# Tissue, Blood and Intra-Brain Distribution of Perfluoroalkyl Carboxylates/Sulfonates, and Perfluorooctane Sulfonate Structural Isomers in East Greenland Polar Bears


Photo: R. Dietz


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## Introduction

Poly- and per-fluoroalkyl substances (PFASs) are some of the newer emerging contaminants that have been detected in Arctic biotic and abiotic compartments, and retrospective temporal reports have shown that in general their levels have been steadily increasing since the 1970s (*1-3*). The two groups of PFASs that have been of notable concern in Arctic ecosystems are perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs) (*1-3*). In general, reports have shown that PFSAs with chain lengths > $C_6$ and PFCAs > $C_8$ tend to bioaccumulate and biomagnify in aquatic food webs.

Polar bears (*Ursus maritimus*) are apex predators and may serve as indicators of pollution effects at higher trophic levels in Arctic marine ecosystems. It has been reported that polar bears, and particularly those from East Greenland, contain some of the highest PFAS concentrations in their liver relative to other wildlife worldwide, and in particular for the highly bioaccumulative perfluorooctane sulfonate (PFOS) and also for some PFCAs (*1-3*). Recent exponential increases (from about 2000 to 2006) have been reported for perfluorodecanoic acid (PFDA), perfluorotridecanoic acid (PFTrDA) and PFOS (as high as 6,340 ng/g ww) in the livers of East Greenland polar bears (*4*).

The majority of PFCA and PFSA (and selected precursor) studies have focused on liver concentrations due to high hepatic concentrations, while a few (including human studies) have examined concentrations in blood. Transport and distribution of PFCAs, PFSAs, and their precursors throughout the body have not been fully elucidated, although PFOS in liver and plasma has been shown to be associated with proteins such as serum albumin and fatty acid binding proteins (*5*). We recently reported that specifically within regions of the brain of polar bears, longer-chain PFCAs ($C_{10}$ – $C_{15}$) are also strongly correlated with extractable lipid content (*6*). **The present study examined the distribution and patterns of PFCAs, PFSAs and selected precursors (*6,7*), as well as PFOS branched and linear isomers (*8,9*), in the body of highly exposed East Greenland polar bears by examination in the liver, blood, muscle, adipose, and brain.**

## Study Design & Methods

### Sample Collection

Liver, blood, muscle, adipose, and brain samples (8 regions), detailed below, were taken from 20 East Greenland Polar Bears (14 males, 6 females), harvested by local hunters from Scoresby Sound, East Greenland, January to March 2006.

- Liver (n=19)
- Whole Blood (n = 19)
- Muscle (n = 20)
- Adipose (n = 20)
- Pons and Medulla (n=14)
- Cerebellum (n=15)
- Frontal Cortex (n=16)
- Occipital Cortex (n=17)
- Temporal Cortex (n=15)
- Striatum (n=11)
- Thalamus (n=8)
- Hypothalamus (n=4)

### Extraction and Clean-up (*6-9*)

0.1 - 1.0 g tissue, spiked with Internal Standards → Extract PFAAs/precursors with 10mM KOH in ACN/$H_2O$ (80/20 v/v) → Clean extracts by SPE using a WAX Cartridge and elute fractions

- Neutral Fraction A (PFAA precursors): Elute FTOHs and FOSA with MeOH → Analyze by LC-APPI-MS/MS
- Acidic Fraction B (PFAAs): Elute PFCAs, PFSAs and FTUAs with 1% $NH_4OH$/MeOH → **Analyze by LC-ESI-MS/MS**
- PFOS linear and branched isomers: Tetrabutylammonium hydroxide (TBAH) derivatization (GC in-port) to butyl PFOS isomer derivatives → **Analyze by GC-MS(ECNI)**

- Evaporate to dryness, reconstituted in $H_2O$, partitioned into TBAH-diethyl ether

| Samples were analyzed for: | Internal Standards Used: |
|---|---|
| **PFAAs:** | $^{13}$C PFHxA, PFOA, PFNA, PFDA, PFUdA, PFDoA |
| -PFCAs ($C_6$ – $C_{15}$) | $^{18}$O PFHxS, $^{13}$C PFOS |
| -PFSAs ($C_4$, $C_6$, $C_8$, $C_{10}$) | |
| **PFAA precursors:** | $^{13}$C PFOSA d-NMeFOSA |
| -FOSA (PFOSA, N-Me-FOSA) | $^{13}$C 6:2, 8:2, 10:2 FTOH |
| -FTOHs (6:2, 8:2, 10:2) | $^{13}$C 6:2, 8:2, 10:2 FTUCA |
| -FTUAs (6:2, 8:2, 10:2) | |

### Analysis and Quantification (*6-9*)

All tissue samples were analyzed for target compounds by LC-MS/MS using an ACE 3, C18, 50 mm L x 2.1 mm i.d., 3 µm particle size column. Samples blanks containing all components except the tissue were run to reduce contamination interference.

Lipid content was also determined for all tissues by extraction in 1:1 Hexane:DCM using an accelerated solvent extractor (ASE), followed by solvent evaporation and gravimetric analysis.

## Results

### ΣPFSA and ΣPFCA Concentrations: Tissue/Blood Distribution

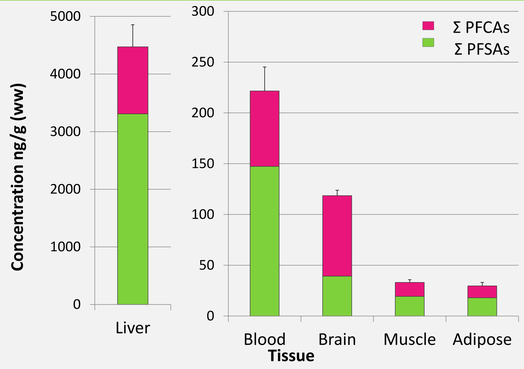


**Figure 1:** Arithmetic mean concentrations (ng/g ww) SE of ΣPFCAs and ΣPFSAs in the liver, blood, brain, muscle, and adipose tissue .

