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Comparison of the plant uptake factor of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) from the three different concentrations of PFOA and PFOS in soil to spinach and Welsh onion

Deuk-Yeong Lee[1] · **Geun-Hyoung Choi**[2] · **Jin-Ho Rho**[2] · **Hyo-Sup Lee**[2] ·
Sang-Won Park[2] · **Kyeong-Yeol Oh**[1] · **Jin-Hyo Kim**[1]

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Abstract The long-chained perfluoroalkyl acids (PFAAs), perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are a potential exposure risk in the environment, specifically for humans due to high levels of bioaccumulation, persistence, and toxicity. In the current study, the plant uptake factors (PUFs) of spinach and Welsh onion were investigated on the three different concentration levels of PFOA and PFOS in soil. Spinach and Welsh onion were divided into three residue groups, a control group and two levels of PFOA and PFOS. The PFAAs spiked soils were aged for six months and the extractable residue of PFOS in the aged soil was reduced to 30-59% of the initial spiked concentrations for PFOS, while PFOA showed almost the same initial spiked concentrations. The PUFs for PFOA and PFOS were 0.111-2.821 and 0.047-3.175 for spinach, and 0.203-0.738 and 0.035-0.181 for Welsh onion, respectively. The highest PUF values in both vegetable were displayed when the residual concentration of PFAAs were part-per-billion (ppb) or sub-ppb in soil.

Keywords Perfluorooctanesulfonic acid · Perfluorooctanoic acid · Plant uptake factor · Spinach · Welsh onion

Introduction

Perfluoroalkyl acids (PFAAs) have been widely used for industrial or consumer products for more than 50 years, and short chain PFAAs are still used to the products [33]. However, the residue risk of the long-chained PFAAs such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in environment and food are constantly raised due to their potent physico-chemical stability, bioaccumulation and toxicity. Especially, PFOA, PFOS and those related compounds have been listed on persistent organic pollutants (POPs) in Stockholm Convention [17,18,29,31,32]. Although the POPs had been banned to produce and use in commercial products, there were frequently detected in environment, crops and wildlife as well as in human [2,14,20,24-26,28,30]. To know the current exposure level of PFOA and PFOS in human, many scientists reported the exposure assessment and scenarios from air, water, food and living environment [1,4,13,37,38]. From these researches, the dietary exposure was identified as a major exposure route for human, and the importance of the risk management in food crops was addressed to reduce the exposure risk of PFOA and PFOS. However, there were still lack of the information for the residues and the exposure risk assessment for human. To study of the advanced research for the risk management of PFOA and PFOS in food crops, firstly, the national survey of the residues could be performed in agricultural environment and in locally produced food crops. Then the bioaccumulation properties in various food crops should be investigated to establish an environmental safety guidelines for irrigation water and soil.

Deuk-Yeong Lee and Geun-Hyoung Choi are contributed equally to this work.

Jin-Hyo Kim (✉)
E-mail: jhkim75@gnu.ac.kr

[1]Department of Agricultural Chemistry, Institute of Agriculture and Life Science (IALS), Gyeongsang National University, Jinju 52828, Republic of Korea

[2]Chemical Safety Division, Agro-Food Safety and Crop Protection Department, National Institute of Agricultural Sciences, RDA, Wanju 55365, Republic of Korea

Recently, the residues of PFOA and PFOS were surveyed in agricultural environment by Choi et al. [6] and most of the residue distribution of the sum of PFOA and PFOS were reported below 0.05 μg L^{-1} (92%) in the irrigation water and below 1.0 μg kg^{-1} (88%) in the farmland soil. In addition, some of the PFAAs residue surveys for food in Korea were investigated by a few researchers [13,27]. However, the exposure risk of PFOA and PFOS for Korean from the recent food survey would be expected to exceed the recent reference doses (RfDs, 0.8 ng kg_{bw}^{-1} day^{-1} for PFOA and 1.8 ng kg_{bw}^{-1} day^{-1} for PFOS) by European Food Safety Authority [35]. However, it would be difficult to propose the environmental guideline for agricultural soil and water unless the study on crops uptake of PFOA and PFOS from soil environment is supported. To solve this problem, plant uptake factors (PUFs) or bioconcentration factors of PFOA and PFOS were reported in several crops such as carrot, cucumber, lettuce, maize, potato, radish, rice and wheat [7,11,19,22,37]. However, the plant uptake potentials of PFAAs were studied limitedly and there was still needed to study on various food crops.

