

**-A NOVEL MASS BALANCE APPROACH TO STUDY PERFLUORINATED CHEMICALS -
ANALYSIS OF TOTAL AND ORGANIC FLUORINE USING COMBUSTION ION
CHROMATOGRAPH FOR FLUORINE (CIC-F)**

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Introduction

In 1966, Taves reported the occurrence of considerable levels of fluoride in human sera and suggested that exposure through drinking water contributed to this occurrence. Furthermore, based on the analysis of total and inorganic forms of fluorine, presence of other forms of fluorine, particularly the organic forms, was suggested. However, due to the lack of analytical methods at that time, no specific identification of organic fluorine was possible. Later, in the 1990s, with the advancement of analytical techniques, specific organic forms of fluorine, particularly perfluorinated chemicals (PFCs) such as PFOS and PFOA were identified. In addition, the number of organofluorine acids detected in environmental and biological matrixes has been increasing over the last few years (e.g., from four in 2000 to more than 15 in recent years). Nevertheless, it is still not clear if all of the organic forms of fluorine have been identified and accounted for in human body yet.

PFOS and PFOA have received worldwide attention because of their distribution in wildlife on a global scale. It is now known that PFOS and PFOA constitute only a portion of the PFCs in the environment and a number of other fluorochemicals such as telomer alcohols and long-chain perfluorocarboxylates (PFCAs) have become increasingly important in monitoring surveys and risk evaluation. Several PFCs including PFOS and PFCAs have been nominated as candidate POPs (persistent organic pollutants) in 2006. However, information regarding environmental dynamics, fate and toxicity of PFCs, particularly those that are still not identified, is still needed for enacting effective regulations on the production and use of PFCs. The production methods and usage patterns of PFCs is complex which results in their release into the environment at multiple forms (e.g., polymers, precursors, degradation intermediaries, metabolites). Because of the lack of appropriate analytical standards and methods for all of the potential forms of PFCs that can occur in the environment, we developed a novel approach that involves the measurement of total fluorine, inorganic fluorine and organic fluorine by combustion ion chromatography (CIC).

Because the total amount of organic fluorine released into environment is not known, a mass balance approach involving the measurement of total fluorine (TF), total organic fluorine (TOF) and inorganic fluorine (IF) is necessary to enable comprehensive understanding of fate, effects and risks of PFCs. The mass balance approach will provide information on potential release of unknown PFCs, if any, into the environment. It is a challenging task because of the difficulties associated with trace level analysis of TOF. For instance, several PFCAs are present at parts-per-trillion or parts-per-quadrillion levels in aqueous media; however, the currently available methods to quantify TF using general type of CIC, can detect fluorine only at sub-parts-per-million or parts-per-billion levels. This is because of the high background levels arising from instrumental or procedural blanks. In this study, we have developed a method for trace level analysis of fluorine using new type of CIC (CIC-F) that enable trace level analysis of fluorine in any kind of matrixes. Sample matrixes were extracted and fractionated by systematic “multiple extraction method” to provide polar and non-polar fractions, and organic and inorganic fluorine fractions. Several matrixes including water, biota, and industrial products were subjected to analysis by this method. Contributions of known perfluorinated acids to total fluorine and the percentage of unknown organic fluorine in total fluorine were discussed.

Materials and Methods

Several kinds of environmental matrixes were subjected to fluorine measurement using combustion ion chromatography for fluorine (CIC-F). Water samples including seawater, run-off water, snow from aqueous film forming foam (AFFF) polluted environment and some biological matrixes including human blood were analyzed. Although the individual PFCs in human blood was analyzed based on ion-pair extraction method described elsewhere, several modifications were made to enable separation of inorganic fluorine (IF), and extractable organic fluorine (EOF). Residue from ion-pair extraction procedure was further extracted in a non-polar solvent (fraction 2) (Figure 1). The final residue from the matrix, after ion-pair solvent and hexane extractions, was expected to contain IF and non-extractable organic fluorine. Bulk analysis of blood sample was also carried out to determine total fluorine (TF). Surface water and wet deposition samples such as snow were subjected to modified solid phase extraction method of Taniyasu et al., 2005. Schematic outline of the extraction procedures is presented in Figure 1.

