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Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plant and Distribution to the Different Plant Compartments Studied in Cultures of Carrots (*Daucus carota* ssp. *Sativus*), Potatoes (*Solanum tuberosum*), and Cucumbers (*Cucumis Sativus*)

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ABSTRACT: A vegetation study was carried out to investigate the carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from soil mixed with contaminated sewage sludge to potato, carrot, and cucumber plants. Analysis was done by liquid-extraction using acetonitrile with dispersive SPE cleanup and subsequent HPLC-MS/MS. In order to assess the transfer potential from soil, transfer factors (TF) were calculated for the different plant compartments: TF = $[PFC]_{plant\ (wet\ substance)}/[PFC]_{soil\ (dry\ weight)}$. The highest TF were found for the vegetative plant compartments with average values for PFOS below those for PFOA: cucumber, 0.17 (PFOS), 0.88 (PFOA); potato, 0.36 (PFOS), 0.40 (PFOA); carrot, 0.38 (PFOS), 0.53 (PFOA). Transfer of PFOA and PFOS into potato peelings (average values of TF: PFOA 0.03, PFOS 0.04) exceeded the carryover to the peeled tubers (PFOA 0.01, PFOS < 0.01). In carrots, this difference did not occur (average values of TF: PFOA 0.04, PFOS 0.04). Transfer of PFOS into the unpeeled cucumbers was low and comparable to that of peeled potatoes (TF < 0.01). For PFOA, it was higher (TF: 0.03).

KEYWORDS: perfluorooctanoic acid, perfluorooctane sulfonate, carryover, carrot, potato, cucumber

INTRODUCTION

Per- and polyfluorinated chemicals (PFC) are widely used as adhesives, antistatic agents, coatings, for emulsions, in fire-fighting foams, as oil and solvent repellent agents for paper, and for several other industrial applications.[1] As perfluorinated chemicals (PFC) are hardly biologically degradable once liberated, they can cause severe contamination of the environment.[2−4] In many cases, PFC are set free with industrial wastewater (e.g., metal plating industries and chemical industries) either directly into the aquatic environment or indirectly via the canalization and sewage treatment plants. As a result of their low biodegradability, without additional treatment or removal (clean up) steps, perfluorinated chemicals are emitted from wastewater treatment plants to one part in the effluent and to the other part adsorbed on the sewage sludge.[5] The use of sewage sludge as fertilizer in agriculture can thus cause contamination of the soil with PFC. Therefore in Bavaria, as in other German states, there is a preventive maximum guideline value for PFC in sewage sludge, which is to be used as fertilizer in agriculture. Since 2008, the sum of 11 specified PFC (among other parameters) has not been allowed to exceed 100 μg/kg dry matter (DM) if the sludge is to be used for farming.[6] In the German sewage sludge regulation, a corresponding limit of 200 μg/kg until the 31st of December 2011 and 100 μg/kg as of January 1st, 2012, is designated to come into force with the next amendment.[7] A survey carried out by the Bavarian Environmental Agency from November 2006 until December 2009 revealed that from sludge samples of 1075 sewage treatment plants, 66 (about 6%) exceeded the Bavarian guideline value for the sum of 11 specified PFC.[8] These sludge deposits had to be disposed of professionally to prevent further distribution of PFC and avoid the contamination of farmland.

Another source of environmental contamination with PFC is the use of PFOS-containing fire-fighting foams. For example, 48 m^3 of fire retardant foam formulation was applied according to literature when extinguishing a fire after a plane accident at Toronto airport. Using a PFOS-containing foam formulation such as that described for the accident on June 8, 2000 at the same airport, would have released an estimated quantity of 240 to 720 kg of perfluoroalkanesulfonate salts into the environment.[9,10] The use of the substance for this purpose has been banned in the meantime, up to a limit of 0.001%,[11] but foams on stock were permitted until June 2011, and applications in the past for training or for an emergency can still be a problem due to the chemical stability of PFOS. Other poly- or perfluorinated chemicals comprised in fire fighting foams may contribute additionally to environmental contamination.[9] Therefore, the question arises whether there is a carryover of PFC from contaminated soil to plants growing on it. A recently published study[12] investigated the uptake of several perfluorinated acids and telomer alcohols from contaminated soil into different kinds of grass. The PFC burden had been caused by the application of industrially contaminated sewage sludge in this area. In Germany, some studies had already been carried out with several crop plants indicating that there is an uptake from the soil, particularly in the vegetative compartments of the crops.[13−15] In the course of the study by Stahl et al.,[15] the uptake of PFC in rye grass, grain, and also potatoes was analyzed in dependence with the concentration of

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Table 1. Vegetation Study: Overview

	plants per tub	amount of PFOA (μg/kg soil DM)[a]	amount of PFOS (μg/kg soil DM)[a]	vegetation duration (days)	number of harvested fruits	harvest weight of the whole plant (g)
			Cucumbers			
untreated control tub 1	1			96	4	1439
untreated control tub 2	1			96	3	1102
contaminated tub 1	1	406 ± 36	10 ± 1	96	2	1065
contaminated tub 2	1	805 ± 63	556 ± 65	96	4	1425
			Carrots			
untreated control tub 1	30			63	30	649
contaminated tub 1	30	681 ± 160	10 ± 3	63	30	485
contaminated tub 2	30	676 ± 88	458 ± 77	63	30	395
			Potatoes			
untreated control tub 1	3			71	10	868
contaminated tub 1	3	276 ± 22	15 ± 3	71	17	856
contaminated tub 2	3	795 ± 105	317 ± 35	71	15	811

[a] Amount measured in soil: mean value of three independent experiments ± standard deviation.

