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

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The source of involatile, anthropogenic perfluorocarboxylate anions (PFCAs) in biota from remote regions is of heightened interest due to the persistence, toxicity, and bioaccumulation of these materials. Large-scale production of fluorinated compounds is carried out primarily by one of two methods: electrochemical fluorination (ECF) and telomerization. Products of the two processes may be distinguished based on constitutional isomer pattern as ECF products are characteristically comprised of a variety of constitutional isomers. The objective of this research was to develop a method for identifying the constitutional isomer profile of PFCAs in environmental samples and to apply the method to polar bear livers from two different locations. Resolution of constitutional isomers of derivatized PFCAs (8−13 carbons) was accomplished via GC−MS. Seven isomers of an authentic ECF perfluorooctanoate (PFOA) standard were separated. The linear isomer comprised 78% of this standard. Isomer profiles of PFCAs in liver samples of 15 polar bears (*Ursus maritimus*) from the Canadian Arctic and eastern Greenland were determined by GC−MS. The PFOA isomer pattern in Greenland polar bear samples showed a variety of branched isomers while only the linear PFOA isomer was determined in Canadian samples. Samples of both locations had primarily (>99%) linear isomers of perfluorononanoate and perfluorotridecanoate. Branched isomers of perfluorodecanoate, perfluoroundecanoate, and perfluorododecanoate were determined in the polar bear samples. Unlike the PFOA isomer signature, only a single branched isomer peak on the chromatograms was observed for these longer chain PFCAs. The presence of branched isomers suggests some contribution from ECF sources. However, in comparison to the amount of branched isomers in the ECF PFOA standard, such minor percentages of branched PFCAs may suggest additional input from an exclusively linear isomer source.

Introduction

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

PFCAs are unlikely to move long distances in the atmosphere due to low volatility and efficient scavenging by wet and dry deposition (*14*). Therefore, it has been hypothesized that volatile precursor compounds, with the ability for long-range atmospheric transport, will eventually break down to the persistent PFCAs (*15*, *16*). Telomer alcohols, which are volatile, have been found to degrade both atmospherically (*17*) and biologically (*18*, *19*) to PFCAs. Given the large-scale production of telomer alcohols worldwide, these compounds are candidates for contributors to global PFCA levels (*20*). In fact, analysis of North American air samples show widespread tropospheric distribution of fluorotelomer alcohols at significant concentrations (*15*, *21*). Ellis et al. (*17*) have indicated that telomer alcohols may be responsible in part for the presence of PFCAs in the Arctic and other nonurban areas where atmospheric levels of peroxy radicals far exceed that of NO_x (NO and NO_2).

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

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

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TABLE 1. Perfluorinated Carboxylates: Acronyms and Molecular Ion of 2,4-Difluoroanilide Derivative Used for Determination

name	acronym	molecular ion of 2,4-difluoroanilide deriv
perfluoroheptanoate	PFHpA	475[a]
perfluorooctanoate	PFOA	525
perfluorononanoate	PFNA	575
perfluorodecanoate	PFDA	625
perfluoroundecanoate	PFUnA	675
perfluorododecanoate	PFDoA	725
perfluorotridecanoate	PFTrA	775
perfluorotetradecanoate	PFTA	825[a]

[a] Not determined in this study.

erization reaction that involves reaction of perfluoroalkyl iodides with perfluorinated alkenes, such as tetrafluoroethene. This synthetic method merely increases chain length of the reacting perfluoroalkyl iodide and does not produce isomeric mixtures (*24*).

