Formation of PFOS and other metabolites from *N*-Ethyl Perfluorooctane Sulfonamidoethanol (*N*-EtFOSE) during an exposure experiment to Japanese Medaka

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Introduction

Per- and polyfluoroalkyl substances (PFASs) have been widely used in industrial applications and consumer products and perfluoalkyl acids (PFAAs) have been recognized as one of the contaminants of emerging concern. Regulations have been strengthened on the representative compounds such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). For example, 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. Recently, on May 2016, U.S. Environmental Protect Agency (U.S. EPA) established the health advisory levels at 70 ng/L of the combined PFOS and PFOA concentrations from drinking water[1]. However, PFAAs are still detected globally. Some polyfluoroalkyl substances are reported that may form PFAAs through abiotic or biologically catalyzed transformation[2,3,4]. Kitao *et al*. (2017) reported that PFOS and *N*-ethyl perfluorooctane sulfonamidoethanol (*N*-EtFOSE) were detected at 64 ng/g-wet and 584 ng/g-wet on average from riverine fish samples (n=15) collected in Okinawa, Japan in 2016[5]. In addition, their discussions based on the comparison of the concentration between body and viscera in analyzed *tilapias* suggested that *N*-EtFOSE might be metabolized into PFOS and/or other perfluoroalkyl sulfonates (PFSAs) during digestion in fishes[5].

The objective of present work was to conduct an exposure experiment of *N*-EtFOSE to a riverine fish in order to understand the behavior of *N*-EtFOSE including uptake by fish and its *in vivo* transformation into PFOS and other metabolites during the experiment.

Materials and methods

Exposure experiment

Exposure experiment was designed as shown in **Fig.1**. A riverine fish, Japanese medaka (*O. latipes,* 7 months old) was prepared for the experiment. The experiment was conducted in two phases: an exposure period (7 days) and a depuration period (7 days). In the exposure period, a water tank made of polypropylene was

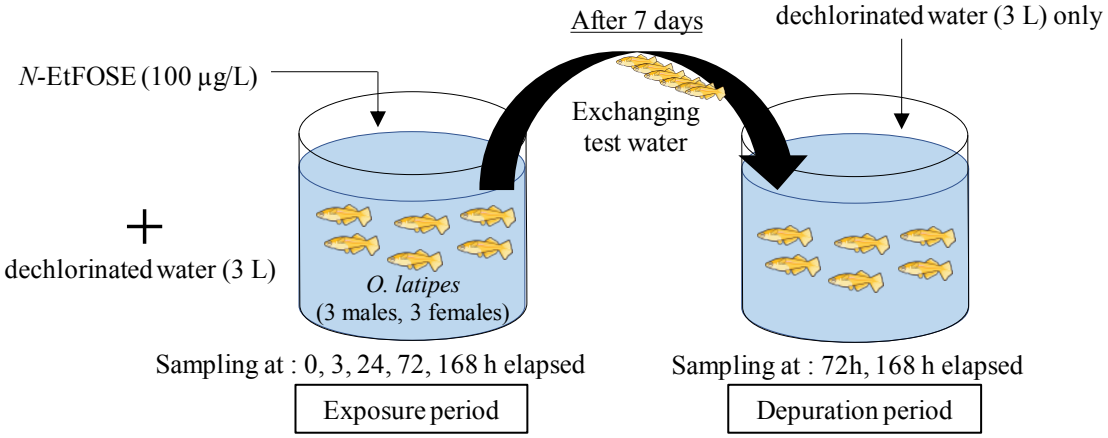

Figure 1 Overview of this study (Exposure experiment)

provided with 100 µg/L of *N*-EtFOSE in 3 L of dechlorinated water. Seven tanks were prepared in this manner and experiment was started just after *O. latipes* (3 female and 3 male) were introduced to each tank. At 5 selected elapsed time (0, 3, 24, 72, 168 hours (h)), exposure for a set of water tank was terminated. At each termination, *O. latipes* (3 male and 3 female) and test water were collected for chemical analyses. In the depuration period, two remaining sets of water tank (after 168 h elapsed in exposure period) were used for the following steps. *O. latipes* were move to a water tank containing 3 L of dechlorinated water only (not *N*-EtFOSE solution) and the experiment was started. At two elapsed time (72 h (240 h in total) and 168 h (336 h in total)), experiment for a set of water tank was terminated. At each termination, samples were collected for chemical analyses in the same way with the exposure period. In each tank, water was aerated and temperature was controlled at 25 □. Each tank was coved by cap to prevent diffusion of *N*-EtFOSE and its transformation products. In addition, laboratory bench was covered by black plate to prevent photolysis of target chemicals.

