composite sample contained measurable concentrations of PFOA and PFDA. PFCs were below the method detection limit in all unprocessed meats, breads, cereals, fruits and beverages.

The concentration of PFCs was below the limit of quantitation in all composite samples with the exception of PFOS in processed cheese. PFHpA and PFOA were detected most frequently (4 and 5 samples respectively). PFOS was detected

in 2 samples and the concentration only exceeded the limit of quantitation in processed cheese (1.14 ng/g). PFOS was detected in processed cheese and regular cheese, but not in the other dairy samples analyzed (i.e. milk, ice cream, cottage cheese or butter).

Longer-chained PFCAs (C_9-C_{11}) were detected infrequently in composite samples. PFNA was detected in cold cuts and cookies and PFDA was detected in peppers. PFUA was not detected in any samples analyzed. 8:2 and 10:2 FTUCAs were not detected in any samples and 6:2 FTUCA was only measurable in the cold cuts and luncheon meats composite samples. 6:2 FTUCA was detected above instrument detection limit in wieners and chicken, but these levels were below the method detection limits.

Dietary Exposure Estimates. There were no statistically significant differences in mean PFC exposure levels for different age and gender groups. Dietary exposure estimates are given in figure 1 for the general Canadian population based on current food habits of Canadians (Gray-Donald et al., 2000). Mean dietary exposure estimates ranged from 2 ng/day (females 61-65 years old) to 59 ng/day (males, 61-65 years old). All age-gender groups were exposed to PFCAs and PFOS. Total PFCAs were found to contribute the greatest quantity of PFCs in both men and women followed by PFOS; FTUCAs contributed less than five percent to dietary exposure. The contribution of individual PFCAs differed for the various age and gender groups, due to different sources of dietary exposure.

Dietary sources of exposure varied somewhat between age and gender groups. Given that very few composite samples were found to contain measurable levels of PFCs, dietary exposure to PFCs was associated with the consumption of only a few foods, namely cakes and cookies, processed cheese, and natural cheese. Different sources of dietary exposure for Canadians are illustrated in figures 2 and 3. Sources of exposure differed more between age groups in females than males, although processed cheese was consistently an important source of dietary

exposure to PFCs for all Canadians (45 to 73 percent of daily exposure). The low daily dietary exposure to PFCs in the oldest women (mean daily exposure 2.4 ± 2.1 ng/day) was associated with the consumption of small quantities of only a few contaminated foods (beef and processed cheese).

Discussion

Levels of PFCs in store-bought foods. Few foods contained PFCs and unprocessed foods were generally free of PFCs. PFCs were most frequently detected in foods that had undergone a form of processing, including industrial preparation (e.g. cookies) or mixing and cooking (e.g. lunch meats, processed cheese). The concentrations of PFCs in samples were very low with total concentrations equal to or less than 5 ng/g in all samples analyzed. Some foods that contributed to dietary exposure in the 2004 Canadian TDS samples (e.g. ground beef, roast beef, beef steak and fish) contained higher levels of PFCs than the 1998 composite samples analyzed in this study (Tittlemier et al., 2007). Mortimer et al. (2006) detected PFOS and PFOA in composite samples that were free of PFCs in our study (e.g. potatoes, eggs, sugar and preserves and canned vegetables). The concentrations of PFCs detected in the UK and Canadian Total Diet Study samples (Mortimer et al., 2006; Tittlemier et al., 2007) were within the range of levels detected in the 1998 Canadian TDS samples analyzed in this study.

Sources of PFCs in 1998 Composite Samples. In general, PFCs were detected in processed foods, suggesting contamination occurs following the initial production or cultivation of various foods. PFCs were not detected in milk, unprocessed meat, beverages and plant products excluding peppers. Some PFCs and PFC-precursors have been shown to migrate from packaging into foods, due to the use of fluorinated grease and water repellent coatings on food packaging (Begley et al., 2005; Sinclair et al., 2007; Tittlemier et al., 2006).

These results are the first reported results of FTUCAs in the food chain and indicate the need for further study of this group of compounds in humans and the identification of the source of these chemicals in foods. To our knowledge, PFCs have not been analyzed in fluorinated cleaning products or food grade lubricants, although 6:2 fluototelomer alcohol was detected in Zonyl ® FSE and other fluorinated materials (Dinglasan-Panlilio and Mabury, 2006). Further study of the presence of PFCAs and FTUCAs in products used by the food industry is warranted given the presence of PFCs in a variety of processed foods.

The detection of PFOA and PFDA in the pepper composite sample suggests that contamination occurred during the cultivation of the peppers, since there is no processing and little packaging of these fruits. PFCAs have been detected in agricultural areas from the application of contaminated sewage sludge (Skutlarek et al., 2006), which could offer some explanation of the contamination observed in pepper. Other possible sources of contamination may be PFCAs in rainwater (Loewen et al., 2005) or the use of contaminated surface water in irrigation. However, the lack of broad contamination of fruits and vegetables with PFCAs suggests that there was a point source of contamination of peppers in the composite sample.

PFOS was only detected in the two cheese samples analyzed but was not detected in any other dairy products. These data suggest that contamination of cheese may have occurred during cheese-production or that PFOS is present in milk but only becomes detectable in cheese when the protein in milk becomes more concentrated.

Dietary Exposure. In general, few foods were responsible for direct dietary exposure to PFCs in this study and these were primarily processed cheese, cakes and cookies and cheese. The mean exposure estimates from this study are lower than the range of exposure estimates derived for the previous Canadian and UK dietary exposure studies (Mortimer et al., 2006; Tittlemier et al., 2007). The

exposure estimates ranged from 1 to 70 ng/kg body weight/day for PFOA and 10 to 100 ng/kg body weight/day for PFOS in the UK study (Mortimer et al., 2006). Our dietary exposure estimates for PFCs were below one ng/kg body weight/day, which is much lower than estimates from the UK exposure study (Mortimer et al., 2006), which is likely due in part to the elevated PFOS and PFOA concentrations measured in the UK potato composite sample. Given that PFCs were not detected in 1998 beef or pork samples purchased in Whitehorse, Yukon Territory, the difference in dietary exposure between 1998 and 2004 is certainly linked to the presence of PFCs in beef samples from 2004 (Tittlemier et al., 2007).

There are limitations in comparing dietary exposure to PFCs from 1998 to 2004, given that the 2004 estimate by Tittlemier et al. (2007) is based on the analysis of fewer samples than this study and uses dietary intake information collected in 1970. However, a direct comparison of dietary exposure estimates suggests that dietary exposure to PFCAs and PFOS may have increased slightly over time. Future monitoring of PFCs in food should include processed foods and cheese to determine if contamination of these foods continues.

We anticipate that the phase-out of perflurooctyl sulfonyl compounds will result in a reduction of sulfonyl-based perfluorinated compounds in store-bought foods, which is supported by the study of perfluorooctanesulfonamides in fast food and packaged foods (Tittlemier et al., 2006). A decreasing trend of N-EtPFOSA levels was observed for fast-foods between 1992 and 2004; however, PFOS was detected in various samples in the 2004 Total Diet Studies in the UK and Canada that were not found to contain PFCs in 1998 (Mortimer et al., 2006; Tittlemier et al., 2007). The presence of PFOS in food samples after the phase-out of POSF production may reflect persistence and bioaccumulation of PFOS in the environment.

Risk Assesment. The World Health Organization and Health Canada have not established guideline levels for chronic PFOS and PFCA exposure; therefore we

compared our exposure estimates to benchmark dose levels and lowest observable effects levels from animal feeding trials. The most appropriate BMDL10 (dose calculated to elicit a response in ten percent of the study population) for PFOA was determined to be 0.6 mg/kg/day based on greater liver weight in rats from a two-generational PFOA feeding study (Butenhoff et al., 2004). This BMDL10 was used for assessing risk associated with PFCA and FTUCA exposure due to the lack of feeding trial data for the individual analytes. The most sensitive endpoints established for PFOS exposure were changes in blood lipid profiles in cynomolgous monkeys and the LOEL was determined to be 0.03 mg/kg body weight/day (Thomford, 2002). A safety factor of 10000 was applied to the BMDL10 and LOEL to assess human health risks associated with PFC exposure estimates for Canadians.

