Supporting Information

Perfluorinated Compounds, and Total and Extractable Organic Fluorine in Human Blood Samples from China

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Details of Materials and Methods

Chemicals and reagents

The potassium salt of perfluorooctane sulfonate (PFOS) was purchased from Tokyo Chemical Industries (Portland, OR). Potassium salts of perfluorohexanesulfonate (PFHxS), perfluorobutanesulfonate (PFBS), and perfluorooctanesulfonamide (PFOSA) were provided by the 3M Company. Perfluorononanoic acid (PFNA) was purchased from Avocado Research Chemicals Ltd. (Lancashire, UK). Perfluorooctanoate (PFOA) was obtained from Strem Chemicals Industries (Newburyport, MA). Perfluorohexanoic acid (PFHxA) was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA) were purchased from Fluorochem Ltd (Derbyshire, UK). Saturated fluorotelomer carboxylate (8:2 FTCA) and unsaturated fluorotelomer carboxylate (8:2 FTUCA) were purchased from Asahi Glass Co Ltd. (Tokyo, Japan). Purities of all the analytical standards were ≥ 95%. Oasis® weak anion exchange (WAX; 6 cc, 150 mg, 30 μm) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Envi-carb graphitized carbon was purchased from Supelco (Supelco, Bellefonte, PA). Milli-Q water was used throughout the experiment. Methanol (residual pesticide and PCB analytical grade), ammonium acetate (97%), ammonium solution (25%), acetic acid (99.9%), tetra-n-butylammonium hydrogen sulfate (TBA), methyl-tert-butyl ether (MTBE), sodium carbonate and sodium fluoride (99%) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Individual PFC analysis

Thirteen individual PFCs were analyzed using the ion-pairing method, the details of which are described elsewhere (*1*), and which is summarized in Supporting Information Figure 2. Briefly, 0.5-1 mL of whole blood was adjusted to 1 mL with 0.5 mL Milli-Q water. The solution was mixed with 1 mL 0.5 M TBA solution and 2 mL 0.25 M sodium carbonate buffer (pH 10) and then added to a 15-mL PP tube for extraction. After mixing, 5 mL MTBE was added, and the mixture was shaken for 20 min at 250 rpm. The organic and the aqueous layers were separated by centrifugation at 3000 rpm for 15 min. Four mL MTBE was removed and transferred to a second 15-mL PP tube. The extraction was repeated twice as described above, except that 5 mL MTBE was removed each time, instead of 4 mL. All three extracts were combined in the second 15-mL PP tube. The final extract was concentrated under nitrogen gas to 1mL after adding 1 mL methanol.

In order to reduce interferences, further Envi-carb (*2*) and solid-phase extraction (SPE) (*3*) cleanup were applied to the extract (Supporting Information Figure 2). Briefly, after ion-pairing extraction, 0.5 mL extract was added onto 25 mg Envi-carb graphitized carbon. The suspension was sonicated for 30 s and then shaken for 20 min at 250 rpm. The extract and Envi-carb were separated by centrifugation at 3000 rpm for 15 min. The supernatant was then transferred into a 100 mL PP bottle. Another 1 mL methanol was then added to the Envi-carb to retrieve all the extract. The mixture was then mixed using a vortex mixer for 30 s and then separated by centrifugation. The above procedure was repeated once. One hundred mL Milli-Q water was then added to the 100 mL PP bottle for further SPE cleanup.