the contaminants in the soil. For the grain, Stahl et al. distinguished between seeds and straw, whereas for the potatoes, PFC-amounts only were quantified for the tubers and for the peelings. Differences were found concerning the PFC uptake of the different plants, with regard to the peeled potatoes, the peelings, and also with regard to the two substances PFOA and PFOS. In the case of grain, higher amounts of PFC were found in the straw than in the seed. Therefore, it looks as if PFC predominantly are dislocated to the vegetative compartments of plants and do not get stored in the storage organs. To our knowledge, further studies on the distribution of PFC in the different plant compartments have not been carried out. In the past, studies addressed the plant uptake of chlorinated chemicals like dibenzo-*p*-dioxins, organochlorine pesticides, or chlorobenzenes. While these substances significantly accumulate in plant roots by diffusion and sorption onto lipophilic root solids, the dislocation into the shoots via the xylem differs depending on the physicochemical properties of the chemical and the plant family.[16] It is unclear if perfluorinated chemicals like PFOA and PFOS behave similarly as they are water-soluble ionic surfactants. Therefore, there is still a lack of data regarding transfer rates of PFC from contaminated soil into different vegetables. As a governmental authority responsible for risk assessment related to food and feed, we need these data when contamination sites are discovered that affect agricultural food production. Transfer factors should help to rapidly deduce the toxicological risk potential of vegetables, when ambient PFC-contamination is detected. Therefore, the aim of this vegetation study was to investigate the carryover of PFOA and PFOS to different types of vegetables. The two plants with edible parts growing below ground (potatoes and carrots) and cucumber plants whose cucumbers had no direct contact with the contaminated soil were chosen for the experiment. The aim was to determine if there is a difference between the uptakes of PFC of the three crops and whether there are preferences for the distribution of PFOA and PFOS to the different compartments of the plants.

MATERIALS AND METHODS

Vegetation Study. Seed potatoes (variety Christa-Z) were exposed to light two weeks before planting, so they could start sprouting. Cucumber seeds (variety Pepinova) were sown, and the seedlings were cultivated for three weeks before they were planted in the contaminated soil. Carrot seeds (variety Flyaway, F1 Hybrid) were treated the same way as the cucumber seeds. Contamination of the soil for our experiment was done by mixing PFC-free soil with PFC-contaminated sewage sludge. For each kind of plant, there was a control tub containing untreated soil and two tubs of contaminated soil containing PFOA and PFOS in varying concentrations. The tubs for potatoes and carrots comprised 43 kg soil (DM), on average, and the ones for cucumbers, 25 kg soil (DM). The sewage sludge used for spiking contained 7000 μg of PFOS per kg DM. DM content was 3%. For some sets, the sewage sludge additionally was spiked with PFOS and PFOA prior to mixing with the soil. An aliquot of the mixture was then analyzed to measure the actual contamination of the substrate for each tub. In Table 1, an overview of the experimental setup is given.

The amount of sewage sludge per tub was calculated according to the German sewage sludge regulation,[17] which restricts the application of sewage sludge in agriculture to a maximum level of 5 tons (DM) per hectare within three years. Plants were cultivated in a greenhouse and were watered daily with water free of PFOA and PFOS (PFOA and PFOS < 0.001 μg/L) from a fountain. Thus, side contamination with PFC was ruled out. The average volume of water applied for each tub was 275 L for cucumbers, 139 L for potatoes, and 151 L for carrots. Holes in the bottom of the tubs were necessary to avoid stagnant moisture. Thus, small amounts of water drained off sometimes, and it is impossible to calculate the transpiration rate of the plants exactly. A minor loss of PFOA and PFOS could have occurred by this as well.

After harvesting, the plant material from each tub was separated according to its respective plant compartment. Thus, mixed samples of vegetative compartments, edible parts, and peelings were created. Potato leaves, stalks, and roots were washed with pure water, dried superficially, and homogenized together using a BUECHI B-400 mixer (Essen, Germany). The tubers were peeled, and the peeled tubers and the peelings were homogenized separately. The carrot foliage was separated, washed with pure water, dried superficially, and homogenized. The carrots were peeled, and the peeled carrots and the peelings were homogenized separately. The cucumber stalks and leaves were separated, washed with pure water, and homogenized. The cucumbers were homogenized unpeeled. Soil samples of the different tubs were freeze-dried using an Edwards freeze-dryer type Modulyo (Crawley, UK). The dried soil samples were extracted and analyzed in triplicate.

Table 2. Mass Transitions Used for Identification and Quantification

compd	transition quantifier (m/z)	transition qualifier (m/z)	approx. retention-time (min)
PFOA	413/169	413/369	6.68
PFOS	499/80	499/99	7.65
^{13}C-PFOA	417/372	417/169	6.7
^{13}C-PFOS	503/80	503/99	7.7

Chemicals. Methanol hypergrade for LC-MS was obtained from Merck (Darmstadt, Germany). Pure water was from an Integral 3 A10 Millipore water purification system (Millipore, Billerica, MA). Nitrogen 5.0 was from Linde (Munich, Germany). Methanolic HCl was supplied by SUPELCO (Taufkirchen, Germany), and sodium hydroxide was from VWR (Darmstadt, Germany). Ammonium acetate, puriss. p.a., ACS reagent, reag. Ph. Eur., ≥98%, was supplied by Sigma Aldrich (Taufkirchen, Germany). Diamino-Carbon Clean-Up-Mix (Quechers Mix VII containing 0.9 g $MgSO_4$, 0.4 g Chromabond Carbon, and 0.3 g Chromabond Diamino) was from Macherey-Nagel (Düren, Germany). Perfluorooctanesulfonic acid potassium salt (KPFOS >98%) obtained from Fluka (Taufkirchen, Germany) and perfluorooctanoic acid (PFOA >96%) from Sigma Aldrich (Taufkirchen, Germany) were used for sewage sludge spiking. As the analytical reference material PFOA (50 μg/mL MeOH), linear perfluorooctanesulfonic acid sodium salt (L-PFOS) (50 μg/mL MeOH), 1,2,3,4-$^{13}C_4$—PFOA (50 μg/mL MeOH), and 1,2,3,4-$^{13}C_4$—PFOS (50 μg/mL MeOH) were obtained from LGC-Standards (Wesel, Germany) as well as acetonitrile of HPLC gradient grade. SPARTAN 13/0.45 RC-filter units were from Whatman (Dassel, Germany) and syringes (2 mL without needle, 6% luer) from Terumo Europe N.V. (Leuven, Belgium)