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

The ratio of linear to branched isomers in PFOSF-derived compounds can vary from 90:10 to 70:30 (*28*, *29*). The Certificate of Analysis for *N*-EtFOSE obtained from 3M (St. Paul, MN) states constitutional isomer composition based on ^{19}F NMR data. The linear:branched isomer ratio in this batch of *N*-EtFOSE was 70:30 (*30*). Table 2 depicts the structures of each of the types of isomers associated with ECF products. The most abundant branched isomers of *N*-EtFOSE were found to be the isopropyl branched isomer (~11%) and the internal monomethyl branched isomer (~17%). Identified branched isomers comprising the remaining ~2% consist of the terminal *tert*-butyl branched isomer, α-methyl branched isomer, and *gem*-dimethyl branched isomer (*30*). A document submitted to the U.S. EPA by 3M provides the Certificate of Analysis describing constitutional isomer composition for PFOA derived from perfluorooctanecarbonyl fluoride, a compound fluorinated by ECF (*30*). These isomers are shown in Table 2. The same classes of branched isomers found in *N*-EtFOSE were identified in this batch of PFOA. Again, the most abundant branched isomers were the isopropyl branched isomer and the monomethyl branched isomer. Contrary to *N*-EtFOSE, the ratio of linear to branched isomers in this sample of PFOA was found to be 78:22 (*30*). This indicates that there is some batch-to-batch variation in the isomer component of ECF products. Like *N*-EtFOSE, PFOS is synthesized from PFOSF. Isomer analysis of a batch of PFOS from 3M has been reported (*31*). The branched isomer content of this sample of PFOS is the same (30%) as the *N*-EtFOSE described above. However, the individual branched isomer content varies by ±0.4%. Although it is believed that the branched isomer composition of ECF products can range from 10% to 30%, this may be an overestimate. Thus, isomer determination of a wider range of compounds of ECF origins is necessary to gain appreciation for batch to batch variation.

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

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

TABLE 2. Identified Constitutional Isomers in ECF-Derived Compounds Using PFOA as an Example[a]

constitutional isomer	structure	% by wt[b]
linear	$CF_3(CF_2)_6COOH$	78.0
internal monomethyl branch	$CF_3(CF_2)_xCF(CF_3)(CF_2)_yCOOH$, where $x + y = 4$ methyl branch can be on any of the internal carbons of chain	12.5
isopropyl branch	$(CF_3)_2CF(CF_2)_4COOH$	9.0
tert-butyl branch	$(CF_3)_3C(CF_2)_3COOH$	0.2
internal *gem*-dimethyl branch	$CF_3(CF_2)_xC(CF_3)_2(CF_2)_yCOOH$ where $x + y = 4$ dimethyl branch can be on any of the internal carbons of chain	0.1
α branch	$CF_3(CF_2)_4CF(CF_3)COOH$	0.1

[a] Table adapted (with permission from 3M) from EPA Public Docket submitted by 3M (*37*). [b] As determined by ^{19}F NMR and ^{1}H NMR.

branched, and 0.12% terminal isopropyl branched isomer (*37*). The percentage of branched isomers in an LPDPF product is significantly less than that of an ECF product.

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

The objective of this study was to qualitatively examine the isomer patterns of PFCAs in environmental samples. Although LC/MS/MS is typical for PFCA determination, a GC-based method was chosen to separate the physically similar constitutional isomers because GC-based methods have greater potential for resolution as compared to LC-based methods. Another benefit of GC application is its avoidance of contamination, which sometimes arises with LC instruments containing perfluoro polymer parts (*39*). GC determination of PFCAs requires derivatization to volatile analogues (*40*). This was accomplished using 2,4-difluoroaniline, which has been used for derivatizing haloacetic acids, and more recently PFCAs (*41*−*43*).

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

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

Experimental Section

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

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

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

Derivatization. The derivatization method described here has been reported elsewhere (*41*−*43*). Reconstituted extracts were acidified to pH 1.0 using concentrated HCl. To this, 1.00 g of NaCl and 20.00 mL of ethyl acetate were added. A catalyst consisting of 1.00 mL of 1.1 M DCC in ethyl acetate and derivatizing agent, 1.00 mL of 1.0 M 2,4-DFAn in ethyl acetate) were then added. The resulting mixture was stirred vigorously for 1 h. After dissolving 5.0 g of NaCl into the mixture, the aqueous phase of the samples was removed using a separatory funnel. The remaining organic phase was washed with 5 mL of 10% HCl, saturated $NaHCO_3$ solution, and saturated NaCl solution, respectively. This phase was passed through a filter containing Na_2SO_4 and evaporated to dryness using N_2. Residues were dissolved in a 2.00 mL of hexane−diethyl ether solvent system (95%/5%). Further cleanup was performed using a silica gel column and elution with 15 mL of the hexane−diethyl ether solution. The eluants were evaporated to 200 μL volumes using N_2.