After Envi-carb cleanup, all extracts were subjected to Oasis WAX cartridge (0.2 g, 6 cm^3) for further cleanup (*3*). The cartridge was firstly pre-conditioned by consecutively passing 4 mL 0.1% ammonium/methanol, 4 mL methanol and 4 mL water through the cartridge at a rate of 2 drops/sec. Approximately 100 mL extract in Milli-Q water was passed through the pre-conditioned cartridges at a rate of 1 drop/sec. In order to remove inorganic fluorine from the samples, 20 mL 0.01% ammonium/water was used (*4*). Three 10 mL Milli-Q water rinses were used to wash off the remaining ammonium in the cartridge. The cartridge was then washed with 4 mL 25 mM acetate buffer solution (pH 4) and this fraction was discarded. The water remaining in the cartridges was completely removed by centrifugation. The target analytes were eluted into two fractions. The first (F1) and second (F2) fractions were eluted by 4 mL methanol and 4 mL 0.1% ammonium/methanol, respectively. Each eluate was concentrated to 0.5 mL under a stream of high purity nitrogen.

Total (TF), inorganic (IF) and extractable organic fluorine (EOF) analyses

An aliquot of each blood sample was subjected to fractionation (Supporting Information Figure 2) for the analysis of TF, IF and EOF, using extraction procedures described elsewhere (*5*). TF was determined by taking 0.1 mL whole blood on a silica boat and placing it directly into the CIC. EOF (Fr1) was quantified from the extract of ion pairing (MTBE fraction), and EOF (Fr2) was quantified after another extraction of the ion pairing with hexane using CIC.

Instrumental analysis and quantification

Concentrations of PFCs in whole blood samples were analyzed by the use of high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS).

Separation of the analytes was performed by an Agilent HP1100 liquid chromatograph (Agilent, Palo Alto, CA) interfaced with a Micromass Quattro Ultima Pt mass spectrometer (Waters Corp., Milford, MA) operated in electro-spray negative mode. A 10 µL aliquot of extract was injected onto a Keystone Betasil C18 column (2.1 mm i.d. x 50 mm length, 5 µm, 100Å pore size, endcapped) with 2 mM ammonium acetate and methanol as the mobile phases starting at 10% methanol at a flow rate of 300 µL/min. The gradient was increased to 100% methanol at 10 min before reverting to the original conditions at 13 min. The desolvation gas flow and temperature were kept at 610 L/h and 450 ºC, respectively. The collision energies, cone voltages and MS/MS parameters for the instrument were optimized for individual analytes, and were reported elsewhere (*3*). PFC concentrations were quantified using external calibration curves including a concentration series of 0, 2, 10, 50, 200, 1000, 5000, 20000 pg/mL, and the deviation of every point from the standard was less than 20%. The linearity and repeatability of these calibration curves were confirmed prior to each set of determinations. Reliability of the above method was further verified through participation in the First International Laboratory Calibration Study coordinated by ASG-RIVO and Örebro University (*6*). Our laboratory reported acceptable results for the blood plasma sample with one of the least coefficients of variation. Thus, our results were judged to be accurate and precise.

Concentrations of TF and EOF were determined using combustion ion chromatography (CIC), and the details of for analyses are given elsewhere (*4,5*). This method involves modifications to the traditional combustion ion chromatography (CIC), by the combination of an automated combustion unit (AQF-100 type AIST; Dia Instruments Co., Ltd.) and an ion chromatography system (ICS-3000 type AIST;

Dionex Corp., Sunnyvale, CA). In this study, we removed possible sources of fluorochemical contamination in blanks by replacing certain parts of the instrument with non-fluorinated materials; the customized instrument, combustion ion chromatograph for fluorine (CIC-F), as described in detail elsewhere (*4,5*), was used for our studies. The sample extract or the blood sample was set on a silica boat and placed into a furnace at 900–1000°C. Combustion of the sample in the furnace converted organic fluorine and inorganic fluoride into hydrogen fluoride (HF). The HF was absorbed into sodium hydroxide solution (0.2 mmol/L). The concentration of F^- in the solution was analyzed using ion chromatography. Sodium fluoride (99% purity; Wako Pure Chemical Industries, Tokyo, Japan) was used as a standard for quantification. Five calibration points were prepared routinely at 0.2, 1, 5, 25, and 100 µg/L, and injected at 1.5 mL to check for linearity of the instrument. Quantification was based on the response of the external standards that bracketed the concentrations found in the samples. The analytical conditions of ion chromatography were reported elsewhere (*4,5*). All solutions were prepared in Milli-Q water, and the fluoride concentration in the Milli-Q water was <0.025 µg/L. The limit of detection (LOD) of organofluorine was evaluated for each sample, based on the maximum blank concentration, the concentration factors, and the injection volume of the sample. The LOD for blood samples was 3 µg F/L (ppb) when 1 mL of blood sample was used for analysis, in a final volume of 0.5 mL. The limit of quantifications (LOQs) were evaluated based on several criteria, including, (i) the smallest concentration of standard on the calibration curve that could be accurately measured within ± 20% of its theoretical value; (ii) a signal-to-noise ratio of equal to or greater than 10; (iii) concentration factor; and (iv) sample volume.

