

Survey on Per- and Polyfluoroalkyl Substances in Aquatic Biota in Okinawa, Japan -Is *N*-ethyl perfluorooctane sulfonamidoethanol related to formation of PFOS in Fish?-

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Introduction

Per- and polyfluoroalkyl substances (PFASs) have been widely used in industrial applications and consumer products and are detected globally in the water environment and wildlife. PFASs are currently recognized as one of the contaminants of emerging concern, and the regulation of representative chemicals has been started. For example, perfluorooctane sulfonate (PFOS) and related substances were listed in the Annex B (restriction of production and use) of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009. In Japan, using and importing PFOS have been prohibited since 2010 under the Chemical Substances Control Law. In addition, on May 2016, U.S. Environmental Protection Agency (U.S. EPA) established the health advisory levels at 70 ng/L of the combined PFOS and PFOA concentrations from drinking water [1]. Meanwhile, on January 2016, it was broadcasted that high PFOS concentration (183-1,320 ng/L) was detected in water samples from Dakujaku-river near Kadena Air Base in Okinawa Prefecture, Japan [2]. Original source of PFOS at this area was largely unknown and there was a possibility that related PFASs have also been discharged and contributed to form PFOS in this ecosystem. Biota can be used to understand the trend of contamination in a target area and to investigate on the possibility of biodegradation from polyfluoroalkyl substances to perfluoroalkyl substances [3,4]. Thus, the main purpose of this study was to conduct further surveys on the occurrences of PFASs in Okinawa and investigate on the source of PFOS contamination.

Materials and methods

Preparation of samples.

Four types of fish samples, namely, swordtail (*Xiphophorus helleri*) ($n=3$), pearl danio (*Danio albolineatus*) ($n=2$), guppy (*Poecilia reticulata*) ($n=4$), tilapia (*Oreochromis niloticus*) ($n=6$) were collected in Hija River near Kadena Air Base during 16th -19th June 2016. Sampling location is shown in **Figure 1**. Tilapia samples were separated into muscle and viscera. Biota samples were stored on ice and kept at -20°C in the laboratory until analysis.

Pre-treatment.

Samples (whole body or muscle and viscera) were freeze-dried and homogenized followed by ion-pairing method. Firstly, 1 g of sample and recovery surrogate (5 ng each of ¹³C₂-labeled PFHxA, ¹³C₄-labeled PFOA, ¹³C₄-labeled PFDA, ¹³C₄-labeled

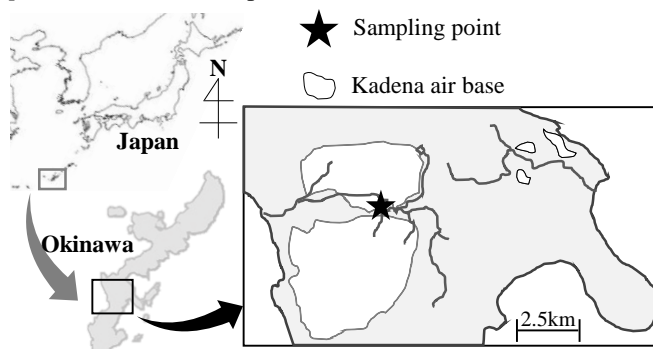


Figure 1 Map of sampling location in Okinawa, Japan

PFOS in methanol) were added to a new PP tube. Then, 1 mL of 0.5M tetrabutyl ammonium hydrogen sulfate, 2 mL of 0.25 M sodium carbonate buffer solution (pH was adjusted to 10 by using NaOH) and 5 mL of methyl *tert*-butyl ether (MTBE) were added to each sample. After shaking for 5 min. and centrifuging at 3,000 rpm for 15 min., the 5 mL MTBE layer was added to the new PP tube. These steps were repeated. The total 10 mL extracts in MTBE was exchanged to 2 mL methanol under N₂ gas purging. After that, extracts passed through 0.2 µm syringe filter (Whatman®) and ENVI™-carb cartridge (Supelco) to remove matrix substances. The final sample volume was 2 mL.

Instrumental Analysis and Quantification.