Analytical Method. In accordance with Powley et al.,[18,19] ultrasonic assisted solvent extraction and cleanup by graphitized carbon black were employed as well as alkaline treatment of soil samples. Contrary to the Powley-protocol, acetonitrile was used for extraction, and sample cleanup was refined by dispersive solid-phase extraction using PSA (primary secondary amine) in accordance with Anastassiades et al.[20]In a polypropylene centrifuge tube, 2.5 g of homogenized carrots, potatoes, or cucumbers, or 1 g homogenized potato peelings, carrot peelings, stalks, leaves, or freeze-dried soil were weighed. ^{13}C-labeled PFOA and PFOS as internal standards were added in solution (150 ng/mL methanol each) and mixed well with the plant material. Acetonitrile (5.0 mL) was added, and the closed tube was shaken vigorously for 5 min before being treated in an ultrasonic bath for 15 min and then centrifuged for 10 min at 3200*g*. The supernatant was removed and placed in another polypropylene tube. The extraction was repeated with another 5.0 mL of acetonitrile. Supernatants were combined and put in a polypropylene tube prefilled with the diamino-carbon-cleanup-mix. The extract was shaken vigorously with the cleanup-mix for 2 min and then centrifuged for 10 min at 3200*g*. An aliquot of the supernatant (5.0 mL) was transferred to a polypropylene falcon tube, and 100 μL of water was added as a keeper. The cleaned extract was reduced to 100 μL under a gentle stream of nitrogen using a commercial evaporation device (Barkey flowtherm optocontrol s, Barkey, Leopoldshoehe, Germany), and 100 μL of methanol was added. Finally, the solutions were filtered using a 0.45 μm RC-filter. According to Powley et al.,[18,19] samples of soil were treated with 1 mL of 200 mM NaOH/methanol solution for 30 min followed by subsequent neutralization with 2 M HCl/methanol before extraction. All samples were analyzed in triplicate. Matrix calibration curves using spiked blank samples that were extracted in analogy to the samples were applied for quantification. Potato tuber matrix calibration was used for the quantification in the peeled tubers and in the potato peelings as well. In the same way, values in the peeled carrots and carrot peelings were determined. Calculated values in the vegetative compartments which exceeded the respective calibration curve were determined by an independent workup using standard addition.

Table 3. Limits of Detection and Limits of Quantification in Different Matrixes[a]

	PFOA LOD/LOQ (ng/g)	PFOS LOD/LOQ (ng/g)	sample weight (g)
cucumber matrix	0.09/0.31	0.15/0.49	2.5 WS
vegetative cucumber matrix	0.14/0.44	0.1/0.32	1.0 WS
carrot matrix	0.03/0.11	0.05/0.17	2.5 WS
vegetative carrot matrix	0.07/0.24	0.11/0.37	1.0 WS
potato matrix	0.05/0.17	0.03/0.10	2.5 WS
vegetative potato matrix	0.08/0.28	0.11/0.37	1.0 WS
soil	0.25/0.8	0.17/0.54	1.0 DM

[a] The data show the mean value of six independent experiments. WS: Wet substance. DM: Dry matter.

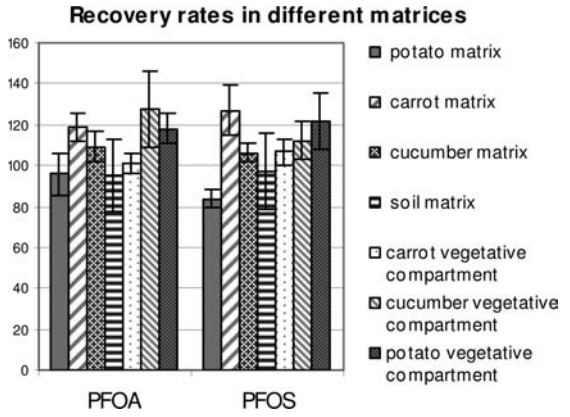

Figure 1. Recovery rates of PFOA and PFOS in different matrixes (determined at the concentration of 1 ng/mL, calculated with internal standard). The data show the mean ± standard deviation of six independent experiments.

Analyses were performed using liquid chromatography—negative electrospray tandem mass spectrometry (LC-MS/MS). Samples (10 μL injection volume) were separated at 25 °C on a 100 mm × 2.0 mm LUNA C_8 analytical column and a 4 mm × 2.0 mm guard column, both 100 Å pore size, 5 μm particle size from Phenomenex (Aschaffenburg, Germany). A 2 mM solution of ammonium acetate in Milli-Q purified water and pure methanol were used as mobile phase solutions A and B, respectively. The following gradient program was applied: Starting with 55% of solvent B, the proportion was increased to 70% B within 1 min; held at 70% for 5 min; increased again within 1 min to 80% B and held at 80% B for 7 min. Solvent B was reduced to 55% in 0.1 min and equilibrated at 55% B for 5 min. The flow rate was held at 0.27 mL/min constantly. The HPLC system was an Agilent 1100 LC binary pump G1312A with 3-line-Agilent degasser G1379A, an Agilent 1100 G1313A autosampler, and Agilent column oven (Agilent, Palo Alto, CA). The liquid chromatograph was connected to an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) for detection. Table 2 shows the mass transitions used for identification and quantification.