Quality assurance/quality control

Individual PFC analyses

To achieve lower detection limits for HPLC-MS/MS, all accessible polytetrafluoroethylene (PTFE) and other fluoropolymer materials were removed from the instruments and apparatus to minimize background signal due to contamination. PP tubes and septa were selected after a thorough checking of blanks. Impurities of each standard chemical were tested at the ng/mL level, and no contamination from chemical reagents was observed. Procedural blanks associated with every 10 samples were tested to check for possible laboratory contamination and interferences. The blank levels in the tube used for sample collection were checked and found to be less than 7 pg/mL in washed methanol for all analytes. All target chemicals were spiked into test samples of blood, and the samples were extracted and analyzed following the same procedures as described above. Recoveries were evaluated by subtracting background levels from detected concentrations. PFC concentrations were not corrected for recoveries.

Total fluorine analyses

The gases used for CIC are a source of fluorine contamination. Therefore, high- purity gases were used (Ar: 99.9999%, O2: 99.9995%). The ion chromatograph tubing, gas lines, valves, and regulator, which contained materials or parts made of polytetrafluoroethylene (PTFE), were replaced with stainless steel, polyetheretherketone, or polyethylene tubing. Furthermore, a gas purifier containing activated carbon was placed in the gas line to remove trace levels of fluorine from the gases. Analyses of total and extractable fluorine were conducted in duplicate. In-

house reference material (pig blood) was injected before and after 10 CIC injections to check the overall combustion efficiency of the combustion process.

References

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Supporting Information Table 1 Details of human blood samples analyzed in 2004