Estimated dietary exposures to PFCs were below 1 ng/kg body weight/day for men and women in all age groups (average body weights of 64 kg for women and 79 kg for men). Mean exposure to PFCAs and FTUCAs ranged from 0.0 to 0.8 and exposure to PFOS ranged from 0.0 to 0.2 ng/kg body weight/day. These exposure levels are not expected to pose a health risk to humans based on the available toxicological data (Butenhoff et al., 2004; Thomford, 2002). However, large interspecies variation in excretion rates for PFCs have been observed (Johnson et al., 1979; Olsen et al., 2005b; Seacat et al., 2002), therefore the use of internal dose data rather than exposure data would reduce some uncertainty associated with extrapolating data from animal feeding trials for human health risk assessments.

A recent study found a small, but statistically significant association between cord blood PFOS and PFOA concentrations and decreased birth weights and head circumference in newborn babies (Apelberg et al., 2007b). These data suggest that current benchmark doses for PFOS and PFOA exposure may not be appropriate for assessing risk, especially for adverse developmental outcomes. There is a lack of monitoring data for PFC concentrations in the Canadian population; only one

small study has been completed to date to measure PFOS, PFOA and PFOSA in serum (Kubwabo et al., 2004). Although exposure levels estimated in this study are not considered to pose a health risk for the Canadian population, future monitoring of PFCs in serum will provide valuable data for reducing the uncertainty associated with assessing the public health risk associated with PFC exposure. Given the long half-lives of PFCs and uncertainty in human health impacts of chronic PFC exposure, we recommend the continued monitoring of PFCs and PAPs in market foods to determine if current regulations will result in a reduction in dietary exposure to PFCs.

Tables and Figures

Table 1. Instrument performance standards used to account for matrix effects for each analyte and MS/MS Multiple Reaction Monitoring Parameters are provided. For analytes in which two transitions were monitored the first transition listed was used for quantitation.

Analyte	Performance Standard	Mass Transition	Cone voltage	Collision energy
Perfluoroheptanoate (PFHpA)	1,2-^{13}C perfluorooctanoate	362.9 → 318.8	10	15
Perfluorooctanoate (PFOA)	1,2-^{13}C perfluorooctanoate	412.9 → 368.8 412.9 → 168.8	10	15
Perfluorononanoate (PFNA)	1,2,3,4,5-C^{13} perfluorononanoate	462.8 → 418.8 462.8 → 218.8	12 16	18 18
Perfluorodecenoate (PFDA)	1,2-^{13}C perfluorodecanoate	512.8 → 468.9 512.8 → 218.9	15 15	18 18
Perfluoroundecanoate (PFUA)	1,2-^{13}C perfluorodecanoate	562.9 → 518.9 562.9 → 268.8	15	15
Perfluorooctane sulfonate (PFOS)	L-$^{18}O_2$ perfluorooctane sulfonate	498.9 → 98.9 498.9 → 79.9	50	60
6:2 Fluorotelomer unsaturated carboxylate (6:2 FTUCA)	2H-Perfluoro-[1,2-$^{13}C_2$]-2-octenoate	356.9 → 292.9	15	17
8:2 Fluorotelomer unsaturated carboxylate (8:2 FTUCA)	2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoate	457 → 392.9	18	20
10:2 Fluorotelomer unsaturated carboxylate (10:2 FTUCA)	2H-Perfluoro-[1,2-$^{13}C_2$]-2-dodecenoate	557 → 493	20	25

Table 2. Summary of composite food samples analyzed for PFCs and FTUCAs. All samples were purchased in Whitehorse, Yukon Territory in 1998 for the Health Canada Total Diet Study.

Food Group	Composite Samples Analyzed for PFCs (N = 65)
Meat, Poultry, Fish, Eggs (N = 9)	Cold cuts and luncheon meats, luncheon meats (canned), canned fish, eggs, beef (steak), beef (roast and stewing), beef (ground), pork, wieners
Dairy (N = 8)	Cheese (processed cheddar), cheese, ice cream, cottage cheese, butter, milk (whole), milk (2%), evaporated milk (canned)
Fast Foods and Pre-prepared Foods (N = 21)	Pizza, French fries, fish burger, chicken, cookies, frozen dinner, frozen dinner (microwave or oven), pasta (plain), pasta (mixed dishes), jams, danish and donuts, potato chips, candy and chocolate bars, baked beans, cooking fats and salad oils, soups (tomato, canned), soups (meat, canned), soups (cream of vegetable, canned), soups (dehydrated), apple pie, pancakes
Fruits, Vegetables, Grains, Mushrooms (N = 20)	Bread (whole wheat), tomato sauce, cauliflower, mushrooms (canned), cabbage, peppers, corn, peas, rice, citrus fruit (raw), lettuce, potatoes (boiled), potatoes (baked), cereal (cooked wheat), cereal (wheat and bran), cereal (oatmeal), cereal (corn), crackers, wheat flour, white sugar
Beverages (N = 7)	Citrus juice (canned), alcoholic drinks (beer), citrus juice (frozen), apple juice (canned, unsweetened), soft drinks, coffee, tea

Table 3. Mean percent recoveries (± standard deviation) for eight pre-extraction fortified market foods. All foods except liver were fortified at 5 ng/g; liver was fortified at 25 ng/g.

Food / Analyte	Vegetable Soup (n=3)	White Bread (n=2)	White Sugar (n=2)	Butter (n=3)	Pizza (n=2)	Raw Ground Beef (n=2)	Arctic Char (n=3)	Liver (n=3)
6:2 FTUCA	43 ± 3	66 ± 2	71 ± 10	126 ± 47	85 ± 24	60 ± 26	66 ± 1	nd
8:2 FTUCA	50 ± 1	68 ± 12	76 ± 4	95 ± 10	77 ± 26	65 ±10	61 ± 3	79 ± 3
10:2 FTUCA	44 ± 15	74 ± 27	67 ± 15	85 ± 4	126 ± 67	63 ± 20	74 ± 7	88 ± 21
PFOS	82 ± 5	76 ± 17	78 ± 5	163 ± 25	113 ± 8	60 ± 13	62 ± 3	81 ± 8
PFHpA	86 ± 7	nd	85 ± 17	121 ± 35	95 ± 16	93 ± 10	72 ± 8	100 ± 12
PFOA	81 ± 6	84 ± 7	na	107 ± 15	88 ± 33	82 ± 13	82 ± 4	129 ± 8
PFNA	98 ± 6	79 ± 14	78 ± 4	95 ± 5	76 ± 4	59 ± 6	70 ± 5	86 ± 12
PFDA	89 ± 27	80 ± 20	84 ± 11	99 ± 7	78 ± 18	72 ± 38	69 ± 3	92 ± 8
PFUA	34 ± 6	48 ± 4	79 ± 11	59 ± 19	nd	75 ± 26	120 ± 34	59 ± 16

na = analyte not spiked into sample

nd = analyte not detected

Table 4. Frequency of detection and concentration of analytes in methanol blanks converted from pg/uL to ng/g for a hypothetical 2 gram sample. Mean method detection limits (± standard deviation) for analytes (mass-labelled and native compounds).

Analyte	No. Detected/ No. Analyzed	Mean Concentration (ng/g)	Standard Deviation	Method Detection Limit
6:2 FTUCA	1/7	nd	0	0.27± 0.16
PFHpA	4/7	0.10	0.05	0.35 ± 0.10
PFOA	1/7	0.02	0.03	0.23 ± 0.10
PFNA	4/7	0.32	0.23	0.97 ± 0.31
8:2 FTUCA	1/7	0.01	0.01	0.05 ± 0.02
PFOS	4/7	0.05	0.03	0.21 ± 0.10
PFDA	1/7	0.06	0.08	0.50 ± 0.15
PFUA	0/7	nd	0	0.44 ± 0.15
10:2 FTUCA	0/7	nd	0	0.54 ± 0.49

Table 5. Blank corrected concentrations (ng/g, wet weight) of PFCs detected in Canadian Total Study composite food samples. Values below the LOQ but above the limit of detection are followed by the LOQ in parentheses.

Description	6:2 FTUCA	PFHpA	PFOA	PFNA	PFOS	PFDA	Total
Cold Cuts	1.26 (3.72)	nd	nd	3.75 (5.65)	nd	nd	5.01
Cookies	nd	0.59 (1.05)	0.36 (0.76)	1.72 (2.74)	nd	nd	2.66
Cheese (processed)	nd	0.55 (1.11)	0.43 (1.09)	nd	1.14	nd	2.12
Peppers	nd	nd	0.77 (1.52)	nd	nd	1.02 (1.33)	1.79
Lunch Meats (canned)	nd	nd	0.52 (1.45)	nd	nd	nd	0.52
Pizza	nd	0.31 (0.90)	0.42 (0.65)	nd	nd	nd	0.73
Cheese	nd	nd	nd	nd	0.71 (0.95)	nd	0.71
Frozen Dinner (beef)	nd	0.48 (1.30)	nd	nd	nd	nd	0.48

Table 6. Estimated mean daily intake of perfluorinated carboxylates, perfluorooctane sulfonate and fluorotelomer unsaturated carboxylates (ng/day) for Canadian males and females in 1998.