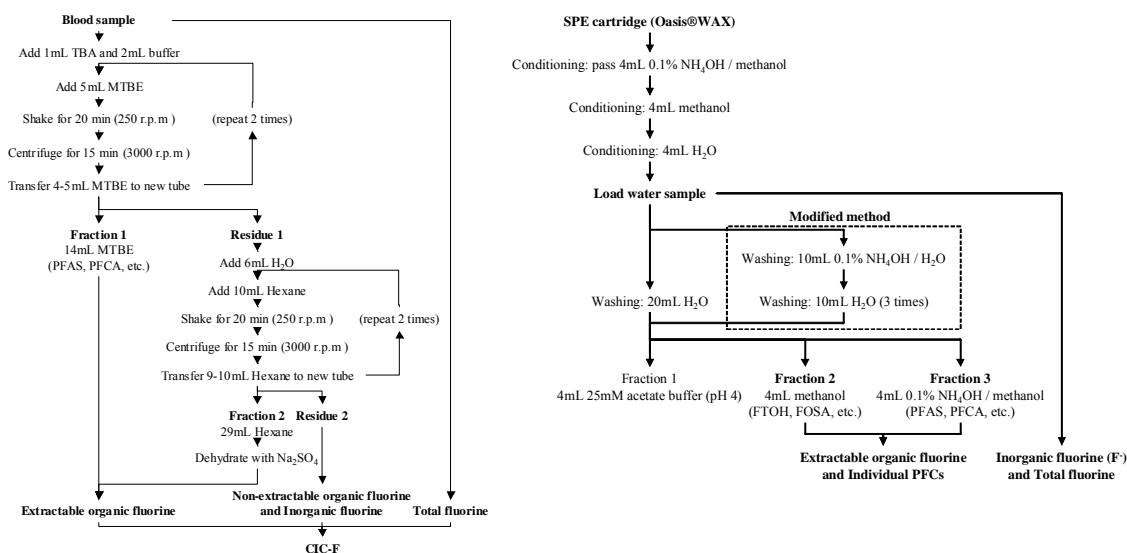


Figure 1. Schematic outline of the multiple extraction methods for total fluorine, organic fluorine, or inorganic fluorine in human blood (left panel) and water samples (right panel) using combustion ion chromatography for fluorine (CIC-F).

Concentrations of TF and EOF were analyzed using combustion ion chromatograph for fluorine (CIC-F), which was developed by our group earlier, by combining an ion chromatograph with a combustion furnace, interfacing HPLC columns for separation, and by modifying several parameters to improve the precision and sensitivity of the analysis. We identified possible source of contamination of blanks by fluorine. This includes, carrier gas and fluoropolymer containing parts within the instrument. Another challenging task was to resolve the co-elution of fluoride and organic acids. This was made possible by the combination of new type of HPLC column and switching system in ion chromatograph. Thus, CIC-F with low levels of blank and improved sensitivity by three orders of magnitude, compared with general CIC was developed. Details of CIC-F method, including the identification of possible sources of contamination of instrumental blanks, have been reported elsewhere (Miyake 2006). The limit of detection (LOD) using CIC-F for water and blood samples was 1 ng-F/L (ppt) and 1 µg-F/L (ppb), respectively. To our knowledge, only above method are applicable for trace analysis of TF in human blood and environmental water samples.

Results and Discussion

The mass balance approach to study PFCs is explained by contribution analysis as shown in Figure 2. TF was quantified at part-per-trillion levels in water sample and part-per-billion levels in biota. EOF was fractionated using solid phase extraction method described in Figure 1.

