TRANSFER OF PERFLUORINATED COMPOUNDS FROM WATER TO SOIL AND UPTAKE BY CROP PLANTS

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Introduction

Over the past 60 years, perfluorinated compounds (PFCs) have been used in a broad range of applications such as surfactants, refrigerants and polymers, and also as components of pharmaceuticals, fire retardants, lubricants, adhesives, paints, cosmetics, food packaging and so on. PFCs have raised global attention since researches have reported on their bioaccumulative potential[1] and various adverse effects on human and wildlife such as hepatotoxicity, immunotoxicity and developmental toxicity.[2] Recently, PFCs have been detected globally in most of the water environment and biota.[3,4,5] PFCs are also found in human serum[6] and brest milk[7] and health risk of human exposure to PFCs are of grear concern. One of the major exposure pathway was considered to be through oral route, mainly diet.[7] Klenow *et al*. conducted diet exposure survey and reported that vegetables were identified to be the most important food category for exposure to PFHxA and PFOA, with up to 69% of the total exposure.[8] Consumption of well water and home grown fruits and vegetables was also found to be correlated to levels of PFOA in human blood in a contaminated area.[9] Moreover, Suzuki *et al.* reported that PFCs contents in vegetation might be influenced by water as well as by carryover in the sediment where contamination in water continued for several years.[10] However, the relationship between input mass of PFCs from water environent and the mass in harvetsed vegetables cannot be investigated by field studies. Transfer of PFCs to agricultural crops has been studied by several literatures altough they focused on translocation from contaminated soil [11,12,13] or contaminated water by hydroponically growing experimennt [14,15] individually. Main objective of this study was to examine transfer of PFCs from water to soil and thier uptake by agricultural crops by plant growth experiment.

Materials and methods

Experiments: Plant growth experiments were designed as shown in **Figure 1**. Two different agricultural crop plants, komatsuna (*Brassica rapa var. perviridis*) and radish (*Raphanus sativus*) were selected. Target chemicals were PFOS, PFOA and PFHxA, having four different treatment levels (①1 ng/day, ②10 ng/day, ③100 ng/day, ④1,000 ng/day). All treatments contained eight replicated growing pots and 70 g (wet wt.) of soil was put in each. Plants were pre-grown in the pots until the seed leaves were developed. Plants were then grown for 24 days under irradiation of LED lights for 18h/day. PFOS, PFOA and PFHxA were treated via irrigation water in accordance with each treatment level (①100 ng/L, ②1,000 ng/L, ③10,000 ng/L, ④100,000 ng/L). The characteristics of growth substrate and irrigation water are shown in **Table 2**. For growth substrate, potting soil was purchase and applied. No PFCs were detected in the soil and it was considered suitable for experiments because of its neutral pH and medium nutrient content. For irrigation, tap water was used and it contained PFOS at 4 ng/L, PFOA at 17 ng/L and PFHxA at 4 ng/L. These original concentrations were included to the calculation of mass transfer of PFCs. Temperature and humidity were controled at 20±5°C and 45±10%

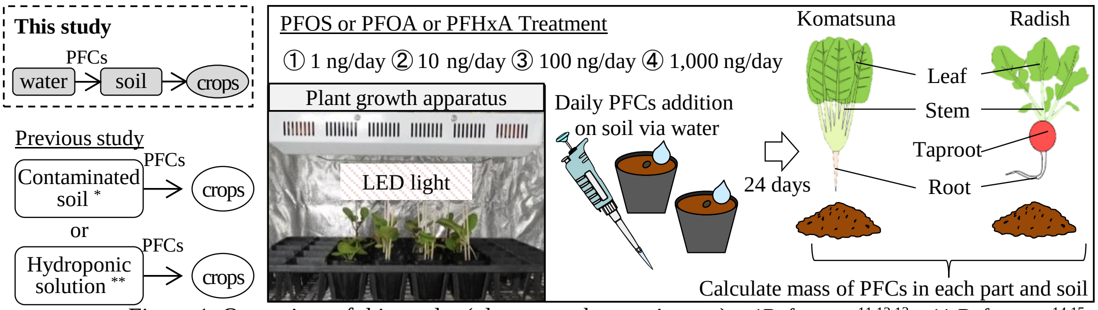

Figure 1. Ovewview of this study (plant growth experiments) *Reference [11,12,13] ** Reference [14,15]

respectively throughout the experiments. After 24 days past, Harvested komatsuna was separated into leaves, stems, roots and soil for, and radish was separated into leaves, stems, taproots, roots and soil. Each part of grown crops and soil were freeze dried by FDU-2200 (EYELA) and crushed into powder by ULTRA-TURRAX®(IKA) or by Wonder Crush/Mill (WDL-1, Osaka Chemical).