Gender/ Age / Analyte	Female				Male			
	13-19 (n=16)	**20-40 (n=80)**	**41-60 (n=112)**	**61-65 (n=13)**	**13-19 (n=17)**	**20-40 (n=31)**	**41-60 (n=44)**	**61-65 (n=13)**
PFHpA	6 ± 13	6 ± 26	12 ± 24	2 ± 6	9 ± 16	13 ± 34	11 ± 32	41 ± 41
PFOA	6 ± 12	4 ± 18	9 ± 22	0 ± 1	5 ± 12	8 ± 23	7 ± 21	10 ± 14
PFNA	0 ± 0	4 ± 30	11 ± 49	0 ± 0	0 ± 0	23 ± 88	19 ± 87	29 ± 105
6:2 FTUCA	0 ± 0	0 ± 0	1 ± 5	0 ± 0	0 ± 0	0 ± 0	1 ± 8	0 ± 0
PFOS	12 ± 23	13 ± 40	16 ± 30	1 ± 2.7	16 ± 32	11 ± 36	14 ± 33	7 ± 14

Figure 1. Mean dietary exposure (± standard error) to PFCs and FTUCAs for Canadians.

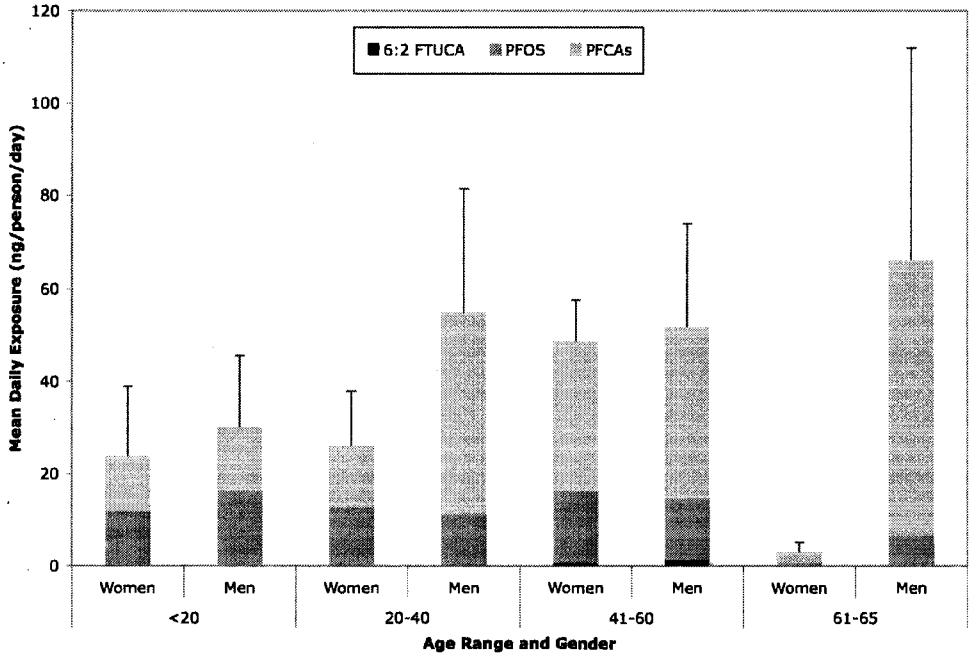

6:2 FTUCA
PFOS
PFCAs
Mean Daily Exposure (ng/person/day)
120
100
80
60
40
20
0
Women
Men
Women
Men
Women
Men
Women
Men
<20
20-40
41-60
61-65
Age Range and Gender

Figure 2. Sources of dietary exposure to PFCs and FTUCAs for Canadian women as percent of mean daily dietary exposure.

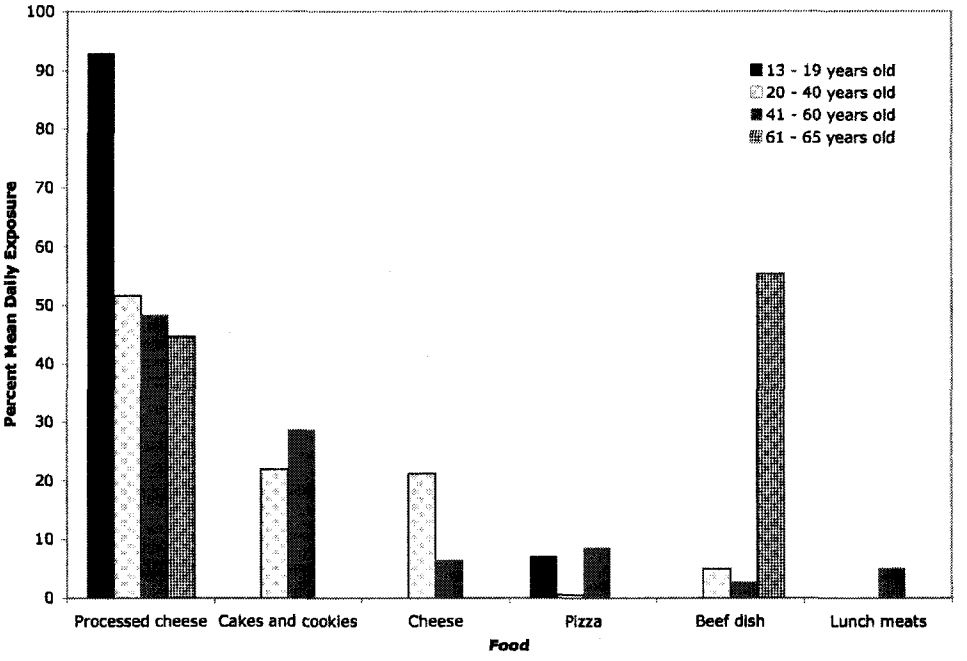
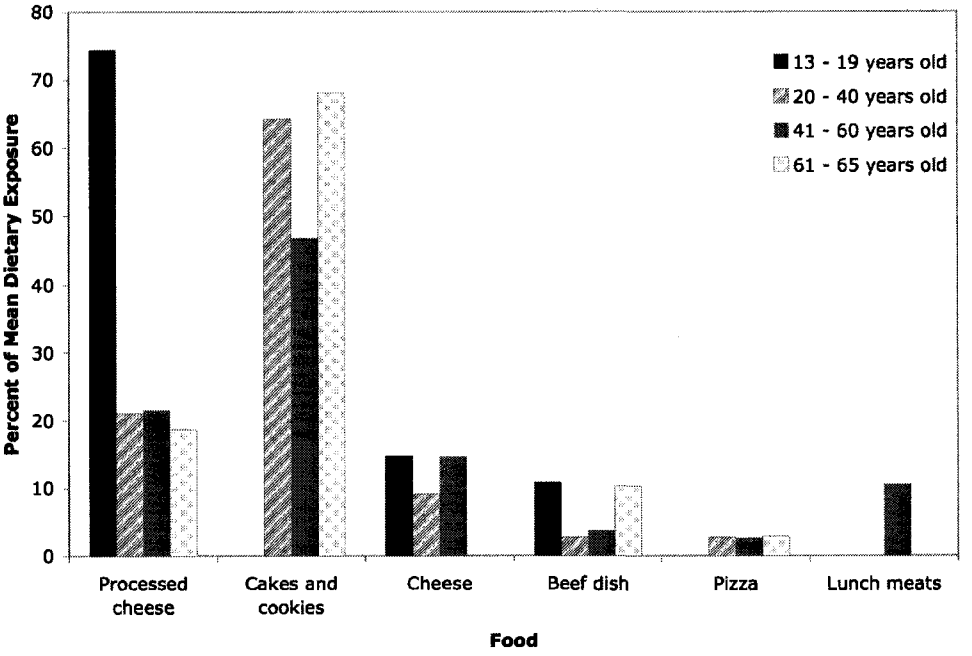

100
90
80
70
60
50
40
30
20
10
0
Percent Mean Daily Exposure
13 - 19 years old
20 - 40 years old
41 - 60 years old
61 - 65 years old
Processed cheese
Cakes and cookies
Cheese
Pizza
Beef dish
Lunch meats
Food

Figure 3. Sources of dietary exposure to PFCs and FTUCAs for Canadian men as percent of mean daily exposure.