Analyses of 22 PFASs

Fish samples were freeze-dried and homogenized followed by ion-pairing pre-treatment method. Firstly, samples and recovery surrogate (5 ng each of $^{13}C_2$-labeled PFHxA, $^{13}C_4$-labeled PFOA, $^{13}C_4$-labeled PFDA, $^{13}C_4$-labeled PFOS in methanol (LC/MS grade, Wako) were added to a new PP tube. Then, 1 mL of 0.5 M 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 a new PP tube. These steps were repeated. The total 10 mL extracts in MTBE was exchanged to 2 mL methanol under N_2 gas purging. After that, extracts passed through 0.2 µm syringe filter (Whatman®) and ENVI^{TM}-carb cartridge (Sigma-Aldrich) to remove matrix substances. The final sample volume was 2 mL. Water samples were extracted by solid phase extraction (SPE) passing through an Oasis® WAX cartridge (Waters). After drying the cartridges via centrifugation at 3,000 rpm for 4 min., Target chemicals were eluted with 1 mL methanol followed by 1 mL methanol with 0.1% ammonium. Samples were reconstituted into a final volume of 2 mL.

Total of 22 PFASs were analyzed by LC-MS/MS (Agilent), namely, 12 Perfluoroalkyl Carboxylates (PFCAs, C4-14 and 16), 3 PFSAs (C4, 6, 8), perflorooctane sulfonamide (FOSA), *N*-methyl perfluorooctane sulfonamide (*N*-MeFOSA), *N*-ethyl perfluorooctane sulfonamide (*N*-EtFOSA), *N*-methyl perfluorooctane sulfonamidoethanol (*N*-MeFOSE), *N*-ethyl perfluorooctane sulfonamidoethanol (*N*-EtFOSE) *N*-ethylperfluoro-1-octanesulfonamidoacetic Acid (*N*-EtFOSAA), Perfluoro-1-octanesulfonamidoacetic acid (FOSAA). Details of separation and quantification of the instrument were explained in a previous publication[6]. Instrumental detection limit was 0.01-0.03 ng/mL and instrumental quantification limit was 0.02-0.09 ng/mL.

Results and discussion:

PFOS formation during exposure period and depuration period

PFOS concentration in test water during exposure period and depuration period was shown in **Fig. 2**. PFOS concentration was steady at 0.5 ng/L until 3 h elapsed. At 24, 72 h elapsed, the concentrations were 3.2 ng/L and 3.4 ng/L. This indicated that PFOS was formed during the first 24 h after the exposure of *N*-EtFOSE started while PFOS concentration was reached steady state by 72 h elapsed. However, at 168 h elapsed, PFOS concentration was increased to 10.7 ng/L. In addition, after exposure period terminated, depuration period was started by using *N*-EtFOSE free test water. As a result, at 240 h and 336 h elapsed, PFOS concentration was 15.4 ng/L and 25.9 ng/L, respectively. This suggested that PFOS was discharged from *O. latipes* into test water, which resulted in the increase of PFOS

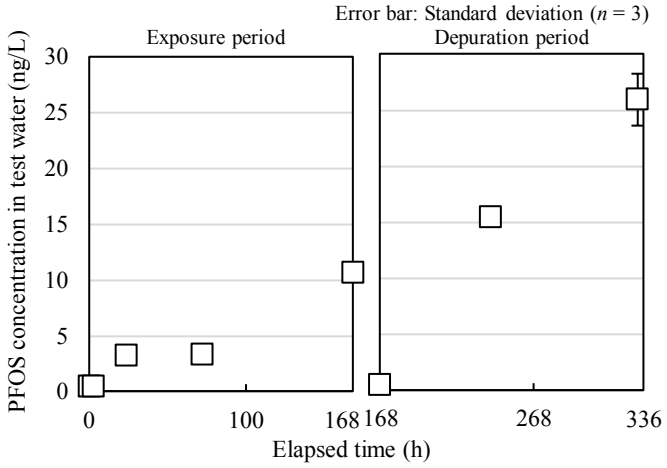

Figure 2 PFOS concentration in test water during experiments

concentration in the test water. Thus, biotransformation of *N*-EtFOSE and/or its transformation into PFOS was confirmed throughout the depuration period. Moreover, the rate of PFOS concentration increase was assumed to be higher during the biotransformation in *O. latipes* than that during the hydrolysis in the test water.