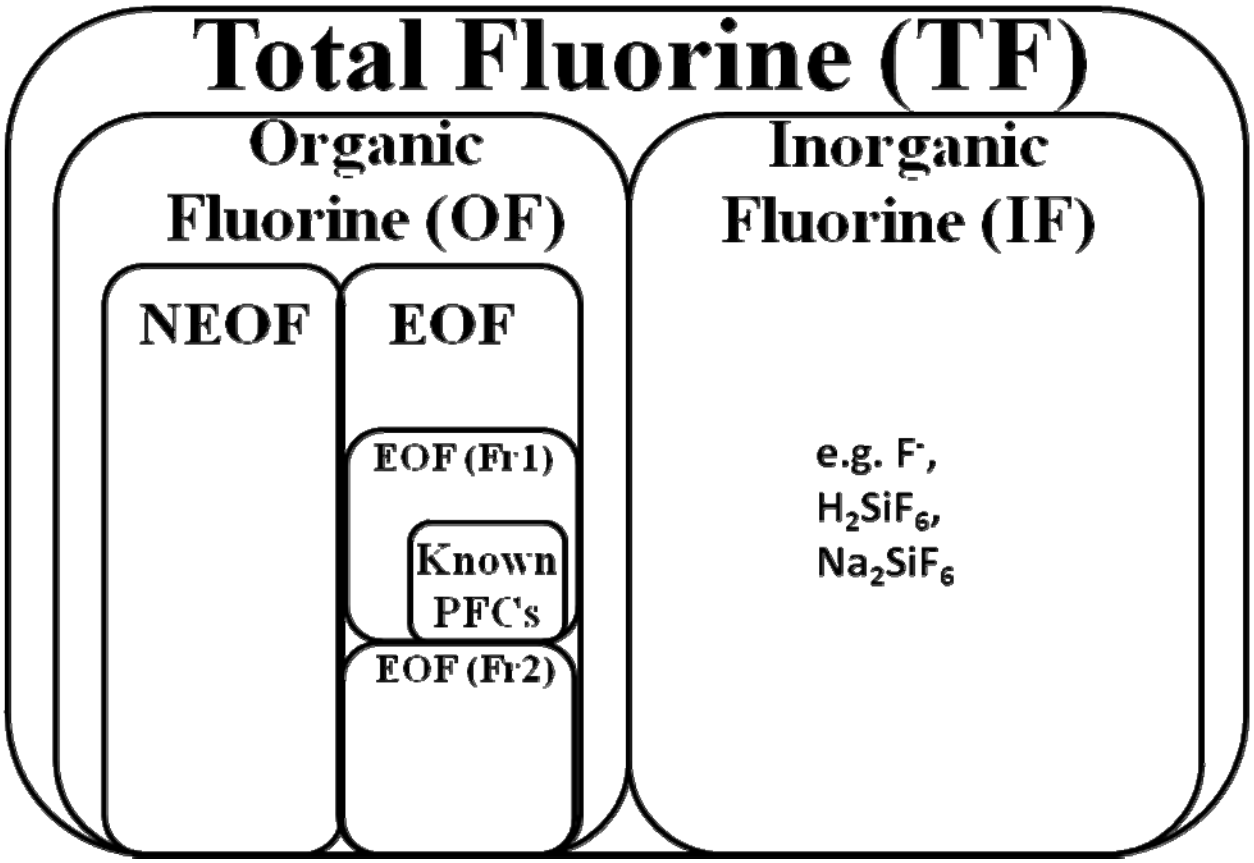
Province	Location	Gender	Age
Liaoning	Shenyang	M:2, F:3	18-40
Beijing	Beijing	M:2, F:3	24-40
Guizhou	Guiyang	M:3, F:2	23-40
Jiangsu	Nanjing	M:5, F:5	23-50
Jiangsu	Jintan	M:3, F:2	25-57

Supporting Information Table 2. Procedural blanks, recoveries and matrix-spiked recoveries for PFCs.