Sample Pre-treatment: PFCs in plant (0.5 g-dry wt.) and in soil (1.0 g-dry wt.) were extracted by ion-pair extraction method similar to that previously reported.[16] Sample was mixed with 1 mL of tetrabutyl ammonium hydrogen sulfate and 2 mL of 0.25 M sodium carbonate. 5 mL of tert-butyl methyl ether was then added to the solution and the mixture was vigorously shaken for 5 min. After centrifugation at 3,000 rpm for 15min., organic layer of extract was taken from the solution. This extraction procedure was repeated again. Extracts were mixed and passed through 0.2 µm syringe filter (Whatman®) and ENVI™-carb cartridge (Supelco) to eliminate matrix substances. Samples were then evaporated with nitrogen gas and reconstituted into a final volume of 1 mL with 40% acetonitrile.

Table 1 Characteristcs of soil and irigation water

	Soil	Irrigation water
pH	6.7	6.9
EC (µS/cm)	240	130
OC (%)	3.8	-
Nutrients	(mg/g-dry)	(mg/L)
N	12.3	1.2
P	0.34	0.02
K	1.3	1.3
Ca	1.7	10.3
Mg	1.6	4.1
Fe	4.06	0.05
PFCs	(ng/g-dry)	(ng/L)
PFOS	*<LOD*	3.6
PFOA	*<LOD*	16.7
PFHxA	*<LOD*	3.4

Table 2 Target chemicals and analytical parameters by HPLC-ESI-MS-MS

Compound	Abbreviation	Molecular structure	Precursor ion (*m/z*)	Daughter ion (*m/z*)	*IDL* * (ng/mL)	*IQL* ** (ng/mL)
Perfluorooctane sulfonate	PFOS	$CF_3(CF_2)_7SO_3^-$	499	80	0.01	0.05
Perfluorooctanoic acid	PFOA	$CF_3(CF_2)_6CO_2^-$	413	369	0.01	0.02
Perfluorohexanoic acid	PFHxA	$CF_3(CF_2)_4CO_2^-$	313	269	0.02	0.06
Perfluoro-1-[1,2,3,4-$^{13}C_4$] octane sulfonate	$^{13}C_4$-PFOS	$CF_3(CF_2)_3(^{13}CF_2)_3{}^{13}SO_3^-$	503	80	0.02	0.06
Perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid	$^{13}C_4$-PFOA	$CF_3(CF_2)_3(^{13}CF_2)_3{}^{13}CO_2^-$	417	373	0.02	0.08
Perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid	$^{13}C_2$-PFHxA	$CF_3(CF_2)_3{}^{13}CF_2{}^{13}CO_2^-$	317	271	0.01	0.03

**IDL* = Instrument Detection Limit, ***IQL* = Instrument Quanitification Limit

Instrumental Analysis and Quantification: Analytical parameters of each taget PFC are shown in **Table 2**. Details of separation and quantification are shown in a previous literature.[10] Recovery rates were calculated by spiking 10 ng of mass-labeled PFCs ($^{13}C_2$-PFHxA, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS, Wellington Laboratories) into each sample prior to pre-treatment. Recoveries of $^{13}C_2$-PFHxA, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were ranged between 43% to 64%, 49% to 74% and 46% to 81%, respectively and relative standard deviation of recovery values for sample vegetables were less than 20%..