Transformation of N-EtFOSE during exposure period

Concentration of *N*-EtFOSE and its transformation products during exposure period was shown in **Fig. 3**. The size of *O. latipes* used in this experiment were in the range of 3 to 4 cm. No mortality nor abnormal swimming behavior were observed in the population of *O. latipes* during the experiment. In the test water during the exposure period, the concentration of *N*-EtFOSE, the original exposure substance, was decreased to 20,300 ng/L at 3 h, 4,700 µg/L at 24 h and 147 ng/L at 168 h elapsed (0.147% of the original concentration). In *O. latipes* during the exposure period, *N*-EtFOSE concentrations were increased to 253 ng/g-wet in female and 158 ng/g-wet in male after 3 h elapsed while those were decreased to 82 ng/g-wet and 139 ng/g-wet after 168 h elapsed. In consideration of the timing of *N*-EtFOSE concentration decrease in test water, the decrease of *N*-EtFOSE concentration in *O. latipes* was suspected due to the metabolism in the body. *N*-EtFOSA concentration in test water was at the maximum after 3 h elapsed (2,000 ng/L). *N*- EtFOSAA, FOSAA, FOSA concentration in test water was at the maximum after 24 h elapsed (116, 5.8, 309 ng/L, respectively). Concentration of *N*-EtFOSAA, FOSAA, FOSA in *O. latipes* was gradually increased until the termination of exposure at 168 h elapsed. Therefore, FOSAA was considered to be formed from *N*-EtFOSE and/or its transformation products in test water and in *O. latipes*. In contrasts, concentration of *N*-EtFOSA in *O. latipes* was not stable during the exposure period. This result indicated that formation of *N*-EtFOSA from upstream precursors and its decomposition into next transformation products were occurred at the same time at the similar rate. PFOS concentration in *O. latipes* was increased until the termination of exposure at 168 h elapsed (268 ng/g-wet in female and 373 ng/g-wet in male). At 168 h elapsed, bioaccumulation factor (*BAF*) of PFOS was calculated as 25,100 L/kg-wet in female and 34,900 L/kg-wet in male. This value was much higher that the reported *BAF* for PFOS in fish under a standard substance exposure test (2,796 L/kg-wet in bluegill sunfish (*L. macrochirus*)). Therefore, most of the PFOS concentration in *O. latipes* was considered to be derived from transformation of *N*-EtFOSE and its transformation products not from transfer from test water.

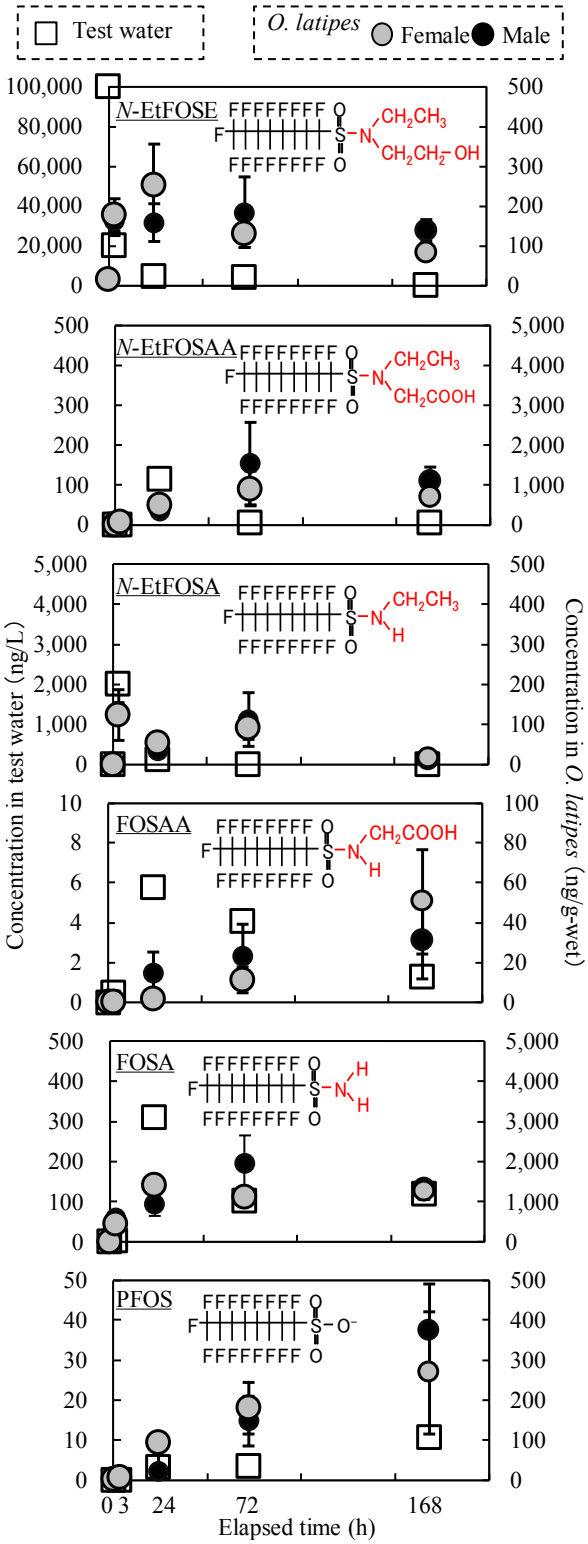

Figure 3 Concentration of *N*-EtFOSE and its transformation products during exposure period