Data Processing and Statistics. Analyst 1.4 (Applied Biosystems, Ontario, Canada) was used for data processing. Statistical data evaluation was carried out with Microsoft Office 2003 (Microsoft Corporation, Redmond, WA).

Table 4. Concentrations of PFOA and PFOS Measured in the Different Compartments of the Cultivated Plants[a]

	potatoes contam. tub 1		potatoes contam. tub 2		carrots contam. tub 1		carrots contam. tub 2		cucumbers contam. tub 1		cucumbers contam. tub 2	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
peeled edible parts	2.9 ± 0.3	<LOD	7.7 ± 0.9	0.7 ± 0.1	31.3 ± 2.5	0.5 ± 0.03	30.8 ± 1.8	18.4 ± 2.5	11.3 ± 0.4	n.d.	23.8 ± 1.0	1.3 ± 0.2
peelings	7.7 ± 0.7	0.2 ± 0.03	17.6 ±1.4	15 ± 2.2	25.1 ± 1.4	0.3 ± 0.1	29.3 ± 3.1	16.4 ± 2.5	*b*	*b*	*b*	*b*
vegetative compartments	103.6 ± 15.8	4.1 ± 0.46	331.1 ± 20.6	141.1 ± 9.9	361.2 ± 55.2	3.2 ± 0.1	356.7 ± 27.7	194.9 ± 7.7	307 ± 38.9	1.2 ± 0.07	796.6 ± 73.6	119 ± 0.7

[a] The data show mean values of three independent experiments. All values are given in μg/kg WS ± standard deviation. n.d.: not detectable. [b] Cucumbers were analyzed unpeeled.

Validation. Blank matrix was obtained from the control tub with PFC-free soil. For every matrix, six calibration points with concentrations of 0.1/0.3/0.5/1.0/5.0/10.0 ng/mL were prepared, and for each concentration point, six extractions were made and analyzed. The spiked samples were extracted as described above.

The limit of detection (LOD) was defined as the lowest concentration that could be distinguished from a sample containing no analyte and calculated with the formula LOD = $y_0 + 3 \cdot s_0$ (y_0, average of measured values for the blank matrix; s_0, standard deviation of the measured values for the blank matrix). Limits of quantification were calculated with the formula LOQ = $y_0 + 10 \cdot s_0$. LODs and LOQs were measured for each matrix on replicate analyses ($n = 6$) of blank samples. LOD and LOQ were determined for each matrix and yielded values well below 1 ppb (see Table 3). The accuracy of the method was assessed by determining the recoveries of PFOA and PFOS from the spiked blank matrix samples in comparison to matrix-matched standards (c = 1 ng/mL). Resulting recoveries were between 75% and 128% with standard deviations ranging from 5 to 19% (see Figure 1).

RESULTS

Vegetation Study. There were no remarkable differences in the growth of the untreated and contaminated sets of the plants except for with the cucumbers. After two weeks, cucumber plants growing on untreated soil needed fertilizer, so an extract of stinging-nettles was self-made and used as fertilizer. There was no PFOA or PFOS detectable in this stinging-nettle extract. Depending on the plant, harvesting started after 63 to 96 days. For the cucumbers, between two and four fruits were harvested from each plant. Thirty little carrots were harvested from each tub, and one potato seed yielded between 10 to 17 new potatoes. An overview on growth duration, yield of fruits, and harvest weights is provided in Table 1.

Uptake of PFOA and PFOS. Concentrations of PFOA and PFOS measured in the different plant compartments of each experimental set are given in Table 4. Transfer factors (TF) were calculated by division of the amount of analyte found in the plant material (μg/kg wet substance (WS), see Table 4) by the concentration determined in the soil (μg/kg DM, see Table 1). For all calculations, the mean value of three independent analyses was used. The transfer rates determined for the two tubs per plant type are presented in Table 5. They fit quite well, even at different concentrations.

For the potatoes, the amount of PFOA measured in the peelings stemming from the lower contaminated tub 1 came to 7.7 ± 0.7 μg/kg WS. For peelings from tub 2, 17.6 ± 1.4 μg/kg WS was determined. These amounts are about twice as high as those in the peeled tubers (2.9 ± 0.3 μg/kg WS and 7.7 ± 0.9 μg/kg WS). PFOS could only be detected in low amounts of 0.7 ± 0.1 μg/kg WS in the peeled tubers, which grew on the highly contaminated soil. The amount in the corresponding peelings was about 20 times higher (15.0 ± 2.2 μg/kg). The resulting transfer rate for PFOS to the potato peelings was 0.05 and even higher than that for PFOA (0.02). The transfer rates to the green parts of the potatoes were found to be about 10 times higher for both compounds (PFOA 0.40; PFOS 0.36, average values).

The transfer rate of PFOA to the peeled carrots was calculated as 0.05 for the two sets analyzed in this study. Thus, it is generally higher than that of the peeled potatoes, for which a factor of 0.01 was determined for both tubs. In the case of the carrots, there is hardly any difference between the concentration of PFOA and

Table 5. Transfer Factors Determined for the Different Plant Compartments[a]

	potatoes contam. tub 1		potatoes contam. tub 2		carrots contam. tub 1		carrots contam. tub 2		cucumbers contam. tub 1		cucumbers contam. tub 2	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
peeled edible parts	0.01	0	0.01	<0.01	0.05	0.05	0.05	0.04	0.03	0	0.03	<0.01
peelings	0.03	0.02	0.02	0.05	0.04	0.03	0.04	0.04	*b*	*b*	*b*	*b*
vegetative compartments	0.38	0.27	0.42	0.45	0.53	0.32	0.53	0.43	0.76	0.12	0.99	0.21

[a] Calculated as the mean value of sample divided by the mean value of soil (μg/kg WS plant/μg/kg DM soil). [b] Cucumbers were analyzed unpeeled.