80
70
60
50
40
30
20
10
0
Percent of Mean Dietary Exposure
13 - 19 years old
20 - 40 years old
41 - 60 years old
61 - 65 years old
Processed cheese
Cakes and cookies
Cheese
Beef dish
Pizza
Lunch meats
Food

Connecting Bridge

In our first study we investigated the extent of dietary exposure to PFCs for Canadians. Our results suggest that very few store-bought foods contained PFCs in 1998 and the levels detected were generally very low (less than 5 ng/g ww). Mean dietary exposure for the general Canadian population was estimated to range from 2 to 59 ng/day, which is not expected to cause adverse health outcomes. Given that PFCs have been detected in Arctic marine mammals and store-bought foods consumed by Inuit, we were interested in investigating the dietary exposure to PFCs for Inuit. In our second study, we measured PFCs in animal and plant-derived foods harvested in Nunavut between 1997 and 1998. These data were combined with the data from our previous study (Chapter 2) to estimate dietary exposure to PFCs for Inuit in Nunavut. In the third chapter we analyze the sources of dietary exposure to PFCs, compare dietary exposure between Inuit and Canadians and assess the health risks associated with these dietary exposure estimates using available toxicological data.

Chapter 3. Estimated Dietary Exposure to Fluorinated Compounds for Inuit in Nunavut, Canada

Manuscript Title:

Estimated Dietary Exposure to Fluorinated Compounds for Inuit in Nunavut, Canada

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Abstract

Dietary exposure to perfluorinated compounds (PFCs) was estimated for Inuit in Nunavut based on the analysis of perfluorooctane sulfonate, perfluorinated carboxylates (C_7-C_{11}) and fluorotelomer unsaturated carboxylic acids (6:2, 8:2 and 10:2 FTUCAs) in archived traditional food samples. Dietary exposure was estimated using PFC concentration data from traditional and store-bought foods consumed by Inuit. Total PFC concentrations were highest in caribou liver (6.2±5.5 ng/g), ringed seal liver (7.7, 10.2 ng/g), polar bear meat (7.0 ng/g), and beluga meat (7.0, 5.8 ng/g). Mean daily dietary exposure estimates ranged from 210 to 610 ng-person^{-1} for 754 individuals. Men in the 41-60 year age group had statistically significantly higher PFC exposure ($p<0.05$) than younger men (<20 years old, 20-40 years old) and women from the same age group. Traditional foods contributed a higher percentage to PFC exposure than market foods in all age and gender groups. In general, caribou meat, arctic char, cookies and beluga muktuk contributed most to dietary exposure for Inuit, with caribou flesh contributing 43 to 75 percent of daily PFC dietary exposure. Health risks associated with these estimated exposure levels are minimal based on current toxicological information available from animal feeding studies.

Introduction

Perfluorinated compounds (PFCs) used in such applications as cosmetics, fire fighting foams, and water and grease repellent coatings for fabrics and food packaging have been detected in whole blood and serum of non-occupationally exposed humans in North America (Calafat et al., 2006; Kubwabo et al., 2004; Olsen et al., 2005b), South America (Calafat et al., 2006; Kannan et al., 2004), Europe (Fromme et al., 2007; Kannan et al., 2004) and Asia (Inoue et al., 2004; Kannan et al., 2004; Taniyasu et al., 2003). Sources of human exposure to PFCs have not been fully elucidated although dietary exposure estimates for perfluorinated compounds range from less than 1 ng/kg body weight/day and 4 ng/kg body weight/day for Canadians (Ostertag et al., in review; Tittlemier et al.,

2007) to 100 ng/kg body weight/day for the British population(Mortimer et al., 2006). Dietary exposure to PFCs may be of particular concern for people consuming wildlife species, given that PFCs have been shown to bioaccumulate and bioconcentrate in aquatic food webs (Martin et al., 2004a; Martin et al., 2004b; Tomy et al., 2004a).

The Inuit diet is composed of market foods purchased from the supermarket and traditional foods obtained from locally harvested plants and wildlife (Kuhnlein et al., 2000). Past studies have shown that traditional foods are a source of elevated exposure to mercury, PCBs and radionuclides for Canadian Inuit (Kuhnlein and Chan, 2000). Inuit dietary exposure to PFCs may be greater than dietary exposure for the general Canadians population, given the low concentrations of PFCs detected in few market foods (Ostertag et al., in review; Tittlemier et al., 2007) and the frequent detection of PFCs in species frequently consumed by Inuit (Martin et al., 2004a; Tomy et al., 2004a).

Adverse effects of chronic exposure to PFCs have not been studied extensively in human populations, although a recent study suggests that PFOA and PFOS exposure *in utero* are associated with decreased weight and decreased head circumference (Apelberg et al., 2007b). Clinical outcomes associated with elevated PFOA exposure in fluorochemical workers include prostate cancer (Gilliland and Mandel, 1996) and increased cholesterol, triglycerides and thyroid (T3) levels (Olsen et al., 2003a). Serum cholesterol was positively associated with PFOS exposure in fluorochemical workers in one year of a two-year study (Olsen et al., 1999). Subchronic dosing with PFOA in laboratory feeding trials have been associated with liver toxicity, increased liver weight and developmental toxicities (Abbott et al., 2007; Butenhoff et al., 2002; Butenhoff et al., 2004; Thomford, 2002; White et al., 2007). Dosing of cynomolgous monkeys with PFOS resulted in decreased weight, increased liver weight, decreased serum total cholesterol and changes in thyroid stimulating hormone (TSH) and total T3 (Seacat et al., 2003).

In this paper, we present the results of the analysis of PFCs and FTUCAs in archived traditional foods from Nunavut and estimate daily dietary exposure to these compounds for Inuit men and women in the late 1990s. Dietary exposure estimates are based on quantities of market and traditional foods consumed and the concentrations of PFCs in archived food samples analyzed previously (Ostertag et al., in review) and in this study. The inclusion of both traditional foods and market foods is necessary because past studies indicate that both food sources contain detectable concentrations of PFCs (Giesy and Kannan, 2001; Martin et al., 2004a; Mortimer et al., 2006; Ostertag et al., in review; Tittlemier et al., 2006; Tittlemier et al., 2007; Tomy et al., 2004a). A preliminary health risk assessment is presented based on benchmark dose and lowest observable effects levels (LOELs) from animal feeding studies.

Experimental Section

Sample description. Samples of traditional foods were selected for analysis from archived samples available from the Center for Indigenous Peoples' Nutrition and Environment (CINE). Locally harvested animal and plant-derived foods samples were collected in Nunavut between 1997 and 1998 for the study 'Assessment of Dietary Benefit/Risk in Inuit Communities' (Kuhnlein et al., 2000). Inuit prepared the foods as per consumption; therefore samples correspond to the species, parts and preparations of traditional foods consumed in Nunavut. All samples selected for this study were stored in chemically cleaned polypropylene Nalgene™ containers and lids at –20° Celsius following homogenization. Food samples were selected to match the animal (species, tissue and preparation methods) cited in 24-hour recalls (Kuhnlein et al., 2000). Frequently consumed foods were analyzed along with foods expected to contain greater concentrations of PFCs such as organ meats, blood, and tissue (i.e. muscle, blubber, muktuk) from animals at high trophic-positions. In total, 68 traditional foods were analyzed and details about the samples (animals, species, tissues and preparations) are given in tables 7 and 8.

Dietary Exposure Estimate and Statistical Analyses. Dietary exposure to PFCs was estimated for 752 Inuit individuals from eight communities in Nunavut that participated in 24-hour dietary recalls between 1998 and 1999 for the study 'Assessment of dietary risk/benefit in Inuit communities' (Kuhnlein et al., 2000). Human ethics approval was obtained to carry out secondary data analysis with dietary intake information collected in Nunavut. Daily dietary exposure to PFCs was estimated for each individual by multiplying the quantity of each food consumed with mean concentrations of PFCs measured in traditional foods (this study) and market foods (Ostertag et al., in review). Concentrations below the MDL were entered as zero. Mean exposure to PFCs was grouped according to gender and four age categories (13-19, 20-40, 41-60, older than 61 years old). A one-way ANOVA was conducted to identify if PFC exposure differed significantly with age and gender. Multiple comparisons were conducted using Bonferonni tests to compare mean exposure levels between age groups for each gender and between genders in each age group. All statistical analyses were carried out using SAS v.9.1.