Molar amount calculation for after depuration period

Molar amount of 22 analyzed PFASs in test water and *O. latipes* during depuration period was shown in **Fig 4**. Molar amout of PFOS, the final product in our assumption, in *O. latipes* was 0.100 nmol at 168 h, 0.115 nmol at 240 h, 0.118 nmol at 336 h elapsed. In contrast, molar amount of PFOS in test water was 0.027 nmol at 168 h, 0.093 nmol at 240 h, 0.156 nmol at 336 h elapsed. These results also indicated that PFOS in *O. latipes* was released into test water during depuration period. In addition, remaining molar amount of total of 22 PFASs was 8.8 nmol (8.1 nmol in test water and 0.7 nmol in *O. latipes*) at the termination of the experiment. This value was only 1.7 % of original molar amount of the exposure substance (*N*-EtFOSE). Thus, it was suggested to be important to understand not only the contamination levels of known PFASs but also the potential contamination of unknown transformation products.

Acknowledgements:

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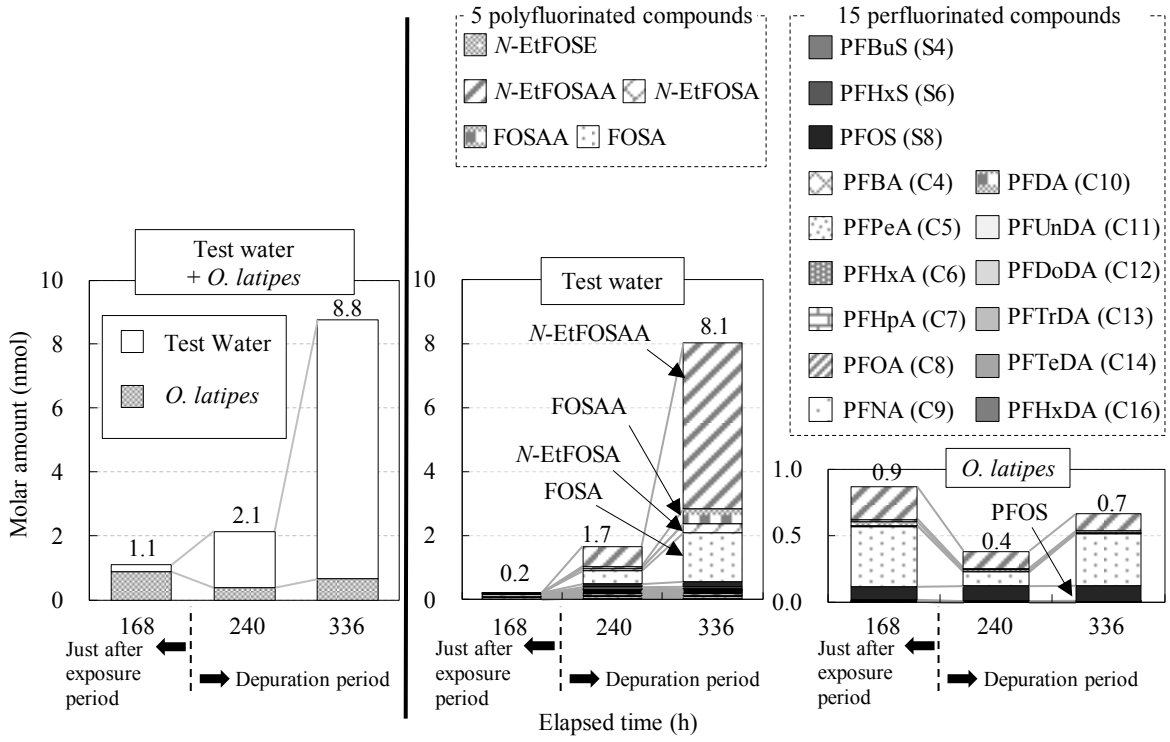

Figure 4 Molar amount of 15 perfluorinated compounds and targeted 5 polyfluorinated compounds in test water and *O. latipes* during depuration period