Table 6. Distribution of the Whole Harvest Weight in the Plant Compartments[a]

	potatoes uncontam. tub	potatoes contam. tub 1	potatoes contam. tub 2	carrots uncontam. tub	carrots contam. tub 1	carrots contam. tub 2	cucumbers unconatm. tub 1	cucumbers unconatm. tub 2	cucumbers contam. tub 1	cucumbers contam. tub 2
peeled edible parts	56.5	58.9	55.6	42.7	43.6	39.1	47.2	39.3	44.9[b]	48.2[b]
peelings	9.6	9.1	8.3	16.1	14.4	15.0	*b*	*b*	*b*	*b*
vegetative compartments	33.9	32.0	36.1	41.2	42.0	45.9	52.8	60.7	55.1	51.8

[a] All values are given in % of the whole harvest weight. [b] Cucumbers were analyzed unpeeled.

PFOS in the peeled roots and in the peelings analyzed separately. Accordingly, transfer rates of PFOA and PFOS for peeled roots and peelings are found in the narrow range between 0.03 and 0.05. As with the potatoes, the highest concentrations were detected in the carrot foliage with transfer factors between 0.32 and 0.53, which were thus about 10 times higher than those in the edible parts.

Concentrations of PFOA measured in the cucumbers were the same as those in the carrots (11.3 ± 0.4 μg/kg WS and 23.8 ± 1.0 μg/kg WS). In contrast to this, only an amount of 1.3 ± 0.2 μg/kg WS PFOS could be found in the cucumbers, which grew on the highly contaminated soil. This amount was comparable to that found in the peeled potato tubers of tub 2. For the vegetative compartments of cucumber plants, the relationship between PFOA and PFOS with regard to the transfer rates was found to be different from that in potato and carrot plants. Whereas the transfer factors for PFOA in the cucumber plants were determined as 0.76 and 0.99 and were thus about twice as high as those in the vegetative compartments of carrots (0.53) and of potatoes (0.38 and 0.42), the respective factors for PFOS were only 0.12 and 0.21, which is about half of the values for PFOS in potatoes (0.27 and 0.45) and in carrots (0.32 and 0.43).

In summary, it can be stated that increasing concentrations of PFOA and PFOS in the soil cause increasing amounts in the plants. For all three kinds of plants, the transfer rates of both compounds to the respective vegetative compartments are about 10 times higher than to the edible parts.

The proportion of biomass of the edible parts (with peelings) calculated as a percentage of the whole biomass at the harvest stage ranged from 39 to 68% with the highest mean value for the potato plant (66%) and the lowest for the cucumber plant (45%). The mean value for the carrots lay in between (57%) (see Table 6). It can be calculated that for all three kinds of vegetables over 80% of the whole amount of PFOA and PFOS taken up by the plant was localized in the vegetative compartments. The calculated distribution to the different plant compartments for the six experimental sets expressed as a percentage of the whole uptake is presented in detail in Table 7. It can be seen that the portion of the whole amount of PFOA in cucumbers was about 2.8%, whereas in the case of the PFOS, it was only 1%. In the case of potatoes, about 4% of PFOA but only 0.6% of PFOS is localized in the edible, peeled tuber. The highest carryover to the edible parts (without peelings) occurred in the carrots (about 7% of PFOA and between 7% and 13% of PFOS).

DISCUSSION

The results of the vegetation study confirm that the carryover of PFC from soil to plants varies with the plant species and with the substance. These findings are in accordance with a comprehensive carryover study carried out by Stahl et al., who investigated spring wheat, oats, potatoes, maize, and rye grass.[15]

Stahl et al. found increasing carryover rates of PFOA and PFOS with rising amounts of the chemicals (0.25−50 mg/kg DM) in the soil.[15] This concentration dependence cannot be evaluated statistically in the present study due to the small number of experimental sets, but nevertheless, the data for PFOS, which was spiked in high levels (317 to 556 μg/kg DM) and in low levels (10 and 15 μg/kg DM) in the soil of different tubs, show a similar tendency for carrots and potatoes even in the smaller overall amounts chosen in this case. For all three kinds of plants, the highest transfer rates of both compounds were found in the respective vegetative compartments with calculated TF from 0.12 (PFOS, cucumber) to 0.99 (PFOA, cucumber). To our knowledge, PFC transfer factors for vegetative compartments of carrots, potatoes, or cucumbers have not yet been published elsewhere. Only recently, Yoo et al.[12] determined grass/soil accumulation factors of several perfluorinated chemicals for different sorts of grass. For PFOA, the mean accumulation factor, on a dry weight basis, was 0.25. For PFOS, it was 0.07. This corresponds well with the factors of 0.25 for PFOA and 0.16 for PFOS, which were calculated for corn shoots from the data given by Stahl et al. In the same study, higher factors were obtained for wheat straw: 3.99 (PFOA) and 0.77 (PFOS).[12,15] TF determined for the vegetative compartments of vegetables in the present study are even higher. To a certain extent, this might be caused by the root material of the potato and cucumber plants that

Table 7. Distribution of the Whole Amount of PFOA and PFOS in the Different Plant Compartments[a]

	potatoes contam. tub 1		potatoes contam. tub 2		carrots contam. tub 1		carrots contam. tub 2		cucumbers contam. tub 1		cucumbers contam. tub 2	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
peeled edible parts	4.9	0.4	3.4	0.7	8.1	13.3	6.7	7.3	2.9[b]	0[b]	2.7[b]	1.0[b]
peelings	2.0	1.6	1.2	2.3	2.1	2.6	2.4	2.5	*b*	*b*	*b*	*b*
vegetative compartments	93.1	98.0	95.4	97.0	89.8	84.2	90.9	90.3	97.1	100.0	97.3	99.0

[a] The data show mean values of three independent experiments. All values are given in % of the whole uptake amount. [b] Ccucumbers were analyzed unpeeled.