Total of 29 PFASs were analyzed by LC-MS/MS (Agilent), namely, 12 Perfluoroalkyl Carboxylates (PFCAs, C4-14 and 16), three Perfluoroalkyl Sulfonates (PFSAs, C4, 6, 8), three fluorotelomer carboxylic acids (n:2 FTCAs, n=6,8,10), three fluorotelomer unsaturated carboxylic acid (n:2 FTUCAs, n=6,8,10), three fluorotelomer sulfonate (n:2 FTSs, n=6,8,10), perfluorooctane sulfonamide (FOSA), *N*-methyl perfluorooctane sulfonamide (*N*-MeFOSA), *N*-ethyl perfluorooctane sulfonamide (*N*-EtFOSA), *N*-methyl perfluorooctane sulfonamidoethanol (*N*-MeFOSE) and *N*-ethyl perfluorooctane sulfonamidoethanol (*N*-EtFOSE). Details of separation and quantification of the instrument were explained in a previous publication [5]. Instrumental detection limit was 0.01-0.03 ng/mL and instrumental quantification limit was 0.02-0.09 ng/mL. Recovery rates were ranged between 36-190%, and the relative standard deviations ($n=15$) were less than 35%.

Results and discussion

PFASs concentrations in fish samples.

PFASs concentrations in fish samples collected from Hija River in Okinawa, Japan were shown in **Figure 2**. Among PFCAs and PFSAs, PFOS concentration was the highest at 64 ng/g-wet on average of 15 fish samples. PFOS levels in fish in this survey were 700 times higher than the monitored data from the survey conducted by Japanese Ministry of the Environment in 2015 [6]. In case of 14 polyfluoroalkyl substances, 6:2 FTS, 8:2 FTS and *N*-EtFOSE were detected and *N*-EtFOSE concentration was 584 ng/g-wet on average. In the best of our knowledge, the occurrence of *N*-EtFOSE in environmental biota samples have not been reported before.

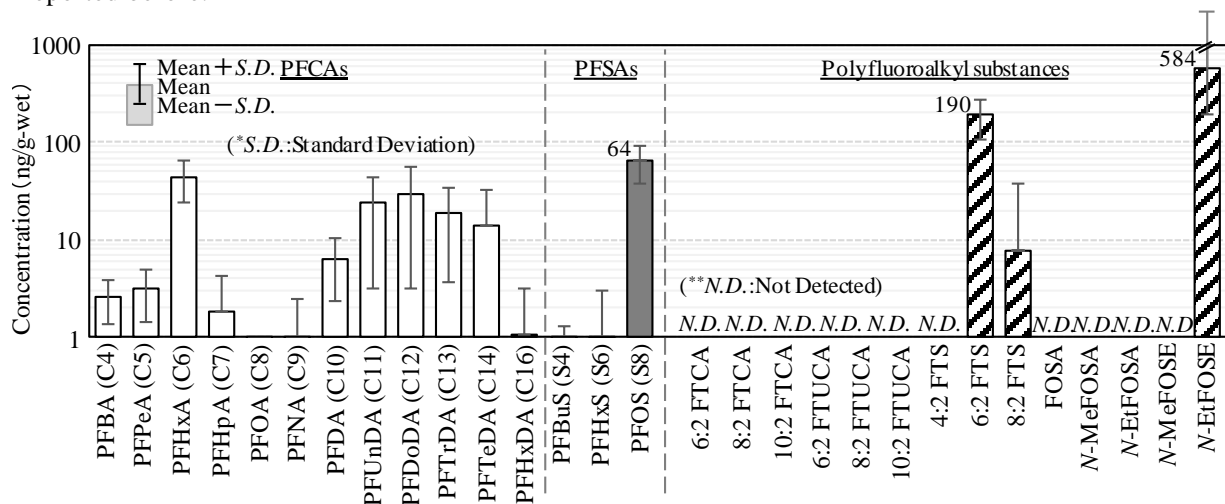


Figure 2 PFASs concentrations in fish samples collected from Hija River in Okinawa, Japan ($n=15$)

Correlation between body length of fishes and *N*-EtFOSE/PFOS concentration ratio

Figure 3 shows the relationship between body length of fishes and the *N*-EtFOSE/PFOS concentration ratio. A significant negative correlation ($r = -0.602$) was found (an outlier was excluded in the calculation). Previous studies have reported that precursors can be transformed to PFASs in the food web [7] [8], so it was indicated that biotransformation of *N*-EtFOSE might be occurred during the retention time in the environment [9] [10].

