Separation and Characterization of Structural Isomers of Perfluorinated Compounds and their Epigenetic Toxicity

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ABSTRACT
Using modifications of current perfluorinated compound (PFC) analytical methods we have separated a technical PFOS mixture into three separate isomer peaks (Fig.1). The largest peak (peak I), presumed to be the linear isomer constitutes greater than 63 % of the materials mass while the earlier eluting peaks, peaks I and II constitute 2.5% and 34% of the mass respectively. The mixture also contained traces of PFOA and shorter chain sulfonates that were not quantified. Using a preparative HPLC column (150mm x 22.2mm) the linear isomer chain peak was separated from the branched chain to greater than 95.5% purity (Fig. 2).

To assess the potential epigenetic toxicity of the purified isomers and other PFCs, a gap junction intercellular communication (GJIC) assay was conducted. In the current study, the linear PFOS isomer was approximately equipotent, on a mass basis, at inhibiting GJIC compared to a technical mixture. The enriched branched and linear isomers had similar potencies (Fig. 3). This indicates the different PFSO isomers have similar potency for inhibition of GJIC. The specific chain length was a determining factor of inhibition, which is consistent with previous studies (Fig. 5). The PFCs with only 5–9 fluorinated carbon units showed inhibition potencies, regardless of their functional groups.

INTRODUCTION
• While current analytical methods are able to separate and quantify different PFCs such as sulfonates from carboxylates and different carbon chain lengths, little attention has been paid to the separation of branched from linear chain isomers and, in general standards used for PFC quantification have not been characterized for their isomer distribution.
• Similarly, all toxicological studies to date have been conducted using relatively crude analytical available PFPC preparations.
• In this study, separation of isomers in the commercial mixture PFOS was achieved using a HPLC-MS and a fraction collector. Potential epigenetic toxicity of purified PFOS and various PFPCs was evaluated using GJIC inhibition in vitro assay.

METHODS and MATERIALS
Preparative Chromatography
Preparative chromatography was conducted using a gradient of MeOH in 5 mM ammonium acetate. Separations were carried out on an HP 1100 HPLC system using a Retail 5 150mmX21.2mm, Sum, C18 column (Thermo Electron, San Jose, CA). The effluent from the column was split approximately 99.1 between a fraction collector and a Micromass Platform II spectrometer running in SIR mode to monitor chromatographic separation.

Analytical Chromatography
Analytical chromatography was conducted using a 250mmX2mm, Sum, 100 Å C18 column (Thermo Electron). Solvent A was 10% MeOH in 5 mM ammonium acetate, solvent B was 100% MeOH. Analytes were quantified by use of a HP 100 HPLC system interfaced to a Micromass Platform II mass spectrometer. The mass spectrometer was run in electrospray negative mode and analytes were determined by single ion monitoring (SIR).

NMR Methods
Purified standards were dissolved in deuterated methanol and analyzed using both proton (64 scans, 1 sec cycle delay, 499.96 MHz) and fluorine (32 scans, 5 sec cycle delay, 470.38 MHz) Varian Unity 300 MHz NMR.

The scrape loading & dye transfer assay for GJIC in vitro measurement

RESULTS and DISCUSSION

PFOS: Analytical chemistry

Inhibition of GJIC by various PFCs

Table 1. EC50 values of PFCs in the SL/DT assay conducted in current study (mg/L)

CONCLUSIONS
• Technical PFOS mixture can be resolved into at least 4 peaks.
• Linear chain PFOS is ‘pure’ as determined by NMR (and 99.9 % by LC/MS).
• Branched chain contains at least 3 components – one appears to be a terminal branch. Branched chain preparations may contain some C11 bond impurities?
• Linear PFOS appears to be only slightly more potent than branched chain in the GJIC assay.
• The effects of PFCs on GJIC depended on the functional groups (carboxylate, sulfonate, or alcohol) Among the PFCs tested, PFOS and FTOHs were the most potent inhibitors of GJIC.
• A probable determinant of inhibition was the chain length of the fluorinated carbon tail. Only PFOS with specific length (C5–C9) inhibited GJIC.
• The substitution of the carboxylic acid group with a sulfonate group increased the inhibitory potency.