Analytical Method. The chemicals and methods used have been described in detail by Ostertag et al. (in review), but in brief, the composite samples were thawed at room temperature and one gram cooked food or two grams raw food (liquid or solid) were taken and placed in methanol-rinsed polypropylene centrifuge tubes. The extraction of perfluorinated carboxylates (C7-C11), PFOS and unsaturated fluorotelomer carboxylates (6:2, 8:2 and 10:2 FTUCAs) was carried out with a methanol extraction modified from a method described previously (Tittlemier et al., 2005). Liquid samples were freeze-dried (Flexi-Dry MP microprocessor controlled bench top lyophilizer made by FTS Systems, Inc., Stone Ridge, NY.) for six hours before the methanol extraction took place. Following the methanol extraction, the supernatants were combined, vortexed and dried to 0.5 mL under a gentle stream of nitrogen gas in a water bath at 37°C. Clean-up involved SPE with OASIS ® WAX cartridges (6cc, 150 mg, 30μm), modified from the method described by Taniyasu et al. (2005). The eluate was dried to 0.5 mL, and a 250

μL aliquot was taken and internal performance standard (20 μL of a 100 pg/μL solution) and 230 μL water were added. Samples were mixed on a vortex mixer and centrifuged at 2200 *x g* for ten minutes. Approximately 450 μL of sample was then transferred to a polypropylene autosampler vial, capped and stored at 4°C until analysis.

Instrumental Analysis. Samples were analyzed using liquid chromatography negative electrospray tandem mass spectrometry (LC-MS/MS). The instrument parameters have been described in more detail elsewhere (Ostertag et al., in review). In brief, samples (10 uL injection) were chromatographed at ambient temperatures on a C_{18} analytical column and guard column installed on an HP 1100 binary pump high performance liquid chromatograph (Agilent, Palo Alto, CA). The mobile phase solutions were 5 mM ammonium formate in Barnstead Diamond water (18 MΩ*cm) (solution A) and a 1:1 (v/v) solution of acetonitrile/methanol (solvent B). The liquid chromatograph was connected to a VG Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK). Samples were analyzed for PFCs in the multiple reaction-monitoring mode and the transitions are provided in the supplemental information.

Quantitation. Positive identification of target analytes was based on retention times being within 2.5 percent of the standard retention time, a signal to noise ratio greater than three and the presence of the confirmation mass transition (when applicable). Quantitation was carried out using the relative response of the target analyte compared to the corresponding mass-labelled internal performance standard (provided in supplemental information). An external five-point calibration curve made up in methanol and water (1:1 ratio) was used for quantitation. The curve for each analyte consistently had an r^2 value > 0.98 for all analytes of interest. Concentrations were blank-subtracted but not recovery-corrected prior to analysis.

Quality Assurance and Quality Control. Quality assurance and control steps included methanol laboratory blanks, fortified matrix samples and the addition of mass-labelled surrogate recovery standards ($^{13}C_4$ PFOA, $^{13}C_2$ PFNA and $^{13}C_4$ PFOS) and internal performance standards to each sample. One methanol blank and one homogenized raw ground beef sample fortified with target analytes (50 µL of a 100 pg/µL standard solution) were included in each batch of samples. The instrument detection limit was estimated for each sample as the concentration for which the corresponding peak had a signal-to-noise ratio of 3. Method detection limits (MDL) were considered as three times the standard deviation of the blanks plus the instrument detection limit (Smithwick et al., 2006) divided by the weight of sample. If the blank was below the detection limit, the MDL was estimated as three times the standard deviation of the lowest concentration standard of the calibration curve (Gomez-Taylor et al., 2003). The limit of quantification (LOQ) was determined as three times the detection limit.

Results

Quality Assurance and Quality Control. Six fortified ground beef samples were analyzed to assess the recoveries of target analytes. The following recoveries were calculated for FTUCAs: 64±8 % for 6:2 FTUCA, 74 ±8 % for 8:2 FTUCA and 77 ±9 % for 10:2 FTUCA. PFCA recoveries decreased with increasing chain-length and were: 70±6% (PFHpA), 110±5% (PFOA), 85 ±7 % (PFNA), 68 ±2% (PFDA), 49±6% (PFUA). Mean recovery for PFOS was 55 ±10%. Recoveries of the internal recovery standards, $^{13}C_4$ PFOA, $^{13}C_2$ PFNA and $^{13}C_4$ PFOS, were calculated for all samples (n=68) and were 88±16 %, 110±43% and 99±27 % respectively. Method blanks were free of detectable concentrations of all FTUCAs, PFOS and PFCAs longer than PFOA. The concentrations of PFHpA and PFOA in method blanks were converted to concentrations for 2 grams of tissue and mean concentrations and standard deviations were 0.16 ± 0.03 and 0.02 ± 0.03 ng/g respectively.

MDLs for each analyte were calculated for each sample and mean MDLs were less than 0.35 ng/g for all target analytes. Mean MDLs and standard deviations were: 0.33±0.14 (6:2 FTUCA), 0.18±0.07 (8:2 FTUCA), 0.13±0.15 (10:2 FTUCA), 0.24±0.19 (PFHpA), 0.19±0.12 (PFOA), 0.31±0.25 (PFNA), 0.20±0.16 (PFDA), 0.24±0.18 (PFUA) and 0.15±0.14 (PFOS).

Concentrations in traditional foods. PFCs were detected in 61 of the 68 traditional food samples analyzed. The highest total PFC concentrations were detected in ringed seal (*Phoca hispida*) liver, caribou (*Rangifer tarandus*) liver, ringed seal blood, polar bear (*Ursus maritimus*) meat and beluga (*dephinapterus leuca*) meat. Total PFC concentrations were below 1 ng/g in 41 traditional foods analyzed. The concentration of PFCs measured in the traditional food samples are given in tables 7 and 8.

PFNA and PFOS were detected most frequently (44 and 39 samples respectively) followed by PFDA (28 samples) and PFUA (23 samples). PFOA was rarely detected in traditional foods (only in nine samples). PFHpA was not detected in any sample analyzed. The sum of PFCAs (PFOA, PFNA, PFDA, PFUA) exceeded the concentration of PFOS in 51 of 68 traditional foods analyzed.

Inuit dietary exposure estimates. Mean exposure was comparable between all Inuit women and the estimated daily exposure ranged from 239 ng/day (women younger than 20 years old) to 443 ng/day (women older than 60). The differences in mean daily exposure were not statistically significant between women of different age groups. PFC exposure estimates for Inuit men ranged from 244 ng/day to 656 ng/day. Men in the 41-60 year age group had statistically significantly higher PFC exposure ($p<0.05$) than younger men (<20 years old, 20-40 years old) and women from the same age group. Mean exposure to PFOS, total PFCAs and total FTUCAs for Inuit men and women are provided in figure 4.

Traditional foods contributed a higher percentage to PFC exposure than market foods as a dietary source of PFCs for Inuit in all age and gender groups. Mean PFC exposure estimates for Inuit from the consumption of market and traditional foods are provided in figure 5. Elevated PFC exposure from market foods was observed in women younger than twenty (92 ng/g) and men between 20 and 40 years old (88 ng/day). Market food contribution to PFC exposure decreased with increasing age for both men and women. In general, caribou meat, arctic char (*Salvelinus alpinus*), cookies and beluga muktuk contributed most to dietary exposure for Inuit, with caribou flesh contributing 43 to 75 percent of daily PFC dietary exposure. Sources of elevated dietary exposure to PFCs were examined for eighteen individuals whose estimated daily exposure levels exceeded 1500 ng/day (data not shown). The elevated exposure in ten individuals was associated with the consumption of polar bear meat and liver from walrus (*Odobenus rosmarus*), caribou and ringed seal.

Discussion

Concentrations of PFCs in Traditional Foods. In marine mammals, total PFC concentrations in muscle tissue were greatest in animals from higher trophic levels (i.e. polar bear, beluga), which is consistent with observations that PFOS and PFOA biomagnify in aquatic food webs (Martin et al., 2004a; Tomy et al., 2004a). The higher concentrations of PFCs detected in caribou liver compared to caribou muscle are consistent with studies that demonstrate that PFOA and PFOS accumulate in liver and blood (Johnson et al., 1979; Vanden Heuvel et al., 1991).

Perfluorinated compounds have been measured previously in ringed seal liver, beluga liver and clams from the Canadian Arctic (Martin et al., 2004a). The concentrations of PFOS, PFNA and PFUA were similar in ringed seal liver samples analyzed by Martin et al. (2004a) and our study. PFDA was detected at a lower concentration in ringed seal liver samples in this study compared to levels previously measured (Martin et al., 2004a). PFOS was not detected in the one

clam sample analyzed in our study, however Tomy et al. observed 0.28±0.09 ng/g PFOS in clams sampled in their study (2004a).