Comparison of the concentrations between body and viscera in analyzed Tilapias

The concentrations in viscera and body in tilapia were plotted in **Figure 4**. *N*-EtFOSE concentration in body was found at 8.8 – 493 times higher than that in viscera. In contrast, concentrations of PFOS and the other PFASs in body were found at the almost same level with those in viscera or less. This suggested that *N*-EtFOSE might be metabolized into PFOS or other PFASs during digestion in fishes.

Investigation on non-targeted PFASs by using HPLC MS/MS MRM analysis

MRM conditions for analyzing non-targeted PFASs in this study was searched from previous studies [11,12]. **Figure 5** illustrated the results of MRM analysis of six unknown compounds in a tilapia. Chromatograms which matched with the MRM condition of perfluorooctane sulfonamidoalkyl amine (PFOSAm) and *N*-ethyl perfluorooctane sulfonamido acetic acid (*N*-EtFOSAA) were detected from body, while those were not found from viscera. PFOSAm is a biodegradation product from perfluorooctane sulfonamidoalkyl amine ammonium salt (PFOSAmS) which is an effective ingredient of aqueous film-forming foams and it was suspected as a parent compound of the series of PFASs [13]. In addition, Ullah et al (2014) demonstrated that *N*-EtFOSAA is a degradation product from *N*-EtFOSE [14]. Thus, it also supported the assumption of transformation from *N*-EtFOSE to PFOS.

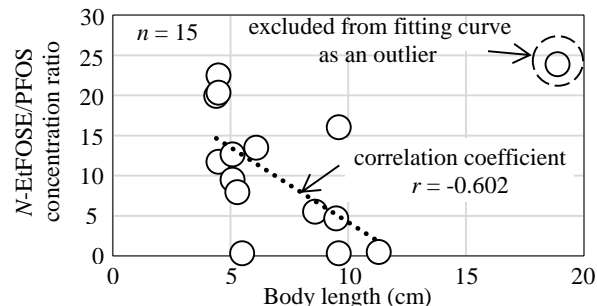


Figure 3 Correlation between body length of fish samples and the *N*-EtFOSE/PFOS concentration ratio

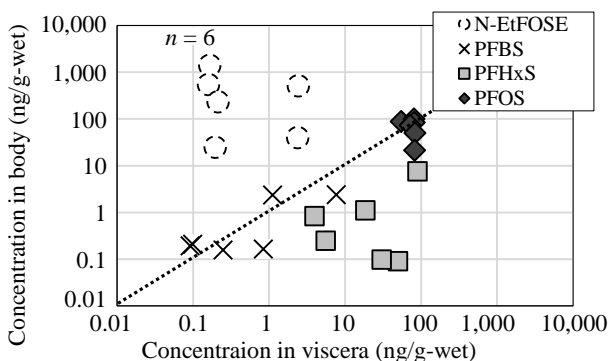


Figure 4 Concentrations in different body parts in tilapias

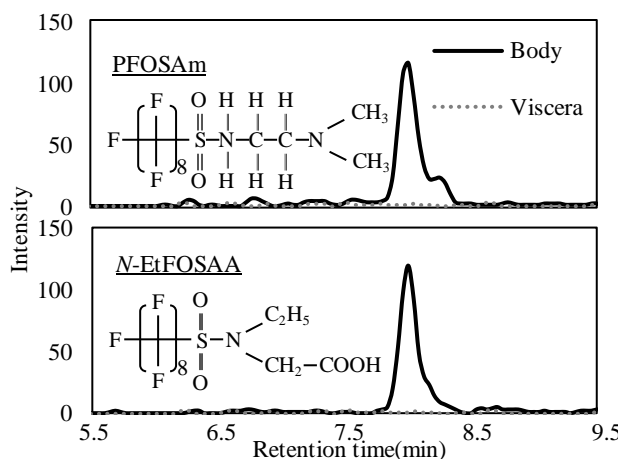


Figure 5 Chromatograms of PFOSAm and *N*-EtFOSAA detected from a fish sample (Tilapia)

Acknowledgements

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