Results and discussion

Mass distribution of PFHxA, PFOA and PFOS in each part of harvested crop plant, soil and planting pot at the end of the experiments are shown in **Figure 2**. PFCs were remained in planting pots less than 2% of treated mass. High proportion of PFCs mass were remained in soil, 64% for PFOS, 71% for PFOA and 70% for PFHxA (Komatsuna experiment) and, 74% for PFOS, 82% for PFOA, 80% for PFHxA (Radish growth). Among each part of harvested komatsuna, proportions of PFCs (of total treated mass) in roots were highest at 26% (PFOS), 19% (PFOA), 14% (PFHxA), while those in leaves were 1% (PFOS), 4% (PFOA) and 9% (PFHxA). Similarly, among each part of harvested radish, proportions of PFCs in

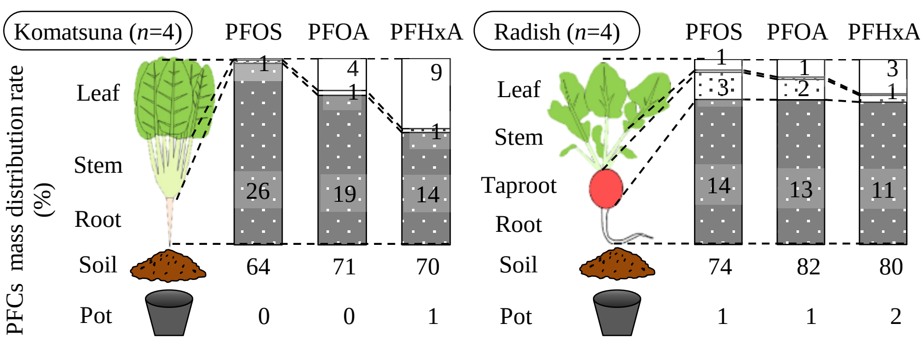

Figure 2. Mass distribution of PFOS, PFOA and PFHxA at the end of the experiments

roots were highest at 14% (PFOS), 13% (PFOA), 11% (PFHxA), while those in leaves were 1% (PFOS), 1% (PFOA) and 3% (PFHxA). In addition, taproot of radish contained 3% of PFOS, 2% of PFOA and 1% of PFHxA. These results indicated that more hydrophobic compounds preferably remained in roots and taproots and less hydrophobic compounds tended to be translocated from roots to leaves (Hydrophobicity: PFOS >PFOA>PFHxA[17]).

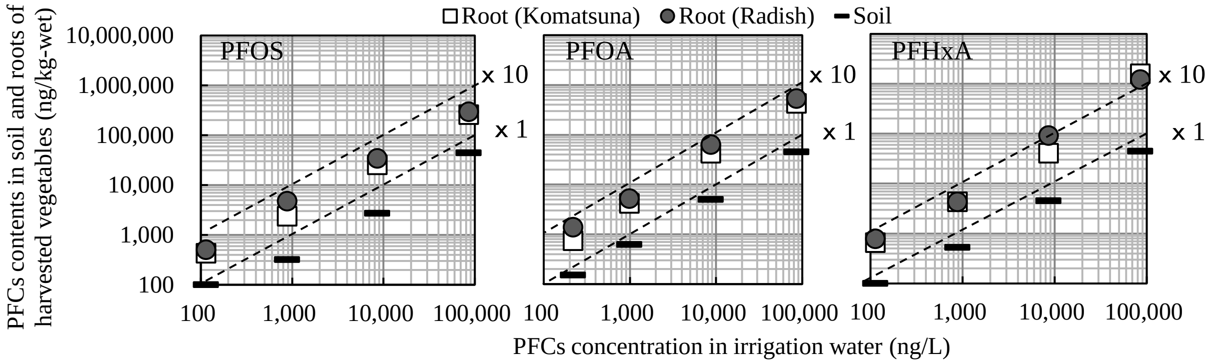

Figure 3. Relationship between PFCs concentrations in irrgation water and PFCs contents in soil and roots of harvested vegetables (Double logarithmic scales)

Concentrations of PFOS, PFOA and PFHxA in soil and roots of harvested vegetables were plotted with those in irrigation water in **Figure 3**. There were linear relationships between exposure concentration of PFOS, PFOA and PFHxA via irrigation water and those contents in the soil and roots of vegetables. Over the applied concentration ranged from 100 ng/L to 100,000 ng/L, these trends in this study were in accordance with the behavior observed by previous studies. [11,15] Concentrations of PFOS, PFOA and PFHxA in roots of vegetables were higher than those in irrigation water while the concentration levels in soil were lower than the levels in irrigation water. These trends possibly indicate that concentrating of PFOS, PFOA and PFHxA in roots of komatsuna and radish was preferably occured by uptake into roots and sorption by surface of roots rather than remaining in soil. On the other hand, the amount of applied soil in this experimental system (70 g-wet wt.) might be influencial to lowering concentration of the compounds since the mass of PFOS, PFOA and PFHxA remained in the soil were more than 60%. In fact, transfer factor of PFCs from water to soil was >1 in a field study reported previsously.[10] To elucidate these behavior, further experiments such as individual sorption experiment to soil and roots.