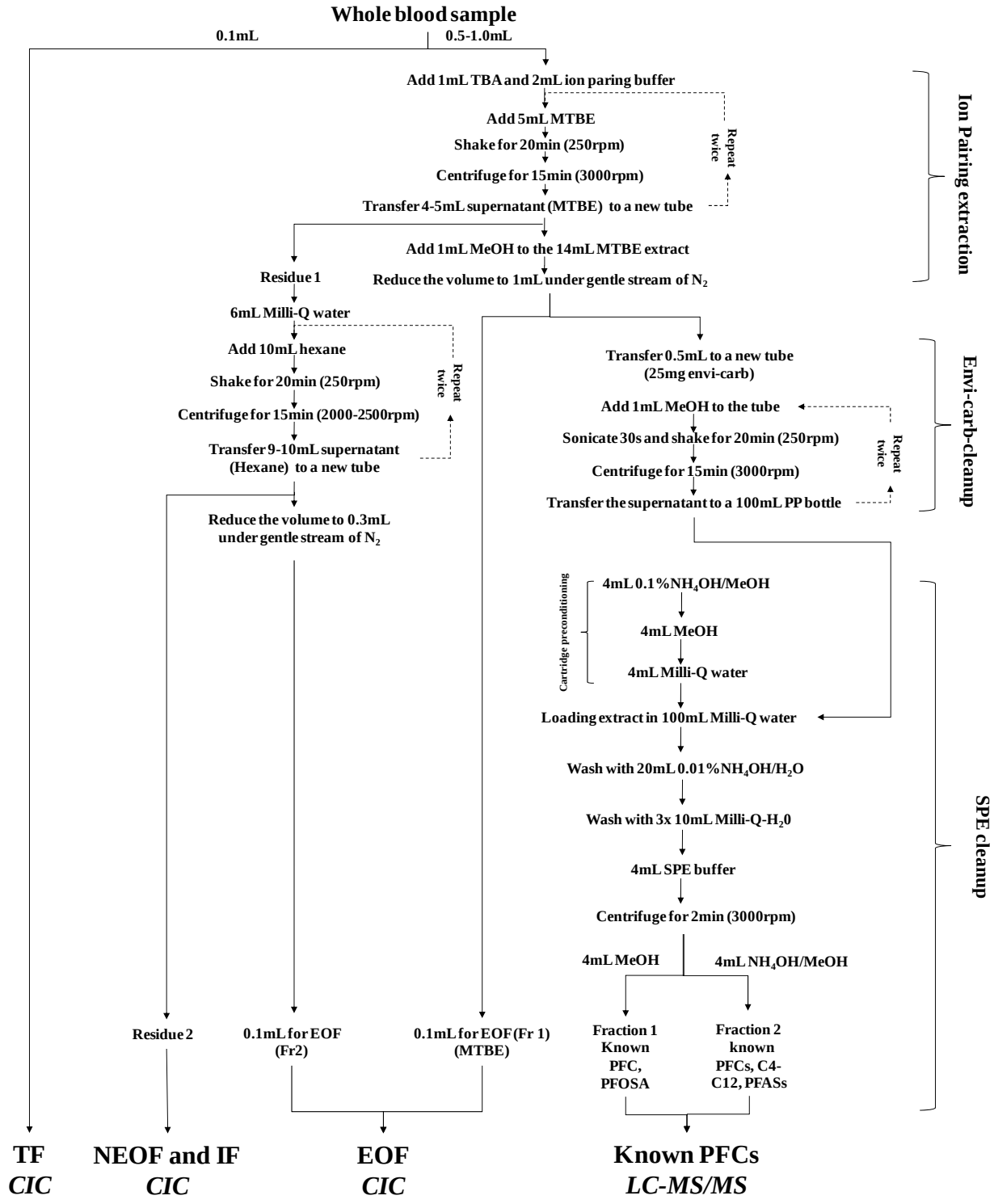
		PFOS	PFHxS	PFBS	PFOSA	PFDoDA	PFUnDA	PFDA	PFNA	PFOA	PFHpA	PFHxA	8:2FTCA	8:2FTUCA
Present study	**Blank ng/mL**	<0.0068	<0.01	<0.02	<0.02	<0.01	<0.05	<0.05	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01
	Mean Rec (%) *N=6*	95	93	90	55	88	88	77	90	89	106	107	73	75
	SD	4	4	6	12	11	15	24	3	15	4	14	11	5
	Mean matrix spike recovery (%), *N=4*	87	97	103	57	101	97	100	98	89	110	117	76	83
	SD	1	5	11	10	7	3	2	4	5	11	6	7	2
Previous study (Yeung et al., 2006)	**Blank ng/mL**	<0.2	<0.1	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-	-
	Mean matrix spike recovery (%), *N=4*	96.4	78.6	-	75.6	86.9	86.0	94.6	94.5	92.5	-	55.1	-	-
	SD	8	3	-	4	3	3	3	6	2	-	6	-	-

"-" not applicable.

Supporting information Figure 1. Components of total fluorine analyses.



Supporting Information Figure 2. Schematic diagram showing extraction and analysis methods for individual PFCs, EOF and TF.



Supporting Information Figure 3. PFC composition profiles in Chinese blood samples.