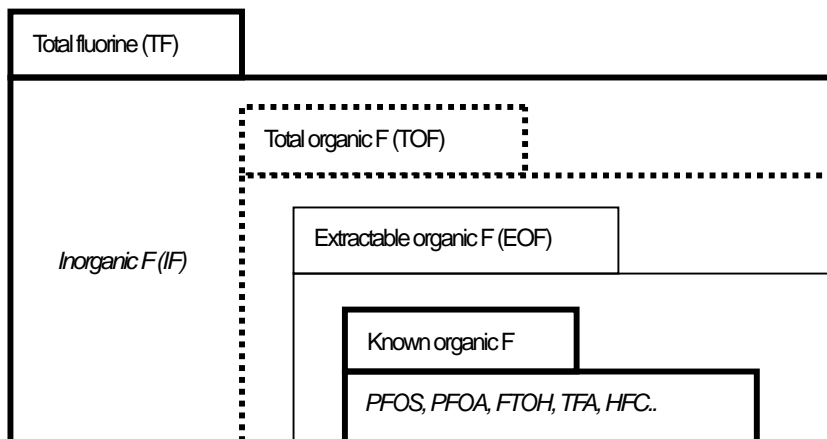


Figure 2. Mass balance approach to study fluorochemicals in the environment

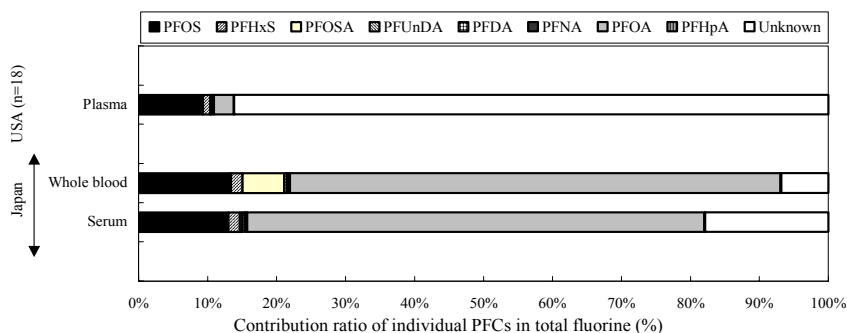


Figure 3. Contribution of individual PFCs to TF in human blood (US general population and occupationally exposed Japanese blood)

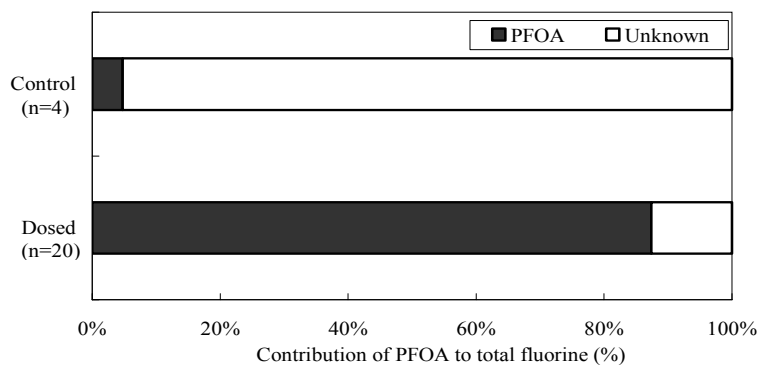


Figure 4. Contribution of individual PFCs to TF in the blood of PFOA-exposed rat

Individual PFCs such as PFOS and PFOA were quantified at part-per-trillion levels in biota and part-per-quadrillion levels in water samples.