PFCs were detected in nearly every traditional food sample analyzed, which was in contrast to the results from the analysis of market foods present in the average Canadian and Inuit diets (Ostertag et al., in review). Many store-bought foods analyzed to date have been free of detectable levels of PFCs (Mortimer et al., 2006; Ostertag et al., in review; Tittlemier et al., 2006; Tittlemier et al., 2007). In general, the levels of PFCs detected in traditional foods exceeded levels detected in store-bought or fast foods (Mortimer et al., 2006; Ostertag et al., in review; Tittlemier et al., 2007). PFCs have been detected in store-bought meats (Ostertag et al., in review; Tittlemier et al., 2007) and the levels have been similar to the levels detected in ringed seal and caribou meat from this study (i.e. below 5 ng/g). However, levels of PFCs in liver (e.g. ringed seal and caribou) and higher trophic level animal meat (e.g. polar bear, beluga) were higher than levels found in most store-bought foods in Canada (Ostertag et al., in review; Tittlemier et al., 2007). Traditional foods are frequently animal-derived foods and include organ meats and blood. The elevated concentrations and increased detection of PFCs in traditional foods compared to store-bought foods reflects the bioaccumulation and biomagnification of PFCs observed in arctic food webs (Martin et al., 2004a; Tomy et al., 2004a).

Dietary Exposure. The primary sources of dietary exposure to PFCs are caribou flesh, arctic char, cookies and beluga muktuk. Elevated dietary exposure to PFCs in males (41-60 years old) was associated with consumption of a larger number of traditional food species, including caribou liver and bearded seal (*Erignathus barbatus*) intestine than other age and gender groups. Caribou liver only contributed to mean daily PFC exposure for men 41 to 60 years old, and bearded seal exposure contributed to PFC exposure for men less than 20 years old and 41 to 60 years old. Beluga flesh contributed a much greater amount to PFC exposure in women under 20 (24 percent) than any other age or gender group (less than 5

percent). Inuit in Nunavut consumed contaminated store-bought foods (e.g. cookies and processed cheese) in smaller quantities than caribou and arctic char meat based on the dietary intake data collected by Kuhnlein et al. (2000).

In general, elevated PFC exposure (greater than 1500ng/day) was associated with the consumption of one traditional food with higher PFC concentrations (i.e. polar bear muscle, caribou liver, ringed seal liver, beluga flesh, bearded seal intestine). Our data suggest that the consumption of large quantities of traditional foods (i.e. ringed seal muscle and broth; beluga muktuk, blubber and caribou meat) or the consumption of only one traditional food with a higher concentration of PFCs leads to more elevated daily exposure to PFCs.

Inuit dietary exposure estimates exceeded average Canadian dietary exposure estimates calculated in Ostertag et al. (Ostertag et al., in review) for all age and gender groups. The greater mean dietary exposure to PFCs for Inuit compared to Canadians is readily explained by the presence of traditional food in the Inuit diet. The importance of traditional foods as a source of exposure was identified in this study, with traditional foods representing more than fifty percent of mean daily dietary exposure to PFCs for Inuit. Caribou meat was the main contributor to dietary exposure for Inuit, due to the large quantities of these foods consumed (Kuhnlein et al., 2000). Very few market foods contain PFCs and the foods containing detectable levels of PFCs are consumed in low quantities (e.g. cookies and processed cheese), therefore dietary exposure is lower for Canadians who only consume store-bought foods.

Significance of Dietary Exposure to PFCs. The dietary exposure estimates derived from 24-hour dietary recalls may be used at the population level to address average intake for a large group of individuals (Beaton, 1992). The World Health Organization and Health Canada have not established guideline levels for chronic exposure to PFOS and PFCAs, therefore we compared our exposure estimates to benchmark dose levels and lowest observable effects levels from animal feeding

trials. The most appropriate benchmark dose lower confidence limit (BMDL10) for PFOA was estimated as 0.6 mg/kg/day for increasing liver weight from a two-generational PFOA feeding study (Butenhoff et al., 2004). This BMDL10 was used for assessing risk associated with PFCA and FTUCA exposure due to the lack of feeding trial data for other compounds. The most sensitive endpoints from PFOS exposure were changes in blood lipid profiles in cynomolgous monkeys with an LOEL of 0.03 mg/kg body weight/day (Thomford, 2002). A safety factor of 10000 was applied to the BMDL10 and LOEL to assess the human health risk associated with exposure estimates for Inuit.

Mean exposure to PFOS and total combined PFCAs and FTUCAs were below 3 ng/kg body weight/day and 60 ng/kg body weight/day respectively, which are levels considered to be of minimal risk based on current toxicological data from animal feeding trials. The highest exposure group (males, 41-60 years old) had an estimated daily PFOS exposure of 2.4 ng/kg body weight/day, which approached the safety margin of 10000 calculated for PFOS (3 ng/kg body weight/day). Certain individuals may occasionally have exposure levels to PFOS that approach or exceed safe exposure levels, but these are not expected to reflect chronic exposure levels. Although our data suggest that Inuit exposure to PFCs is lower than that considered as a concern for public health, our study does not address exposure to PFCA and PFOS precursors, which are expected to be present in both market and traditional foods (D'Eon J and Mabury, 2007; Sinclair et al., 2007; Tomy et al., 2004a). The use of an internal dose of PFCs (i.e. concentration of PFCs in blood or serum) would reduce the uncertainties associated with extrapolating animal exposure data to humans and would also take into account exposure to direct and indirect sources of PFOS and PFCAs.

A recent study found a small, but statistically significant association between cord blood PFOS and PFOA concentrations and decreased birth weights and head circumference in newborns (Apelberg et al., 2007b). Although these results must be used cautiously given that they have not yet been replicated and their

significance on the future health of the individuals is unclear, this study suggests that developmental effects may occur *in utero* at exposure levels of non-occupationally exposed women in industrialized countries. These data suggest that current benchmark doses for PFOS and PFOA exposure may not be appropriate for assessing risk, especially for adverse developmental outcomes. PFOS has also been shown to increase the genotoxicity of cyclophosphamide *in vitro* (Jernbro et al., 2007), which suggests that exposure to PFOS may enhance the toxicity of xenobiotics. This property of PFOS should be investigated further and may have significant consequences, given that Inuit are exposed to multiple environmental contaminants (Kuhnlein and Chan, 2000).

The contamination of the Arctic with PFCs has resulted in increased dietary exposure of Inuit in Nunavut to PFCs compared to the general Canadian population (Ostertag et al., in review). Decreasing PFOS levels have been observed in marine mammals in the Canadian Arctic, however PFCA concentrations have continued to rise in recent years (Butt et al., 2007); therefore, PFCA exposure is expected to increase for Canadian Inuit. Greater efforts to understand the sources of PFCAs to the Arctic and to reduce these inputs are necessary to reduce Inuit exposure to PFCs. The PFC exposure estimates derived in this study do not indicate that dietary exposure to PFOS, PFCA or FTUCA is exceeding levels that should be considered a health risk for Inuit in Nunavut. Comunication of health risk associated with PFC exposure should be undertaken with caution considering the significance of traditional foods in Inuit culture and diet.

Tables and Figures

Table 7. Concentration of PFCs detected in aquatic traditional foods in Nunavut. Concentration is given as ng/g wet weight and when three samples were analyzed the mean ± standard deviation is provided. N refers to the number of samples analyzed and nd refers to concentrations below the method detection limit (MDL).