Spinach and Welsh onion are important vegetables in Korea and Asia. Their daily dietary intakes were reported to 6.38 g for spinach and 10.98 g for Welsh onion in Korea National Health and Nutrition Examination Survey in 2017 [16] and they were listed on top 20 crops in Korea. Spinach was cultivated 73,239 metric tons in 2018 and it was a major food source for supplying vitamin A and vitamin C [21]. Unlike lettuce, spinach was grown in soil and had a smaller growth rate and weight than lettuce [5]. Welsh onions, along with garlic and onions, are representative crops belonging to the *Allium* genus and are produced at an annual rate of 490,000 metric tons in Korea [21]. And strong antimicrobial, antioxidative and anti-obesity activities of the onion extract had been studied recently [23]. Due to the importance of these vegetables, PUF studies on various POPs have recently been conducted, however, there have been no reports of research on the PFAAs. In here, the PUFs on the three different PFOA and PFOS concentrations in soil were investigated and compared in spinach and Welsh onion.

Materials and Methods

Standards and reagents

^{13}C-labelled and native analytical standards for PFOA and PFOS were from Wellington Laboratories, Inc. (Guelph, ON, Canada) and reagent grade of PFOA and PFOS for soil contamination was purchased from TCI Co., Ltd. (Tokyo, Japan). Acetonitrile (ACN), acetic acid, ammonium acetate, methanol and tetrahydrofuran were used a high-performance liquid chromatography grade from Merck (Darmstadt, Germany). Powdered ENVI-Carb^{TM} (Supelco, Bellefonte, PA, USA), hydrophilic-lipophilic balance (HLB) cartridges (0.5 g, 6 mL, Oasis, Waters Co., Dublin, Ireland) and membrane filter and syringe filter (nylon, Silicycle Inc., Quebec, Canada) were purchased.

Soil preparation and vegetable cultivation

The experimental soil was collected on the top soil from an aerial field and used below 5 mm particle for pot preparation. The physicochemical properties of the experimental soil were described in Table 1. The experimental pot was divided to three groups; non-spiked, low-contaminated, and high-contaminated groups. The initial residues of PFOA and PFOS were ideally spiked to 1000 and 5000 μg kg^{-1} in spinach cultivation soil (Group SB and SC), and 50 and 500 μg kg^{-1} in Welsh onion cultivation soil (Group WB and WC) with the reagent grade standards. After the soil homogenization for 4 days, aging process was performed for six months in 1/5000 a of Wagner pot. For prevention of the leaching of the contaminants, the pot was closed on the bottom. For spinach and Welsh onion cultivation, the seeds were purchased from Asia Seed Co. (Seoul, Korea). The vegetables were cultivated in green house. Spinach was sown in February and harvested in June, and Welsh onion was sown in March and harvested in July. The pot cultivation of each treatment was performed with eight replications. The spinach and Welsh onion were harvested whole plant and the residue soil was washed under running tap water gently. The harvested vegetable blended with dry ice and stored at −20 °C until the residue analysis.

Analytical sample preparation for residual PFAAs in soil

The analytical soil sample preparation was followed by Choi et al. [6]. Briefly, 1% aqueous acetic acid (10 mL) was added to 1.0 g air-dried soil sample, then it was shaken for 30 min, sonicated for 15 min and centrifuged at 3000 rpm. The supernatant was collected and the original soil was re-extracted three times using 10 mL of 90% (*v/v*) methanol in 1% acetic acid. All the extracts were combined and concentrated under nitrogen, then diluted with dH_2O. The diluted extract was loaded to pre-cleaned HLB cartridge, then washed with 30% methanol (5 mL) and eluted with methanol (10 mL). The eluent was concentrated and re-dissolved in 1.0 mL of methanol then 20 mg of powdered ENVI-Carb^{TM} was added. The mixture was vortexed and filtered using nylon