The degree of PFOS, PFOA and PFHxA uptake by harvested vegetables were assessed by root concentrstion factor (*RCF*) and eddible parts concentation factor (*ECF*) as following formula:

RCF_{water} (L/kg-wet) = C_{root} (n g/kg-wet) /C_{water} (ng/L) ... (1) ,where C_{root} and C_{water} are concentration in root and irrigation water respectively,
RCF_{soil} (kg-wet /kg-wet) = C_{root} (ng/kg-wet) /C_{soil} (ng/kg-wet)... (2) ,where C_{soil} is concentration in root
ECF_{soil} (kg-wet /kg-wet) = C_{ep} (ng/kg-wet) /C_{soil} (ng/kg-wet)... (3), where C_{ep} is concentration in edible parts (Foliage of komatsuna and Taproot of radish)

Calculated RCF_{water}, RCF_{soil} and ECF_{soil} values are shown in **Figure 4**. Graph was plotted with average values of each treatment level since there were minor influences of treatment levels in the range of this experiment as explained above. RCF_{water} values were 3.1 (PFOS), 4.3 (PFOA) and 5.1 (PFHxA) for Komatsuna and 4.3 (PFOS), 6.3 (PFOA) and 8.4 (PFHxA) for radish, which indicated that PFCs were taken up from water into roots

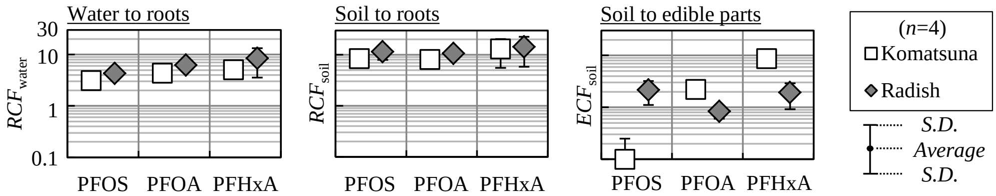

Figure 4. *RCF* (Root Concentration Factor) and *ECF* (Edible parts Concentration Factor) of crop plants (Komatsuna and Radish) to water and soil

in the order of water solubility (PFOS<PFOA<PFHxA). On contrast, RCF_{soil} values were 8.4 (PFOS), 8.1 (PFOA), 12.9 (PFHxA) for komatsuna and 11.4 (PFO S), 10.6 (PFOA) and 14.2 (PFOS) for radish, which were not clearly different among each PFC. This implies that the PFCs selectivity of soil and the roots were similar in this study. PFCs in irrigation water cannot be utilized by vegetation directly, therefore, the relationship between characteristics of soil and the roots possibly have great influences on PFCs transfer and uptake from water to agricultural crops.

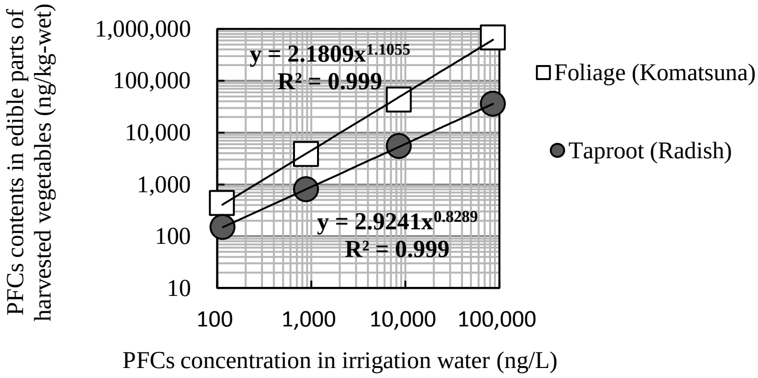

Figure 5. non linear *Freundlich* isotherm between PFCs concentrations in irrgation water and contents in edible parts of harvested vegetables (Double logarithmic scales)