Gas Chromatographic−Mass Spectrometric (GC−MS) Analysis. 2,4-Difluoroanilide derivatives of PFOA, PFNA, PFDA, PFUnA, PFDoA, and PFTrA in the tissue extracts were determined by GC−MS using an HP 6890 series GC system with an HP 5973 MSD (Hewlett-Packard, Palo Alto, CA). The 1.00 μL pulsed pressure injections of the samples and standards were performed using an HP 7683 series injector. Chromatography of the derivatives was performed using a ZB-35 column (90.0 m × 0.25 mm, 0.50 μm film thickness) (Phenomenex, Torrance, CA) with He carrier gas at 12 psi. The injector was held at 250 °C for the duration of each run. Initial temperature of the oven was 40 °C and held for 2 min. The temperature was then increased to 125 °C at a rate of

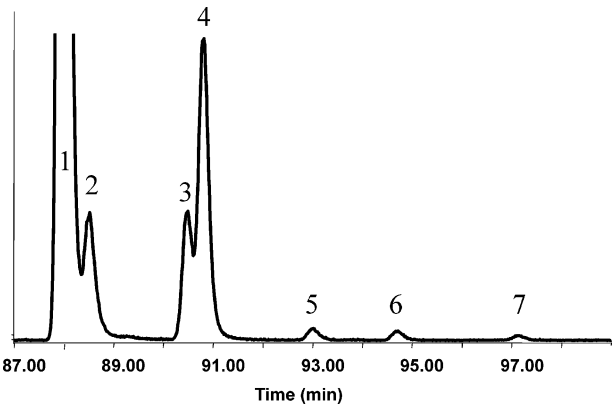

FIGURE 1. GC−MS determination of constitutional isomers of an authentic ECF PFOA standard where peak 1 corresponds to the linear isomer (77% peak area) and peaks 2−7 correspond to branched isomers.

1 °C /min and then held for 15 min. Using the same ramp, the temperature was raised to 140 °C at which point the oven was rapidly heated to 275 °C at 30 °C/min for cleaning. Transfer line and source were kept at 280 °C. All chromatograms presented are based on monitoring of the molecular ions using SIM (shown in Table 1) of the derivatized PFCAs. However, confirmation of PFCA identity was accomplished by monitoring two additional fragments 128 and 156 m/z, which correspond to the 2,4-difluoroaniline fragment and the 2,4 difluoroanilide fragment of the derivatized PFCAs, respectively.

Results and Discussion

Resolution of constitutional isomers of PFCAs by chromatography was accomplished using an ECF-derived PFOA standard provided by 3M. Detection of its 2,4-difluoroaniline derivative was by MS via the molecular ion. Separation of seven constitutional isomers was achieved as presented in Figure 1. In this particular batch of PFOA, 77.0% was determined to consist of the linear isomer based on peak integration of the GC−MS chromatogram. This value closely corresponds to the linear isomer composition (78.0%) of ECF PFOA determined by ^{19}F NMR analysis by 3M (*37*); our own ^{19}F NMR analysis is consistent with that reported by 3M. Further studies are necessary to identify the branched isomers identified in Figure 1.

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

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

Resolution of Isomers, Detection Limits, and Precision for GC−MS of Derivatized PFCAs. Resolution (Rs) was calculated for adjacent isomer peaks of ECF PFOA in Figure 1 using peak width and retention time. Rs was found to be

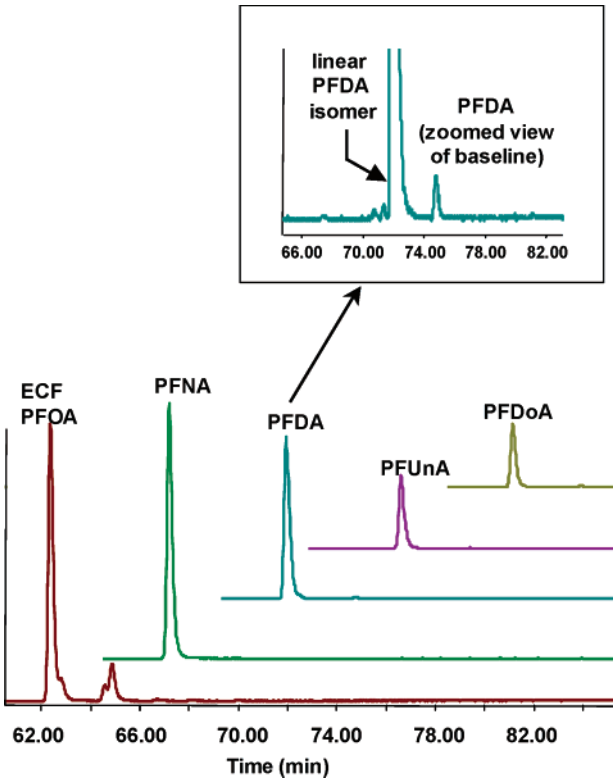

FIGURE 2. GC−MS determination of long-chain PFCA standards. All PFCA standards synthesized via LPDPF except for PFOA, which was derived from ECF. Inset shows magnified baseline of PFDA chromatogram.