### $C_6$ to $C_{15}$ PFCA Chain Length Patterns in Tissues/Blood

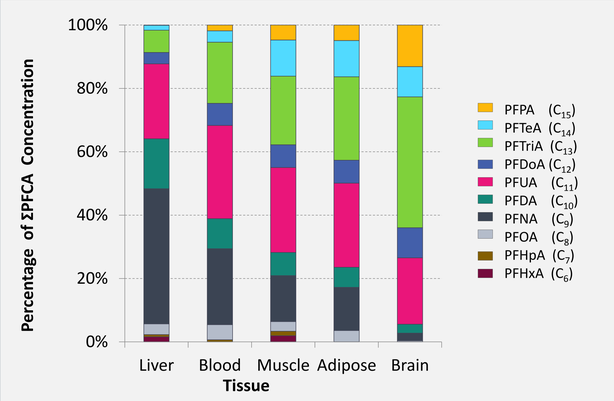


**Figure 2:** Arithmetic mean percentage composition of individual PFCA to ΣPFCA concentrations in the liver, blood, brain, muscle, and adipose tissue .

## Key Findings/Discussion

- Concentrations of ΣPFCA + ΣPFSA decreased in the following order: Liver (4466 ng/g ww) >> Blood (221 ng/g ww) > Brain (115 ng/g ww) > Muscle (33 ng/g ww) ≈ Adipose (29 ng/g ww) (**Fig. 1**) (*7*).
- The dominant PFCA varied by tissue: liver and blood samples contained proportionally more short chain PFCAs (PFNA ($C_9$) and PFUA ($C_{11}$)),whereas muscle, adipose, and brain samples contained more long chain PFCAs (PFUA ($C_{11}$), PFTriA ($C_{13}$)) (**Fig. 2**) (*7*).
- The concentration ratios between PFOS and its precursor perfluorooctane sulfonamide (FOSA) varied among tissues from 9 (±1) :1 (muscle) to 36 (±7) :1 (liver) (not shown) (*8*).

### ΣPFCA Concentation and Extractable Lipid Content

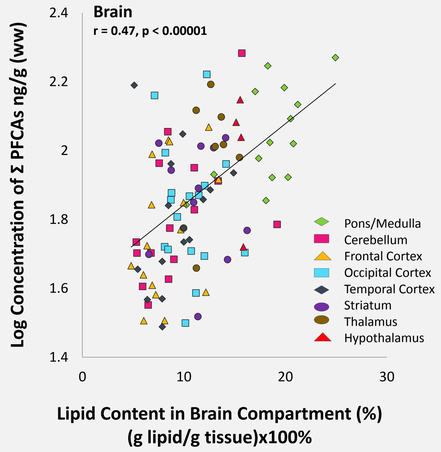


**Figure 3:** Correlation between lipid content and ΣPFCA concentrations in the brain regions of individual bears.

### Linear and Branched PFOS Isomer Patterns

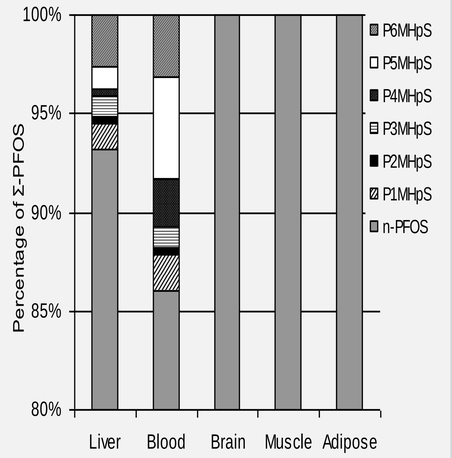


**Figure 4:** Arithmetic mean percentage composition of individual linear and branched PFOS isomers found in the liver, blood, brain, muscle, and adipose.

- A strong and highly significant correlation existed between the concentration of the ΣPFCA and lipid content for all regions of the brain (**Fig. 3**) (*6*). This correlation was not observed in the other tissues and blood (not shown). Lipid normalized (lw) PFOS and PFCA ($C_{10}$-$C_{15}$) concentrations were not significantly ($p$>0.05) different among brain-regions.
- Brain regions with both the highest PFCA, PFOS and FOSA concentrations and the highest lipid content (pons/medulla, thalamus, and hypothalamus), and are also the brain regions that receive the freshest supply of blood (i.e.: the most oxygen and nutrient-rich, closest to the incoming internal carotid arteries and vertebral arteries). A similar result was found for a comparable PFAS data for the same brain regions, but from bears collected in winter 2011 (R.J. Letcher, unpublished results).
- Linear PFOS (n-PFOS) accounted for 93.0 ± 0.5 % of Σ-PFOS isomer concentrations in the liver, whereas the proportion was significantly lower ($p$<0.05) in the blood (**Fig. 4**) (*8*). n-PFOS was the only PFOS isomer consistently detected (≥ 50% of samples). Branched isomers were quantifiable in the liver and blood, and 6-perfluoromethylheptanesulfonate (P6MHpS) was the dominant branched isomer.

The tissue- and blood-specific concentrations and patterns of PFCAs, PFSAs (PFOS), FOSA and PFOS structural isomers suggested PFAS-specific pharmacokinetics, and due to differences in e.g. protein affinities and interactions, and also lipid association in the brain, as well as transport, absorption, and/or metabolism in the body. This in turn suggests that there may be tissue-specific , PFAS-related toxicities and effects.

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