First, for the study of mass balance analysis, IF needed to be separated from TOF because more than 95% of fluorine is expected to be present as IF in water samples. Secondly, it was necessary to confirm the differences in the levels of TOF and EOF. CIC-F was used to analyze Fractions 1 and 2, as shown in Figure 1. In EOF analysis, solid samples often showed unstable extraction efficiencies because of the interaction between particulate matter and individual PFCs. Similar phenomenon was found for TOF. It is presumed that there are non-extractable organic forms of fluorine that exist even after ion pairing or solid phase extraction. The bulk measurement of TF is expected to reflect actual concentration in samples because of its invariably higher concentration than that of known PFCs such as PFOS. Experiments to clarify the difference between TOF and EOF are necessary for sample matrices to validate the results.

As a result, approximately 180 ppb (ng/mL) of fluoride was found in the general population in the US. Although the method and the matrix used by Taves in the 1960s was different from what was used in our study, fluoride concentrations

found in our study are ten times higher after forty years.

Figure 3 shows the contribution analysis of individual PFCs to total fluorine in human blood. Approximately 30% of the PFCs in the US

general population is known and the rest is unknown, and this unknown fraction also includes IF. Human blood from occupationally exposed samples showed the predominance of PFOS and PFOA in TF. A similar contribution of PFOA to TF was found in the blood of rats exposed to PFOA. Figure 4 shows the results of contribution of known organic fluorine to total fluorine in the blood of rats exposed to PFOA. Blood from control rats showed only 8 % of TF to be contributed by PFOA. In PFOA exposed rat, 88 % of TF was PFOA. These results suggested not only the usefulness of mass balance analysis in risk evaluation but also the presence of elevated levels of TF in the blood of experimental rats and in occupationally exposed humans.

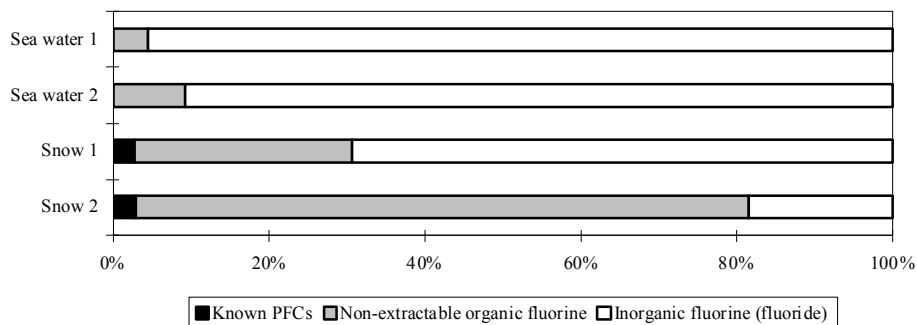


Figure 5. Contribution of known PFCs to TF in water and snow samples

Contributions of known PFCs (perfluoroalkylsulfonates- C2 to C18 and PFCAs and some FTOHs) to TF in surface seawater collected far from known sources of contamination and in snow samples collected from a location affected by AFFF discharge are presented in Figure 5. Greater than 90% of the TF was contributed by IF in relatively less-contaminated seawater samples. Estimated percentages of known PFCs in seawater were less than 0.0005%. On the other hand, snow samples from AFFF polluted environment contained significant contribution of TF by known PFCs such as PFOS and PFOA. It is also worth to mention that 30% to 70% of the TF were contributed by non-extractable organic fluorine in snow. Further studies are needed to identify these unknown and unextractable organic forms of fluorine in this matrix.

Although the CIC-F with multiple extractions is the only applicable method for determination of trace level TF in human blood and environmental waters to our knowledge, further works involve refinement of the quantification approaches. Further, this is a preliminary study conducted to evaluate the potential occurrence/importance of unknown PFCs in the environment. Comprehensive studies involving all kinds of fluorinated chemicals including source materials (e.g. industrial products) and environment and biological samples are subjected for further analysis of similar approach. This project of mass balance analysis of PFCs using CIC-F is underway with support by the Ministry of Economy, Trade and Industry, Japan. This new approach is also useful for risk evaluation for the joint projects that are underway at AIST and NIAH, Tsukuba, Japan and for several international participants.

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