0.8 (peaks 1 and 2), 0.7 (peaks 3 and 4), 3.0 (peaks 5 and 6), and 4.1 (peaks 6 and 7).

Detection limits were determined based on the lowest mass of ECF PFOA injected onto the GC−MS in which the isomer profile depicted in Figure 1 could be determined. This was found to be 10 pg. In other words, 2 ng of ECF PFOA was derivatized and concentrated to a 200 μL volume. The least abundant isomer was isomer 7 which, based on chromatographic peak area, comprised 0.2% of the total PFOA. By this, the detection limits for each isomer was 0.02 pg.

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

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

Constitutional Isomers of PFCAs in Polar Bear Liver Samples. The contamination profile of PFCAs in polar bear livers initially observed by Martin et al. was confirmed using this method where PFCAs with an odd number of carbons exceeds that of the even-numbered PFCA preceding (*12*). In

TABLE 3. Distribution of Branched Isomers of PFCAs in Arctic Polar Bears of Two Locations: Greenland and Canada

	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTrA
			Greenland			
average (%)	5.0	0.3	1.4	2.8	2.1	0.4
maximum (%)	9.8	1.1	2.5	3.4	4.0	0.9
minimum (%)	2.8	nd	nd	2.3	nd	nd
			Canada			
average (%)	nd	0.0	2.9	1.2	3.6	nd
maximum (%)	nd	0.4	3.4	1.5	3.9	nd
minimum (%)	nd	nd	2.3	0.9	3.2	nd

[a] nd = no branched isomers detected.

other words, [PFNA] > [PFOA], [PFUnA] > [PFDA], and [PFTrA] > [PFDoA]. This pattern is consistent both with the atmospheric degradation mechanism of fluorotelomer alcohols proposed by Ellis et al. (*17*), which yields a homologous series of PFCAs, and with the bioaccumulation potential reported by Martin et al. (*47*).

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

In general, the PFCA isomer profiles in polar bear liver samples were dominated by the linear isomers. This is unsurprising considering both telomerization and ECF yield an abundance of the linear isomer. At least 90% of the isomer distribution for each PFCA in the samples was linear. The distribution of branched isomers of each PFCA is presented in Table 3. Branched PFCA isomer composition in the polar bears varied with location. The most striking differences between locations pertained to the PFOA isomer pattern. Compared to other PFCAs, the PFNA isomer profile was quite distinct due to the consistent dominance of its linear isomer. A discussion of the data obtained for PFOA, PFNA, and the remaining PFCAs (PFDA, PFUnA, PFDoA, and PFTrA) follows.

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

The profile of PFOA isomers in the Greenland bears varied from sample to sample. A chromatogram of the most PFOA isomer-laden bear sample is presented in Figure 3. As indicated, the pattern of PFOA isomers in the sample matches well with that of the ECF PFOA standard.

In all Greenland bear samples, branched isomers 3 and 4 were present. These two isomers were the most abundant branched isomers in the standard. The absence of isomers 2 and 5−8 does not necessarily imply a non-ECF source of the PFCA. The method applied for isomer resolution was developed using a highly concentrated ECF PFOA standard. Although good resolution between isomers 1 and 2 was obtained with the standard, it is possible that baseline resolution will not be achieved when the relative amount of isomer 2 is significantly less than that of isomer 1. In fact, isomer 1 in the liver samples was broad in shape, which may

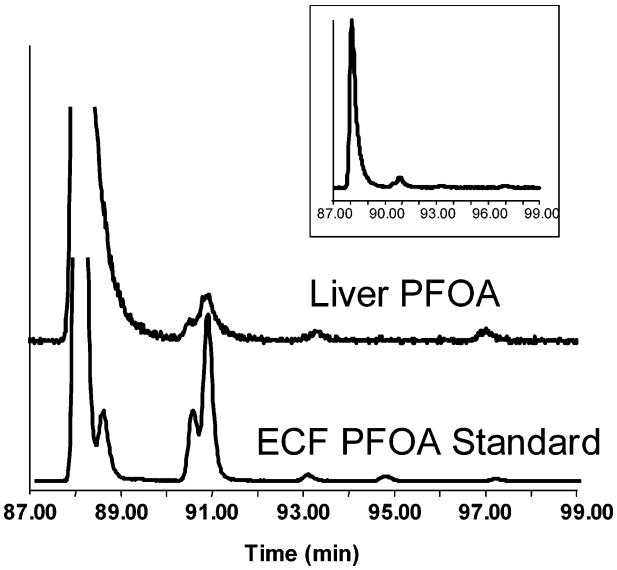

FIGURE 3. GC−MS determination of constitutional isomers of PFOA in polar bear liver sample from eastern Greenland (top) compared to those of an authentic ECF PFOA standard (bottom). Inset displays full view of PFOA isomer profile, normalized to linear PFOA, in polar bear liver sample (*17*).