Food	Part	Preparation	N	PFOS	PFOA	PFNA	PFDA	PFUA	Total
Ringed Seal	Liver	Raw	2	3.8, 7.6	0.3, nd	1.9, 2.1	0.5, nd	1.2, 0.6	7.7, 10.2
	Blood	Raw	3	2.9±2.1	0.1±0.1	1.2±0.3	0.2±0.2	0.6±0.6	5.0±2.9
	Meat	Boiled	1	0.5	nd	0.5	nd	nd	1.0±2.9
		Raw	3	0.2±0.3	nd	0.9±0.6	nd	nd	1.1±0.5
	Blubber	Raw	2	0.1, nd	nd	0.2, nd	0.1, nd	0.2, nd	0.6, nd
Polar Bear	Meat	Frozen	1	4.0	nd	0.8	0.6	1.6	7.0
Beluga	Blubber	Raw	1	1.5	nd	0.4	0.3	0.7	3.1
	Meat	Dried	2	3.6, 1.6	nd	0.6, 0.9	0.7, 0.8	2.0, 2.5	7.0, 5.8
	Muktuk	Raw	1	0.4	nd	nd	nd	0.8	1.2
Narwhal	Blubber	Raw	1	0.2	nd	nd	nd	0.2	0.4
	Muktuk	Raw	2	nd	nd	0.5, 0.5	nd	nd	0.5, 0.5
	Muktuk	Frozen	2	1.6, 0.4	nd	nd	nd, 0.3	nd, 0.3	1.6, 1.0
Bearded Seal	Intestine	Boiled	2	0.5, 0.6	nd	0.3, 1.2	0.2, nd	0.2, 0.4	1.2, 2.2
	Meat	Boiled	1	0.2	nd	nd	nd	nd	0.2
Walrus	Blubber	Aged, raw	1	nd	nd	0.4	0.2	nd	0.6
	Kauk	Raw	1	0.2	nd	0.6	nd	nd	0.8
	Meat	Raw	2	nd	nd	0.6, 0.8	0.2, nd	nd	0.8, 0.8
Eider Duck	Whole	Boiled	1	1.5	0.4	nd	0.6	0.3	2.8
Black Duck	Meat	Boiled	1	0.3	nd	nd	0.3	0.1	0.7
Arctic Char	Whole	Raw	3	nd	nd	0.2±0.3	0.1±0.1	nd	0.3±0.4
Lake Trout	Whole	Raw	2	0.4, 0.1	nd	nd	0.2, nd	0.1, 0.1	0.7, 0.2
Seaweed	Whole	Raw	1	nd	nd	nd	0.1	nd	0.1
Clams	Whole	Raw	1	nd	nd	0.5	nd	nd	0.45

Table 8. Concentration of PFCs detected in terrestrial traditional foods in Nunavut. Concentration is given as ng per gram wet weight and when three samples were analyzed the mean ± standard deviation is provided. N refers to the number of samples analyzed and nd refers to concentrations below the method detection limit (MDL).

Food	Part	Preparation	N	PFOS	PFOA	PFNA	PFDA	PFUA	Total
Caribou	Liver	Baked	1	5.0	0.7	1.6	0.6	1.1	9.0
		Raw	3	2.7±2.3	0.1±0.1	2.0±1.7	0.7±0.7	0.7±0.8	6.2±5.5
	Meat	Boiled	2	nd, 0.1	nd	0.8, nd	nd	nd	0.8, 0.1
		Dried	2	nd	nd	nd, 1.0	nd	nd	nd, 1.0
		Raw	2	nd	nd	0.3, 0.3	nd	nd	0.3, 0.3
		Roasted	1	nd	nd	1.0	0.4	nd	1.4
	Bone marrow	Boiled	1	0.2	nd	0.6	0.2	0.2	1.1
	Heart, blood	Raw	1	0.17	nd	0.80	0.22	nd	1.2
	Kidneys	Raw	2	0.1, 0.1	nd	0.2, 0.2	nd	nd	0.3, 0.3
	Stomach	Raw	1	0.1	0.8	0.1	nd	nd	1.0
	Tongue	Raw	3	0.2±0.2	0.0±0.1	0.6±0.6	nd	nd	0.8±0.8
Ptarmigan	Whole	Raw	3	nd	nd	nd	0.0±0.1	nd	0.2±0.02
Arctic Hare	Meat	Raw	2	0.4, nd	nd	nd, 0.2	nd, 0.2	nd	0.0, 0.3
Snow Goose	Meat	Raw	1	nd	nd	1.2	nd	nd	1.2
Berries	Whole	Raw	3	nd	nd	nd	0.1±0.1	nd	0.1±0.1

Figure 4. Estimated daily dietary exposure to PFOS, total PFCAs and FTUCAs for Inuit men and women (A – men; B- women). Bars represent arithmetic mean (±standard error) and N refers to the number of respondents to 24-hour recalls.

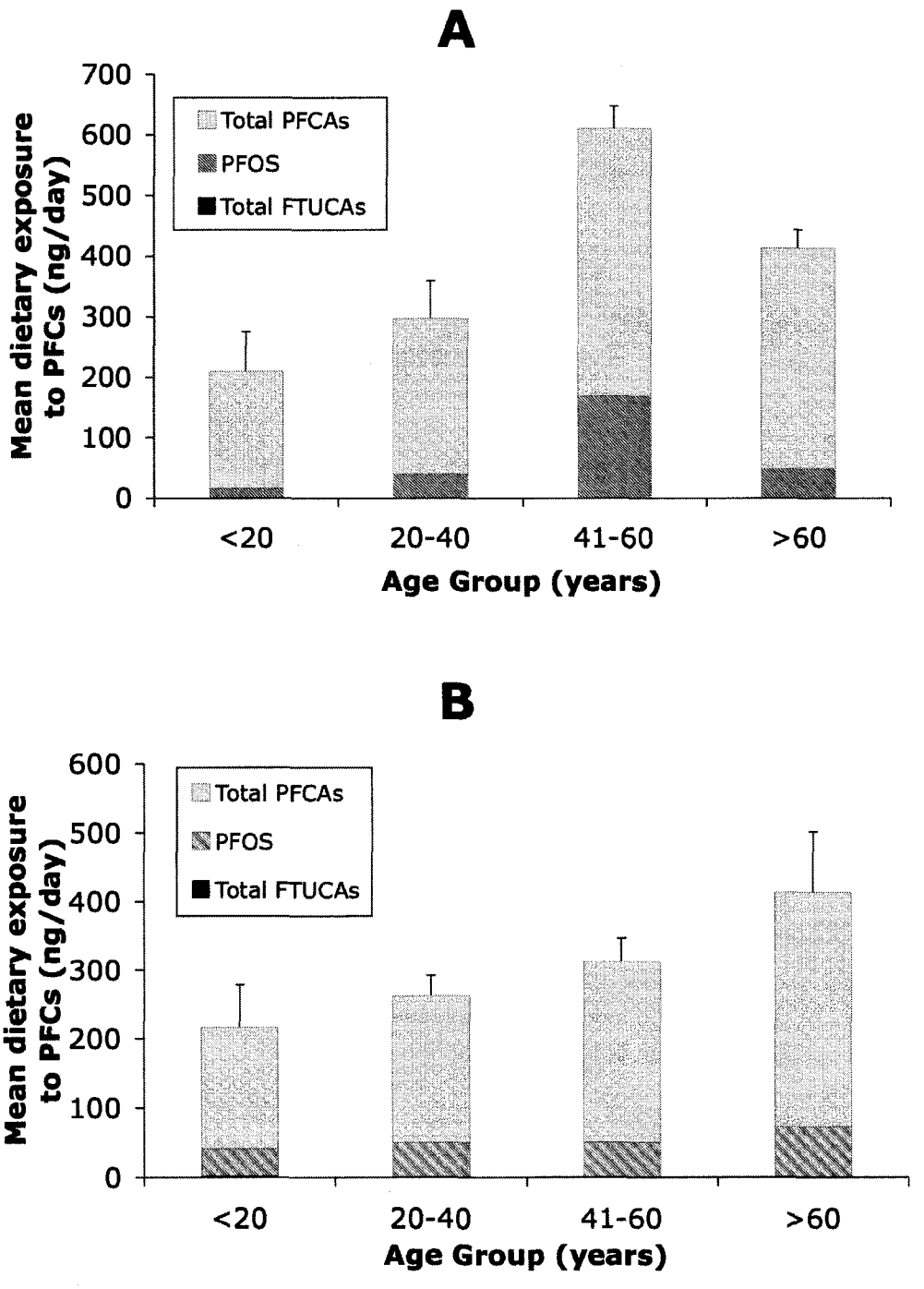
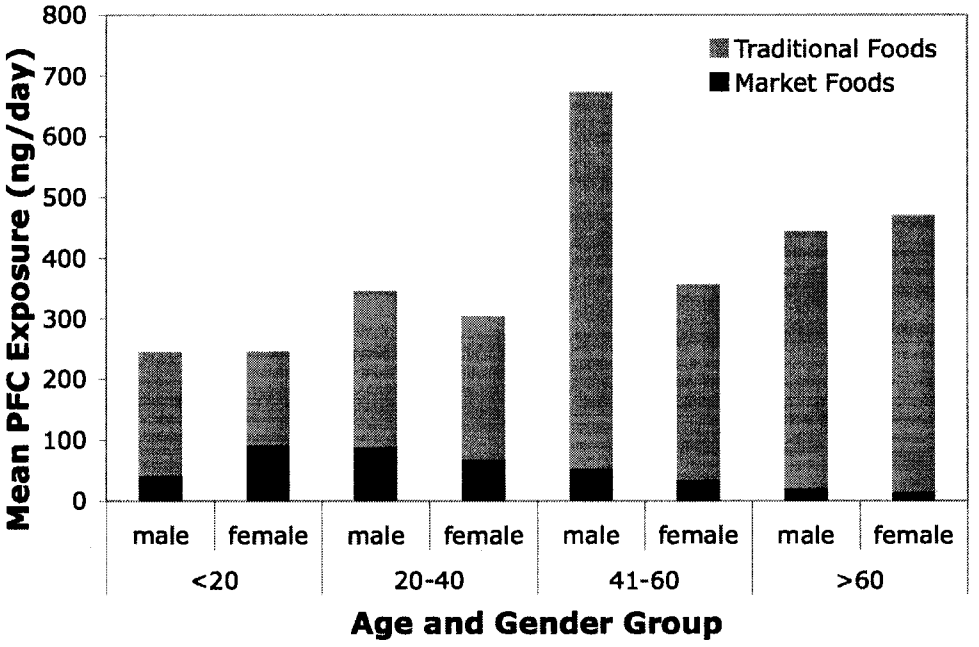