was analyzed together with the shoots and leaves as vegetative compartments, whereas for the determination of the grass accumulation factor by Yoo et al., the grass was cut above ground. However, the root fraction in comparison to the green parts of the plants was very small, and the TF values are the same as those found for pure carrot foliage; therefore, the root fraction is not expected to be an influencing factor. A calculation for the TF given in Table 5 on a dry weight basis for comparison with the literature data shows that the average accumulation factors for the potato vegetative compartments are 4.21 (PFOA) and 3.85 (PFOS) and for the carrot foliage 4.13 (PFOA) and 2.94 (PFOS). An extraordinarily high factor of 8.23 is yielded for the transfer of PFOA to the vegetative compartments of cucumber plants, whereas the accumulation factor of PFOS in this case is comparably low with a value of 1.56. In this respect, PFOA behaves like nonionic hydrophobic organic chemicals for which raised accumulation factors and comparably high translocation in shoots of Cucurbitaceae like cucumbers were reported.[16] Murano et al. suggest that roots of Cucurbitaceae produce protein-like materials in xylem sap that enforce the transportation of hydrophobic organic chemicals like Dieldrin through the roots to the shoots.[21] This mechanism is important for the accumulation of hydrophobic, nonionic substances like the dibenzo-*p*-dioxines or organochlorine pesticides that generally are hardly translocated from the roots to the shoots in the water phase of the xylem sap.[16] Contrary to these hydrophobic substances, PFOA and PFOS are tensides, which are water-soluble to a certain extent. Therefore, transport in the water phase of xylem or phloem sap should be possible. Our study strongly suggests an uptake from the soil through the roots via transpiration of soil water. Alternative pathways such as direct contact of the above ground plant compartments with contaminated soil or contamination by airborne particles containing PFC can be ruled out for our study because the experiment was carried out in a greenhouse, and watering was done carefully so that soil did not spatter. The soil−air−plant pathway, discussed as significant for PAHs, PCBs, and some kinds of PCDDs/Ds,[16] should be ruled out as well, as no contamination with PFOA and PFOS was observed for the blank experiments where the plants were grown near the experimental sets with contaminated soil. Furthermore, we assume that PFOA and PFOS predominantly exist in the ionic species in the soil, and therefore, the vapor pressure should be low.

Transfer to the edible parts (fruits, roots, and tubers) was lower than in the vegetative parts with calculated TF (wet substance basis) from not detectable (PFOS, cucumber) to 0.05 (PFOA and PFOS, carrot). This allocation was also found by Stahl et al. in the comparative study between grain and straw for spring wheat, oats, and maize.[15] A comparison of transfer factors of PFC to vegetables is only possible to our knowledge with data provided in the studies by Stahl et al.[15] and Fischer et al.,[13] which both analyzed potatoes. The TF is calculated on a wet substance basis in this case because for risk assessment, values for ready-to-eat food are needed and not for the dry matter of vegetables. The average PFOA transfer factor calculated for peeled potato tubers in the present study is 0.01. Fischer et al. determine an average value of 0.015 (calculated based on wet substance),[13] whereas the transfer rate calculated from the data given by Stahl et al. is comparatively lower (0.0007).[12,15] The TF found for PFOS by Stahl et al. is the same as that for PFOA (0.0007),[15] whereas in Fischer et al. and in the present study higher values are deduced (Fischer, 0.0025; Lechner and Knapp, 0.01).[13] Fischer et al. did not examine potato peelings. Stahl et al. provide data to calculate a TF of potato peelings for a PFOA of 0.002 (Lechner and Knapp, 0.02; all values based on wet substance).[15] For PFOS, the TF is determined as 0.03 in the present study and as 0.01 in the study by Stahl et al.[15] Therefore, in two autonomous experiments with different PFC levels in soil, PFOS uptake in potato peelings seems to override the uptake in the peeled tubers. According to literature, this behavior was also observed in uptake experiments with PAHs and was ascribed to the higher lipid contents of the peelings in comparison to the pulp.[22] As PFOS is not as soluble in water as PFOA, its lipophilicity might be responsible for the predominant accumulation in the peelings that is observed in the studies. For carrots, no difference in TF between peelings and peeled roots was detected. It is also noticeable that the calculated transfer factors in the carrots were up to five times higher than those of the peeled potatoes. This is not dependent on the water content as in the present study, the dry matter content of the peeled carrots was determined to be 9.4% and of the peelings, 11.0%, which is not a factor of 5 different from that of potatoes and potato peelings (16.2% and 13.9%). One reason for the raised transfer of organics to carrots according to the literature is the presence of oil channels and the higher content of lipids in the root.[23] For water-soluble PFOA and PFOS, the morphological origin of the carrot could have the greater impact. As carrots are storage organs and roots at the same time, water absorption during growth has to take place through the carrot itself, so that PFC solved in soil water have to be transported through the root compartments. The potato tuber is located at the end of a stem and serves as a storing compartment alone. PFC transported by the transpiration stream within the xylem probably had to pass to the phloem in order to reach the tuber together with the assimilation products from the leaves.

In the present study, PFOA could also be detected in cucumbers that did not have any direct contact with the contaminated soil in amounts similar to those of carrots and in

much higher amounts than those in potatoes. It cannot be clarified if the special composition of the xylem sap of Cucurbitaceae mentioned above accounts for the TF of PFOA observed in cucumbers, but obviously there has to be an uptake through the roots and transport in the plant presumably via the water. PFOS that is not as soluble in water as PFOA and that is found to be less mobile in soil[24] behaves quite differently in the vegetation experiment. Transfer of PFOS in the unpeeled cucumbers is comparable to peeled potatoes (TF below 0.01), and the calculated TF of 0.12 and 0.21 in the vegetative compartments of cucumber plants are comparatively low in comparison to the findings for potatoes and carrots (TF ranging from 0.27 to 0.45). It remains unclear whether a different uptake or transport characteristic is responsible for this. Contrary to cucumbers in carrot and potato plants, a significant transport of PFOS as well has to take place because the substance was found in the green parts with the highest TF.