Table 1 Physico-chemical properties of experimental soil for pot experiment

Crop	Texture	pH (1:5)	Organic matter (g kg^{-1})	Avail. P_2O_5 (mg kg^{-1})	Exchangeable Cations ($c$mol$_c$ kg^{-1})		
					K	Ca	Mg
Spinach	Sandy clay loam	5.38	19.6	138.1	1.1	6.9	3.2
Welsh onion	Sandy clay loam	6.28	55.1	497.3	2.0	16.2	4.7

syringe filter then $^{13}C_4$-PFOA and $^{13}C_4$-PFOS were added to the filtrate as internal standards.

Analytical sample preparation for residual PFAAs in vegetables

The cleanup and quantitation procedure for PFAAs was followed by Choi et al. [7] with slight modification. Briefly, 10.0 g vegetable was extracted with 75% methanol (10 mL×3) then centrifuged at 3000 rpm and the supernatant was collected. The extracted vegetable was re-extracted using 75% of aqueous tetrahydrofuran (10 mL). All extracts were combined and concentrated to 10 mL under nitrogen stream. The extract was diluted to 50 mL dH_2O then extracted with HLB cartridge as follow as the above described. The eluent was concentrated and re-dissolved with 1.0 mL methanol-then added 20 mg of powdered ENVI-Carb^{TM}. The mixture was vortexed and filtered using syringe filter then isotope labeled internal standards were added to the filtrate.

Instrumental analysis

The instrumental analysis of PFOA and PFOS was performed with liquid chromatography (LC)-triple quadrupole mass spectroscopy (API 3200 QTRAP, AB Sciex LLC., Framingham, MA, USA) with a C18 column (3 µm, 150 mm×2.1 mm, Silicycle Co., Quebec, Canada) for analysis and a C18 column (5 µm, 50 mm×2.1 mm, Phenomenex. Inc., Torrance, CA, USA) for separation of solvent impurity. The eluent and detailed instrumental conditions were described in Table 2.

Quality assurance

Linearities of PFOA and PFOS from concentrations of 0.010 to 5.000 µg L^{-1} were 0.9992 and 0.9993, respectively. Both of the limit of quantification (LOQ) for PFOA and PFOS were 0.010 µg kg^{-1} for soil and 0.001 µg kg^{-1} for vegetables. Recovery were measured two spiked concentrations on each of 0.05 and 0.5 µg kg^{-1} of the $^{13}C_8$-PFAAs in spiked soil and vegetables and ranged from 69 to 78%. The precisions of PFOA and PFOS were determined to 9.1-9.8% for soil, and 8.4-9.9% for vegetables, respectively.

PUF of vegetables for PFAAs

PUF was calculated with the followed equation. The PFAAs concentration was used an initial level as before plantation in soil and a harvest level in vegetables.

$$\mathrm{PUF}=\frac{C_{vegetable}}{C_{soil}}$$

Results and Discussion

PUFs for PFOA and PFOS residues in soil

The estimated soil contamination level was designed to three groups; non-spiked, low-concentration spiked and high-concentration spiked of PFOA and PFOS groups were prepared. Before the contamination of PFOA and PFOS in the collected soil, the initial residues of PFOA and PFOS were analyzed and the residues were determined to 0.381 and 0.099 µg kg^{-1} in spinach cultivation soil (Group SA), and 4.230 and 3.251 µg kg^{-1} in Welsh onion cultivation soil (Group WA), respectively.