A
Mean dietary exposure to PFCs (ng/day)
700
600
500
400
300
200
100
0
Total PFCAs
PFOS
Total FTUCAs
<20
20-40
41-60
>60
Age Group (years)
B
Mean dietary exposure to PFCs (ng/day)
600
500
400
300
200
100
0
Total PFCAs
PFOS
Total FTUCAs
<20
20-40
41-60
>60
Age Group (years)

Figure 2. Estimated mean daily exposure to total PFCs from market and traditional foods for Inuit men and women from different age groups.


Mean PFC Exposure (ng/day)
800
700
600
500
400
300
200
100
0
Traditional Foods
Market Foods
male
female
male
female
male
female
male
female
<20
20-40
41-60
>60
Age and Gender Group

Conclusions

The concentrations of perfluorooctane sulfonate, perfluorinated carboxylates (C_7-C_{11}) and FTUCAs were measured in 133 food samples in order to estimate levels of dietary exposure for Inuit in Nunavut and average Canadians. In total, 65 store-bought and restaurant foods from Whitehorse, Yukon Territory and 68 traditional foods from Nunavut were analyzed. PFCs were detected in few Canadian store-bought foods, however most traditional foods analyzed contained detectable levels of PFOS and PFCAs. Canadian Total Diet Study samples found to contain detectable levels of PFCs included processed meats, pre-prepared foods and peppers. 61 of the 68 traditional food samples analyzed contained PFCs. In traditional foods analyzed, elevated concentrations of PFCs were found in caribou liver (6.2±5.5 ng/g), ringed seal liver (7.7, 10.2 ng/g), polar bear meat (7.0 ng/g), and beluga meat (7.0, 5.8 ng/g). 41 of the traditional food samples analyzed contained PFC concentrations below 1 ng/g.

Dietary exposure to perfluorooctane sulfonate, perfluorinated carboxylates (C_7-C_{11}) and FTUCAs was estimated for the Canadian population prior to the phase-out of perfluorooctyl-sulfonyl production by 3M and voluntary reductions in PFOA emissions. PFC exposure was estimated for the general Canadian population and for Inuit in Nunavut in 1998 using dietary intake data collected via 24 hour recalls in two studies. Estimated exposure to PFCs ranged from 2 to 59 ng/person/day for average Canadians and 210 to 610 ng-person^{-1} for Inuit from Nunavut. Inuit men between the ages of 41 and 60 had statistically significantly higher levels of exposure than younger men (<20 years old and 20 to 40 years old) and women from the same age group.

Our results indicate that caribou meat contributed the most to dietary exposure of PFCs for Inuit men and women in all age groups. Both market foods and traditional foods contributed to dietary exposure for Inuit, however traditional foods contributed more than half of mean daily PFC exposure compared to market

foods. In the general Canadian population, dietary exposure to PFCs was associated with the consumption of processed cheese and cakes and cookies.

PFC exposure estimates derived in this study do not indicate that dietary exposure to PFOS, PFCAs or FTUCAs are exceeding levels that should be considered a health risk for Inuit in Nunavut or the general Canadian population. However, more data are required to assess health risks due to chronic PFC exposure. Considering the elevated dietary exposure to PFCs for Inuit in Nunavut, greater efforts to understand the sources of PFCAs to the Arctic and to reduce these inputs are required.

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Instrument performance standards used to account for matrix effects for each analyte and MS/MS Multiple Reaction Monitoring Parameters are provided. For analytes in which two transitions were monitored the first transition listed was used for quantitation.

Analyte	Performance Standard	Mass Transition	Cone voltage	Collision energy
Perfluoroheptanoate (PFHpA)	1,2-13C perfluorooctanoate	362.9 → 318.8	10	15
Perfluorooctanoate (PFOA)	1,2-13C perfluorooctanoate	412.9 → 368.8 412.9 → 168.8	10	15
Perfluorononanoate (PFNA)	1,2,3,4,5-C13 perfluorononanoate	462.8 → 418.8 462.8 → 218.8	12 16	18 18
Perfluorodecenoate (PFDA)	1,2-13C perfluorodecanoate	512.8 → 468.9 512.8 → 218.9	15 15	18 18
Perfluoroundecanoate (PFUA)	1,2-13C perfluorodecanoate	562.9 → 518.9 562.9 → 268.8	15	15
Perfluorododecanoate (PFDoDA)	1,2-13C perfluorodecanoate	612.8 → 568.8 612.8 → 318.9	12	30
Perfluorotetradecanoate (PFTeDA)	1,2-13C perfluorodecanoate	712.9 → 668.9 712.9 → 168.8	17	22
Perfluorooctane sulfonate (PFOS)	18O2 perfluorooctane sulfonate	498.9 → 98.9 498.9 → 79.9	50	60
6:2 Fluorotelomer unsaturated carboxylate (6:2 FTUCA)	2H-Perfluoro-[1,2-$^{13}C_2$]-2-octenoate	356.9 → 292.9	15	17
8:2 Fluorotelomer unsaturated carboxylate (8:2 FTUCA)	2H-Perfluoro-[1,2-$^{13}C_2$]-2-decenoate	457 → 392.9	18	20
10:2 Fluorotelomer unsaturated carboxylate (10:2 FTUCA)	2H-Perfluoro-[1,2-$^{13}C_2$]-2-dodecenoate	557 → 493	20	25

Daily dietary exposure to PFOS, PFCAs (C7-C11) and FTUCAs (ng/day) for Inuit in Nunavut based on intake of traditional and market foods. Mean, median, range and 95% Confidence Interval for daily dietary exposure to PFCs were calculated using 24-hour recalls (Kuhnlein et al., 2000) and PFC concentrations in market foods (Ostertag et al., in review) and traditional foods (this study).

Gender	Age Group	N	Mean	Median	95% CI	Range
Females	< 20	34	220	130	140 – 340	0 – 1950
	20-40	232	260	160	250 – 350	0 – 5010
	41-60	106	310	270	300 – 400	0 – 1530
	> 60	46	410	270	300 – 590	0 – 3730
Males	< 20	29	210	100	140 – 350	0 – 1120
	20-40	176	300	200	270 – 400	0 – 5010
	41-60	96	610	450	500 – 810	0 – 6560
	> 60	33	410	360	330 - 550	0 – 1600

Sources of PFC exposure for Inuit males and females, given as the exposure of PFCs from each food as a percentage of mean daily exposure for each age-gender group. Foods that did not contribute at least 5% to mean daily PFC exposure to at least one gender/age group are not shown.

Gender/Age Group / Food	Females				Males			
	< 20	20-40	41-60	>60	<20	20-40	41-60	>60
Caribou (flesh)	45	54	58	67	75	56	43	56
Arctic Char	<5	7	11	22	<5	9	12	24
Cookies	10	7	6	<5	<5	8	5	0
Beluga (muktuk)	5	5	7	<5	0	<5	7	<5
Caribou (liver)	0	0	0	0	0	0	8	0
Seal (intestine)	0	0	<5	0	8	<5	7	0
Beluga (blubber)	0	<5	5	0	0	<5	5	<5
Beluga (flesh)	24	<5	0	0	0	0	0	0
Processed Cheese	6	<5	<5	0	<5	<5	<5	0