For a toxicological risk assessment, the concentrations of PFC found in food have to be set in relation to the respective tolerable daily intakes (TDI) of the compounds. The European Food Safety Agency (EFSA) has published a benchmark confirming 0.15 μg/kg body weight as TDI for PFOS and 1.5 μg/kg body weight as TDI for PFOA.[24] PFC concentrations in the soil chosen for the present experiment were of the same size as in the soil in several fields in the Hochsauerland district of the State of North-Rhine Westphalia after the illegal distribution of a contaminated waste mixture as organic agricultural fertilizer.[2] The elevated soil levels of the present study resulted in maximum concentrations of PFOA at a level of 30 μg/kg in the edible compartments of carrots and cucumbers. PFOS accumulated most in carrots with levels of 20 μg/kg. Without any additional PFC intake, a man of 60 kg would have to eat about 3 kilos of the contaminated carrots or cucumbers every day to exceed the TDI for PFOA. The lower TDI of PFOS would be exceeded in 450 g of the highly contaminated carrots every day. As these amounts of vegetables are comparably high in a normal diet, the risk of exceeding the TDI for PFOA and PFOS is fairly low for adult humans even when consuming produce such as those in the study presented, grown on highly contaminated soil. However, it was shown that the vegetative plant compartments are particularly affected by the PFC carryover, which is in accordance with two other studies.[13−15] Because of this finding, feeding crops could possibly account for the main uptake into the food chain via plants. This assumption is affirmed by investigations of the governmental food control of Lower Saxony (LAVES). It was able to show that farmland contaminated by PFOS with 3300 μg/kg DM resulted in amounts of 154 μg/kg PFOS in meat and 1332 μg/kg PFOS in a cow's kidney in a cow fed on corn grown on the respective farmland. For the corn, an amount of 215 μg/kg DM PFOS had been determined.[25] Taking into account the difference between the transfer rate into carrots and potatoes, for example, it seems difficult to make general estimations for the amount of carryover from soil to plants, as the carryover depends on the type of plant and on the histology of the compartments, which are destined to be eaten or to be fed. Furthermore, it should not be forgotten as well that besides PFOA and PFOS other perfluorinated compounds could account as well for the overall PFC uptake. An investigation of sewage sludge of six wastewater treatment plants in Ontario, Canada, showed detectable concentrations of polyfluoroalkyl phosphates (PAPs) with varying chain length in the same magnitude as those measured for PFOS (about 100 ng/g).[26] Consequently, the European Commission has issued a recommendation to further monitor perfluorinated compounds like PAPs in food.[27] Because of the varying behavior observed for PFOA and PFOS in the present study, possible carryover of the compounds mentioned cannot be predicted at all and would have to be addressed in further studies.

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REFERENCES

(1) Kissa, E. Applications. In *Fluorinated Surfactants and Repellents*, 2nd ed.; Marcel Dekker: New York, 2001; pp 349−389.

(2) Barkowski, D.; Günther, P.; Raecke, F.; Wind D. Investigations concerning occurrence and impacts of PFC in waste, liable to the biowaste regulation: final report (in German), 2007. North Rhine-Westphalia State Office for Nature, Environment, and Consumer Protection (LANUV) Web site. http://www.lanuv.nrw.de/verbraucher/pdf/ifua_pft_bericht.pdf (accessed Sep 7, 2011).

(3) Frisbee, S. J.; Brooks, A. P., Jr.; Maher, A.; Flensborg, P.; Arnold, S.; Fletcher, T.; Steenland, K.; Shankar, A.; Knox, S. S.; Pollard, C.; Halverson, J. A.; Vieira, V. M.; Jin, C.; Leyden, K. M.; Ducatman, A. M. The C8 Health Project: Design, Methods, and Participants. *Environ. Health Perspect.* **2009**, *117*, 1873–1882.

(4) Lindstrom, A. B.; Strynar, M. J.; Delinsky, A. D.; Nakayama, S. F.; McMillan, L.; Libelo, E. L.; Neill, M.; Thomas, L. Application of WWTP Biosolids and Resulting Perfluorinated Compound Contamination of Surface and Well Water in Decatur, Alabama, USA. *Environ. Sci. Technol.* [Online early access]. DOI: 10.1021/es1039425. Published Online: April 22, **2011**. http://pubs.acs.org/doi/full/10.1021/es1039425 (accessed Sep 7, **2011**).

(5) Kissa, E. Toxicology and Environmental Aspects. In *Fluorinated Surfactants and Repellents*, 2nd ed.; Marcel Dekker: New York, 2001; pp 456−461.

(6) Bavarian Environment Agency. Compulsory PFC Analysis: Information on Data Transmission (in German). Bavarian Environment Agency Web site. https://www.klaerschlamm.bayern.de/doc/pft.pdf (accessed Sep 7, 2011).

(7) Second Draft Standard for the German Sewage Sludge Regulation (in German), 2010. Federal Ministry for the Environment, Nature Conservation and Nuclear Safety Web site. http://www.bmu.de/files/pdfs/allgemein/application/pdf/klaerschlammverordnung_neufassung.pdf and http://www.bmu.de/files/pdfs/allgemein/application/pdf/klaerschlammverordnung_grenzwerte_schadstoffe.pdf (accessed Sep 7, 2011).

(8) Ulrich, H.; Hübner, G.; Gierig, M.; Müller, R. PFC in Bavarian Sewage Sludge [Online]. Environment Special; Bavarian Environment Agency: Augsburg, Germany, December 2010; p 8 (in German). http://www.lfu.bayern.de/analytik_stoffe/analytik_org_stoffe_perfluorierte_chemikalien/doc/pft_bericht_klaerschlamm_2008_2009.pdf (accessed Sep 7, 2011).