ECF_{soil} for komatsuna, showed a remarkable difference among PFCs, which were 0.1 (PFOS), 2.2 (PFOA) and 8.7 (PFHxA). ECF_{soil} for radish, on the other hand, were 2.2 (PFOS), 0.8 (PFOA) and 1.9 (PFHxA). These results indicated that leafy vegetables such as komatsuna uptake less hydrophobic, shorter-chain PFCs such as PFHxA preferably to edible parts while root vegetables such as radish remain more hydrophobic PFCs such as PFOS in its edible parts. According to the European Food Safety Authority, tolerable daily intake values (TDIs) for PFOS is 150 ng/kg body weight.[18] **Figure 5** shows the nonliniar *Freundlich* isotherm between PFCs concentrations in irrigation water and PFCs contents in edible parts of harvested crops aquired from this study. The model calcuated that exposure of PFOS via comsumption of 300 g-wet wt. radish will exceed the TDI if irrigation water contains approximately 16.9 µg/L. This estimation was based on that PFOS exposure occurs only via radish and the amount of radish consumption was set to be understandable range of daily diet, thus, more exposure pathways may be assumed in such a contaminated region. In addition, there is no specific tolerable daily intake values for PFHxA, although as reported in a previous literture,[10] there is some region where contamination of PFHxA has been ongoing at a range of µg/L and transfer of PFHxA to leafy vegetables are of great concern.

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References:

1. Conder JM, Hoke RA, De Wolf W, Russell MH, Buck RC. (2008); *Environ. Sci. Technol.*, **42**, 995-1003.
2. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. (2007); *Toxicol. Sci*., **99**, 366–94.
3. Giesy JP, Kannan K. (2001); *Environ. Sci. Technol.*, **35**, 1339–1345.
4. Saito N, Harada K, Inoue K, Sasaki K, Yoshinaga T, Koizumi A. (2004); *J. Occup. Health*., **46**, 49–59.
5. Yamashita N, Kannan K, Taniyasu S, Horii Y, Petrick G, Gamo T. (2005); *Marine Poll. Bull*., **51**, 658-668.
6. Harada K, Saito N, Inoue K, Yoshinaga T, Watanabe T, Sasaki S, Kamiyama S, Koizumi A. (2004); *J. Occup. Health*, **46**, 141-147.
7. Fromme H, Tittlemier SA, Völkel W, Wilhelm M, Twardella D. (2009); Int. J. Hyg. Environ. Health., **212**, 239–270.
8. Klenow S, Heinemeyer G, Brambilla G, Dellatte E, Herzke D, de Voogt P. (2013); *Food Addit. Contam. Part A*−**30** (12), 2141−2151.
9. Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. (2006); *J. Occup. Environ. Med*., **48**, 759-770.
10. Suzuki Y, Tanaka S, Fujii Shigeo, Ando H, Ishikawa K, Kunacheva C, Boontanon SK, Saito N. (2013); *Organohalogen Compounds*, **75**, 1053-1056
11. Stahl T, Heyn J, Thiele H, Hu¨ther J, Failing K, Georgii S, Brunn H. (2009); Arch. Environ. Contam. Toxicol., **57**, 289–298.
12. Lechner, M., Knapp, H. (2011) *J. Agric. Food Chem*., **59** (20), 11011−11018.
13. Blaine, A. C., Rich, C. D., Hundal, L. S., Lau, C., Mills, M. A., Harris, K. M., Higgins, C., P. (2013). *Environ Sci Technol.*, **47** (24), 14062-14069.
14. Felizeter, S., McLachlan, M. S., de Voogt, P. (2012) *Environ Sci Technol.*, **46** (21), 11735−11743.
15. Felizeter, S., McLachlan, M. S., de Voogt, P. (2014) *J. Agric. Food Chem.*,**62** (15),3334–3342.
16. Hansen, K.J., Clemen, L.A., Ellefson, M.E., Johnson, H.O. (2001). *Environ Sci Technol.*, **35** 766–770.
17. de Voogt P., Zurano, L., Serné, P., Haftka, J. J. H. (2012) *Environ Chem.*, **9**, 564–570.
18. EFSA, (2008). *EFSA J.* **653**, 5-131.