suggest that the ratio of isomer 2 to the total PFOA in the samples is much less as compared to the 5.1% contribution of isomer 2 to total PFOA in the ECF standard. Similarly, it is likely that detection limit is an obstacle in determining isomers 5−7. In the ECF PFOA standard, isomers 5−7 accounted for only 2.1% of the total PFOA. The similarity of the pattern of peaks 3 and 4 in the standard to that observed in all seven Greenland samples suggests that the presence of these two isomers may be diagnostic of identifying an ECF source.

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

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

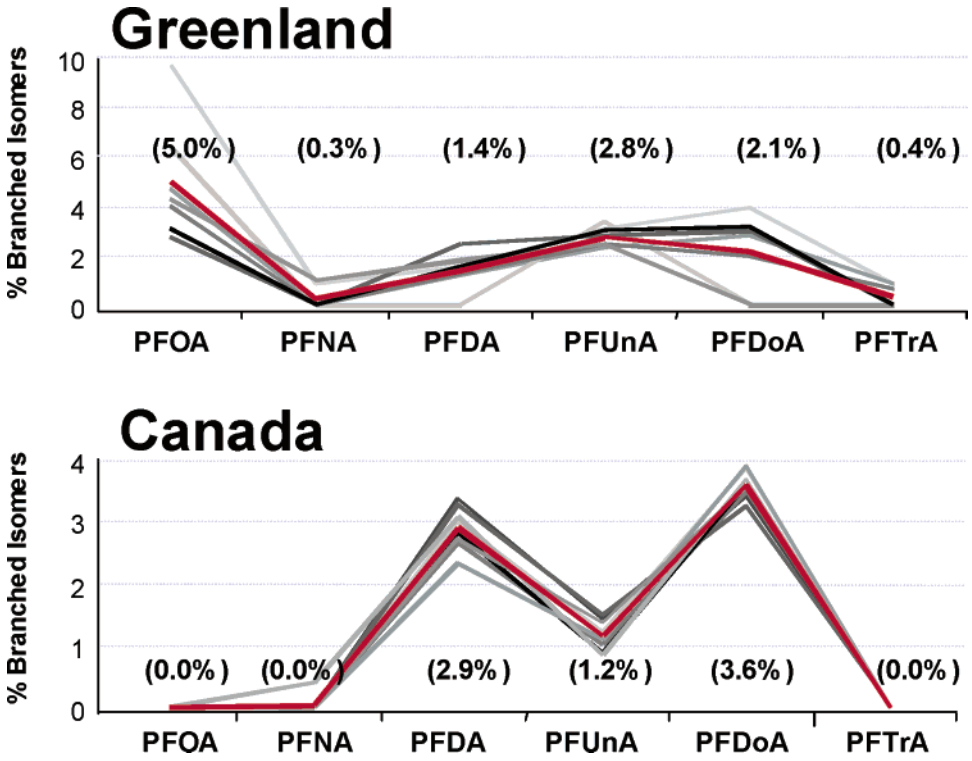

FIGURE 4. Branched isomer distribution of PFCAs in polar bears from Greenland (top) and Canada (bottom). Numbers in parentheses refer to average percentage.

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

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

Constitutional Isomer Profile of Longer-Chain (C > 9) PFCAs in Polar Bears. The constitutional isomer profiles of PFOA and PFNA found in polar bear liver tissue were consistent with the atmospheric degradation mechanism proposed for 8:2 FTOH. Both locations were characterized by a dominance of the linear isomer form, >90% linear PFOA and >99% linear PFNA, as compared to an authentic ECF PFOA standard (77% linear isomer). Atmospheric degradation mechanism of 8:2 FTOH to PFOA and PFNA observed by Ellis et al. is expected to apply to longer chained fluorotelomer alcohols (*17*). Thus, a potential source of PFDA and PFUnA in the Arctic is 10:2 FTOH while 12:2 FTOH could be responsible for PFDoA and PFTrA. The pattern of constitutional isomers for each of these PFCAs was determined to investigate this hypothesis.