For the spinach cultivation, each of PFOA and PFOS was

Table 2 The instrumental condition of LC-MS/MS for quantitative analysis of PFOA and PFOS

LC	Agilent HP1100 (Agilent Technologies, Inc., Santa Clara, CA, USA)	
Column	Siliachrom C18 (3 µm, 150 × 2.1 mm)	
Injection volume	10 µL	
Mobile phase	A: 2 mM NH_4OAc in 5% MeOH B: 2 mM NH_4OAc in 75/25/5 (*v/v/v*, MeOH/ACN/dH_2O)	
Flow rate	0.2 mL/min	
	Time (min)	Mobile phase
Gradient	0	100% A
	2	100% A
	3	90% A/ 10% B
	5	50% A/ 50% B
	9	10% A/ 90% B
Mass spectrometry	API 3200 system (AB Sciex LLC., Framingham, MA, USA)	
Source temperature	600 °C	
Ion spray voltage	−4.2 kV	
Ionization mode	ESI negative	
Detection	412→369 for native PFOA; 417→372 for $^{13}C_4$-PFOA; 421→376 for $^{13}C_8$-PFOA 498→79 for native PFOS; 503→99 for $^{13}C_4$-PFOS; 507→99 for $^{13}C_8$-PFOS	
MS/MS condition	Declustering potential -15V (PFOA), -80V (PFOS); Collision cell enterance potential -20V (PFOA), -28V (PFOS); Collision energy -14V (PFOA), -68V (PFOS); Collision cell exit potential -4V (PFOA), 0V (PFOS)	

spiked on a part-per-million (ppm) level of concentrations to 1000 μg kg^{-1} for low concentration spiked group (Group SB) and 5000 μg kg^{-1} for high concentration spiked group (Group SC) in the soil. And the real concentrations of PFOA and PFOS were detected to 816.3 and 392.6 μg kg^{-1} on Group SB and 4916 and 1521 μg kg^{-1} on Group SC, respectively (Table 3). On the other hand, the soil for Welsh onion cultivation were spiked on a part-per-billion (ppb) level of concentrations to 50 and 500 μg kg^{-1} as initial spiked concentrations for Group WB and Group WC; this spiked concentrations were designed based on the current environmental residue concentrations and the worst contamination scenario. The PFOA and PFOS residues were 51.80 and 27.62 μg kg^{-1} on Group WB and 483.1 and 295.4 μg kg^{-1} on Group WC. Interestingly, the PFOS residues after aging process were detected lower than the initial spiked concentration, respectively (Tables 3, 4). And this residue reduction of PFOS in soil was expected to the adsorption and immobilization of PFOS in soil clay from the previous immobilization studies [10,12,36,39]. These studies reported on the adsorption mechanism of clay minerals like montmorillonite and/or kaolinite with perfluorinated sulfonate and the interaction Al/Fe ions with PFOS, while PFOA didn't show these immobilizaiton process [3,39]. Thus, the diminution of extractable PFOS residue could be expected in aged soil without change of the total residue in soil due to its potent stability in soil [39]. In this experiments, the soil aging for six months decreased the extractable PFOS to 30-59% of the initial spiked concentration, while the PFOA residue was recorded to almost of the initial spiked concentration in soil. These immobilization effect of soil might expect to help the diminution of plant uptake ratio from soil to crop.

PUFs for PFOA and PFOS residues in spinach

The PFOA and PFOS residues in spinach were detected 1.077 and 0.316 μg kg^{-1} on Group SA, 171.5 and 18.32 μg kg^{-1} on Group SB and 544.5 and 73.58 μg kg^{-1} on Group SC, respectively. Based on the residue in spinach and soil, the PUFs of PFOA and PFOS were estimated to 0.111-2.821 and 0.047-3.175, respectively (Table 3). Interestingly, the highest PUFs were recorded on the lowest PFOA and PFOS residues in the soil, and the PUFs for PFOA were gradually decreased on the increasing of the residue concentration in soil. On the other hand, the PUFs for PFOS showed a consistent value, not dependent on over hundreds ppb of the residues in soil. In addition, the residue in spinach and their PUFs for PFOS showed lower than those of the PFOA. In the cultivation experiments, the experimental PFAAs concentration in soil didn't affect to spinach growth and production in all of the groups.