(9) Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A. Monitoring Perfluorinated Surfactants in Biota and Surface Water Samples Following an Accidental Release of Fire-Fighting Foam into Etobicoke Creek. *Environ. Sci. Technol.* **2002**, *36*, 545–551.

(10) Awad, E.; Zhang, X.; Bhavsar, S. P.; Petro, S.; Crozier, P. W.; Reiner, E. J.; Fletcher, R.; Tittlemier, S. A.; Braekevelt, E. Long-Term Environmental Fate of Perfluorinated Compounds after Accidental Release at Toronto Airport. *Environ. Sci. Technol.* [Online early access].

DOI: 10.1021/es2001985. Published Online: July 20, 2011. http://pubs.acs.org/doi/full/10.1021/es2001985 (accessed Sep 7, **2011**).

(11) Commission Regulation (EU) No 757/2010 of 24 August 2010 Amending Regulation (EC) No 850/2004 of the European Parliament and of the Council on Persistent Organic Pollutants As Regards Annexes I and III; *Off. J. Eur. Union* **2010**; *L 223*, 29-36.

(12) Yoo, H.; Washington, J. W.; Jenkins, T. M.; Ellington, J. J. Quantitative Determination of Perfluorochemicals and Fluorotelomer Alcohols in Plants from Biosolid-Amended Fields using LC/MS/MS and GC/MS. *Environ. Sci. Technol.* [Online early access]. DOI: 10.1021/es102972m. Published Online: July 20, 2011. http://pubs.acs.org/doi/full/10.1021/es102972m (accessed Sep 7, **2011**).

(13) Fischer, R.; Kördel, W.; Weinfurtner, K. Final Report on the Project "Investigations on the Carry over of PFC from Contaminated Soil to Plants" (in German), 2008. North Rhine-Westphalia State Office for Nature, Environment, and Consumer Protection (LANUV) Web site. http://www.lanuv.nrw.de/verbraucher/pdf/transfer_pft.pdf (accessed Sep 7, 2011).

(14) Fischer, R.; Kördel, W.; Weinfurtner, K. Final Report on the Project "Investigations on the Carry over of PFC from Contaminated Soil to Plants", Results of Complementary Analyses in 2008 (in German), 2009. North Rhine-Westphalia State Office for Nature, Environment, and Consumer Protection (LANUV) Web site. http://www.lanuv.nrw.de/verbraucher/pdf/transfer_pft_2008.pdf (accessed Sep 7, 2011).

(15) Stahl, T.; Heyn, J.; Thiele, H.; Hüther, J.; Failing, K.; Georgii, S.; Brunn, H. Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plants. *Arch. Environ. Contam. Toxicol.* **2009**, *57*, 289–98.

(16) Collins, C.; Fryer, M.; Grosso, A. Plant Uptake of Non-Ionic Organic Chemicals. *Environ. Sci. Technol.* **2006**, *40*, 45–52.

(17) German Sewage Sludge Regulation with Last Amendment November 9, 2010; BGBl. I, 1992; 912 (in German).

(18) Perforce Method Protocols for Analysis of Perfluorinated Compounds, 2: Analytical Method for Perfluoralkyl Substances (PFAS) in Sediment, Powley Method, 2007. Perforce Project University of Amsterdam Web site. http://www.science.uva.nl/perforce/index.htm?http%3A//www.science.uva.nl/perforce/protocols2.htm. (accessed Sep 7, 2011).

(19) Powley, C. R.; George, S. W.; Ryan, T. W.; Buck, R. C. Matrix Effect-Free Analytical Methods for Determination of Perfluorinated Carboxylic Acids in Environmental Matrixes. *Anal. Chem.* **2005**, *77*, 6353–6358.

(20) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. *J. AOAC Int.* **2003**, *86*, 412–431.

(21) Murano, H.; Otani, T.; Seike, N. Dieldrin-Dissolving Abilities of the Xylem Saps of Several Plant Families, Particularly *Cucurbita pepo* L. *Environ. Toxicol. Chem.* **2010**, *10*, 2269–2277.

(22) Fismes, J.; Perrin-Ganier, C.; Empereur-Bissonnet, P.; Morel, J. L. Soil-to-Root Transfer and Translocation of Polycyclic Aromatic Hydrocarbons by Vegetables Grown on Industrial Contaminated Soils. *J. Environ. Qual.* **2002**, *31*, 1649–1656.

(23) Wang, M.-J.; Jones, K. C. Uptake of Chlorobenzenes by Carrots from Spiked and Sewage Sludge-Amended Soil. *Environ. Sci. Technol.* **1994**, *28*, 1260–1267.

(24) Opinion of the Scientific Panel on Contaminants in the Food Chain on Perfluorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA) and Their Salts. *EFSA J.* **2008**, *653*, 1-31.

(25) Report on the sanitary consumer protection 2008 (Online); Lower Saxony Ministry for Food, Agriculture, Consumer Protection and Regional Development, Lower Saxony State Office for Consumer Protection and Food Safety: Hannover, Germany, 2009; pp 32−33 (in German). http://www.laves.niedersachsen.de/live/live.php?navigation_id=20150&article_id=73752&_psmand=23 (accessed Sep 7, 2011).

(26) D'eon, J. D.; Crozier, P. W.; Furdui, V.; Reiner, E. J.; Libelo, E. L.; Mabury, S. A. Observation of a Commercial Fluorinated Material, the Polyfluoroalkyl Phosphoric Acid Diesters, in Human Sera, Wastewater Treatment Plant Sludge, and Paper Fibers. *Environ. Sci. Technol.* **2009**, *43*, 4589–4594.

(27) Commission Recommendation of 17 March 2010 on the Monitoring of Perfluoroalkylated Substances in Food (2010/161/EU). *Off. J. Eur. Union* **2010**, *L 68*, 22.