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

Subtle differences in branched isomer distribution among PFCAs in both locations are apparent in Figure 4. There is a contrast in percent branched PFCA values between each location. The Greenland samples show the greatest degree of branched isomers in PFOA, PFUnA, and PFTrA as compared to PFNA, PFDA, and PFDoA. The opposite trend is observed in the Canadian samples. It is unknown what the cause of variation is between locales. In a recent study by Smithwick et al., perfluoroalkyl substances (PFAs) including PFCAs were quantified in polar bear samples from the same Greenland location reported here (*13*). Smithwick et al. compared PFCA concentrations in samples from East Greenland with those from southeastern Hudson Bay and other locations in the Canadian Arctic as well as Alaska and Svalbard (*49*). The samples from southeastern Hudson Bay and East Greenland had the highest levels of PFCAs of all locations (*49*). However, the average concentrations of PFCAs (8−13 carbons) were greater in Greenland polar bear liver samples as compared to the Canadian polar bear liver samples (*49*).

It is hypothesized that the source of PFCAs in the Arctic is atmospheric processing of fluorotelomer alcohols, which have the potential for long-range atmospheric transport. This does not necessarily imply a uniform Arctic PFCA concentration or isomer profile. Complex spatial patterns have been observed for many Arctic contaminants (*44*, *45*, *50*−*52*) as exemplified by chlorinated hydrocarbon profiles in Arctic biota (*44*, *45*, *50*−*52*). One factor may be due to air currents.

The dominant direction of air flow across eastern North America is north or east (*50*). Thus, contaminants in Greenland can reflect contribution from both North America and Europe sources, which may account for the consistent and distinct pattern in PFCA isomer distribution between Canadian and Greenland polar bear samples. This implies that there are differences in production and/or application of fluorinated materials between these two regions.

The source of the branched isomers of PFDA, PFUnA, PFDoA, and PFTrA is of considerable interest. Industrial production of perfluoroalkyl compounds with C > 8 would have been minor as it was not apparently pursued commercially (*29*). Historical large-scale production of perfluorooctyl compounds using ECF is documented, and byproducts of ECF are homologous compounds and their constitutional isomers (*53*). In the standard of ECF PFOA analyzed here, impurities of PFDA (0.1%) and PFDoA (0.2%) were determined. Therefore, it is possible that the branched isomers found of the longer chain PFCAs in the liver samples are a result of byproducts in the production of perfluorooctyl chemicals using ECF. If this is the case, it is curious as to why only one branched isomer peak is present in PFDA, PFUnA, PFDoA, and PFTrA when ECF produces a range of branched isomers as evidenced by the branched isomer profile of PFOA in the Greenland polar bears. It may be that the resolution of branched isomers of PFCAs with increasing chain length will be increasingly challenging as the physical property differences among the isomers will correspondingly diminish.

Differences in physical properties will affect not only resolution but transport also. Volatility alone is not sufficient in predicting transport as other physical properties (such as water solubility and octanol−water partitition coefficient) are also relevant. Physical properties of branched versus linear perfluorinated chains are difficult to predict. Smart reported little effect of branching on boiling points for various perfluorinated compounds (*54*). However, it is believed that branching will likely yield differential physical and chemical properties and, by inference, their respective environmental fate. We are actively pursuing these measurements.

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

The presence of branched isomers does not exclude a telomer source since telomerization conserves the geometry of the starting material. A branched telogen, for instance, will result in branched telomer compounds. In fact, a patent describes the deliberate synthesis of an isopropyl branched telogen, heptafluoroisopropyl iodide (*55*). Furthermore, a patent details the production and use of terminal isopropyl branched perfluorocarboxylic acids as surfactants (*56*). These branched perfluorocarboxylic acids were synthesized from the corresponding telogen, ranging from 5 to 16 carbons, and it is stated that the isopropyl branched acid is better at reducing surface tension of the liquid medium it is employed in than its linear counterpart (*56*). Irrespectively, identification of the type of branched isomer observed in the longer chain PFCAs of polar bears is necessary as it may further clarify potential sources. Furthermore, an exploration for branched isomers, within the large suite of telomer-derived materials currently in use, is certainly warranted since there remains the possibility that even minor quantities of these could contribute to what we observe in biological samples.

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