PUFs for PFOA and PFOS in Welsh onion

In the cultivated Welsh onion, the highest PFOA and PFOS were detected 124.0 and 24.19 μg kg^{-1} on Group WC that was found the highest soil residues, respectively. Based on the residue in Welsh onion, the PUFs of PFOA and PFOS were calculated to 0.203-0.738 and 0.035-0.181, respectively (Table 4). As with the results of spinach, the highest PUFs on Welsh onion were recorded on the several ppb-level of PFOA and PFOS concentrations in the soil, and the Welsh onion uptake and accumulate PFOA more than PFOS.

The highest PUF for PFOA and PFOS in the test vegetables was found 2.821 and 3.175 in spinach, respectively. Although both of the plant uptake experiments were not performed on the same soil condition, the PUFs for PFOA and PFOS in spinach were similar values with the previously reported in rice straw (0.769-1.474 for PFOA and 1.749-4.700 for PFOS) [7]. In addition, the PUFs for the PFAAs were at least 10-fold higher than those for endosulfan and other organochlorine POPs [5,8,9,15]. Due to the ionic functional group of PFOA and PFOS, the PFAAs have higher hydrophilicity and ionic charges than the organochlorine POPs and these chemical properties would result to higher PUFs in crops through a different pathway for the uptake and accumulation with non-polar organochlorine POPs [22,34].

The highest PUFs for PFOA and PFOS in spinach and Welsh onion showed up to 2.821 and 3.175 in ppb or sub-ppb level of the residues in soil, respectively. And the PUFs of PFOA and PFOS

Table 3 PFOA and PFOS residues (average ± SD) in the soil, spinach and the PUFs

	Soil (μg kg^{-1})		Spinach (μg kg^{-1})		PUF	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
Group SA	0.381±0.021	0.099±0.003	1.077±0.245	0.316±0.058	2.821	3.175
Group SB	816.3±54.89	392.6±27.49	171.5±28.35	18.32±2.315	0.210	0.047
Group SC	4916±342.2	1521±118.5	544.5±95.12	73.58±10.77	0.111	0.048

Table 4 PFOA and PFOS residues (average ± SD) in the soil, Welsh onion and the PUFs

	Soil (μg kg^{-1})		Welsh onion (μg kg^{-1})		PUF	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
Group WA	4.230±2.867	3.251±1.957	3.122±0.606	0.589±0.033	0.738	0.181
Group WB	51.80±7.61	27.62±6.07	10.53±2.06	0.961±0.147	0.203	0.035
Group WC	483.1±87.4	295.4±46.71	124.0±35.27	24.19±4.117	0.256	0.082

differed up to 20- and 60-folds in spinach, and 3- and 5-folds in Welsh onion according to the soil contamination level. The highest PUFs were found in the popular residues in Korean agricultural environment, and the residues of the cultivated vegetables in Korea would be below 2.821 and 1.588 $\mu g\,kg^{-1}$ in spinach and 0.738 and 0.181 $\mu g\,kg^{-1}$ in Welsh onion, respectively. Actually, the similar residues were reported the previously studied PFAAs residue monitoring of the retailed Korean foods ($<1.0\ \mu g\,kg^{-1}$) by Heo et al. [13]. However, we were still not sure of the maximum and average residues of the PFAAs in crops due to the complexation of effects on the various factors such as the irrigation water contamination, soil condition, and vegetable growth condition. Recently, some of the exposure assessments for PFAAs were reported that the exposure risk of PFAAs from food and tap water was not exceeded the previously reported RfDs (1500 ng kg_{bw}^{-1} day^{-1} for PFOA and 150 ng kg_{bw}^{-1} day^{-1} for PFOS). However, the recent RfDs of the PFAAs were strengthened to 0.8 ng kg_{bw}^{-1} day^{-1} for PFOA and 1.8 ng kg_{bw}^{-1} day^{-1} for PFOS in 2018, and we didn't have the national residue survey data after the implementation of the strengthen of POPs Control Acts for PFAAs in Korea. Thus, the further approach of this research is needed the national level of the residue survey in crops and in soil for the dietary exposure risk assessment of crops and the establishment of agricultural environmental guidelines for the PFAAs.

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