JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Long-Term Lysimeter Experiment To Investigate the Leaching of Perfluoroalkyl Substances (PFASs) and the Carry-over from Soil to Plants: Results of a Pilot Study

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ABSTRACT: To study the behavior of perfluoroalkyl substances (PFASs) in soil and the carry-over from soil to plants, technical mixtures of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) at a concentration of 25 mg/kg soil were applied to 1.5 m^3 monolithic soil columns of a lysimeter. Growth samples and percolated water were analyzed for PFASs throughout a period of 5 years. In addition to PFOA/PFOS plant compartments and leachate were found to be contaminated with short-chain PFASs. Calculation showed significant decreasing trends ($p < 0.05$) for all substances tested in the growth samples. Short-chain PFASs and PFOA pass through the soil much more quickly than PFOS. Of the 360 g of PFOA and 367.5 g of PFOS applied to the soil, 96.88% PFOA and 99.98% PFOS were still present in the soil plot of the lysimeter after a period of 5 years. Plants accumulated 0.001% PFOA and 0.004% PFOS. Loss from the soil plot through leachate amounted to 3.12% for PFOA and 0.013% for PFOS.

KEYWORDS: *PFASs, lysimeter, spiking, carry-over, plant, leachate*

INTRODUCTION

Perfluoroalkyl substances (PFASs) have been manufactured for more than 60 years and due to their unique physical and chemical properties have found a myriad of uses both in industrial processes and in consumer products.[1] PFASs are chemically very stable and resistant to biological degradation, properties that place them in the class of persistent substances. Perfluorooctane sulfonate (PFOS) has been added to the list of Persistent Organic Pollutants (POP Regulation) (Stockholm Convention on Persistent Organic Pollutants. Perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOS-F). Listed under Annex B with acceptable purposes and specific exemptions (decision SC-4/17). Stockholm, Sweden, 2011). These exclusively anthropogenic substances can potentially bioaccumulate and undergo biomagnification. Substances with a chain length of up to eight carbon atoms have elevated water solubilities in water.[2,3] Water, in addition to dissemination of volatile precursor molecules via the air,[2] is presently considered the main pathway for propagation of PFASs in the environment.[4,5] Vegetables or grains that have been grown on agricultural lands that were irrigated with PFAS-contaminated water can take up these molecules as has been shown in a number of studies.[6−9] Another possibility for the carry-over of PFASs from soil to plants is the uptake from agricultural lands that have been fertilized with effluent sludge or the legal deposition of waste material containing PFASs in landfills.[10−12] Illegal disposal can, however, also result in considerable local contamination. For example, in various German provinces thousands of tons of PFAS-contaminated ameliorant (a mixture of effluent sludge from the food, beverage, and tobacco industry mixed with rock flour) have been deposited on farmlands from 2002 to 2006.[13] Aside from direct PFAS uptake, natural precipitation such as rain may wash highly water-soluble PFASs out of soil or from landfills, therefore making these substances available to plants for uptake via the water pathway.[14−16] If these plants serve as human food or animal fodder, this cycle will provide a direct or indirect entry point for PFASs into the human food chain as shown in a study with PFAS-contaminated corn silage fed to sheep.[17] In particular, the consumption by or feeding of fodder to animals from so-called hot-spot regions in which soil or water contains much higher concentrations of PFAS than the ubiquitous background contamination could represent a potential health risk to humans. In addition, PFASs that rapidly pass through the soil may reach the groundwater and thus find their way into tap water or bottled mineral water,[18] thereby contributing to human PFAS exposure. In the meantime, PFASs have been detected in various human matrices including blood,[19,20] plasma,[21,22] and breast milk.[23,24] Toxicological evaluation of this contamination has also been undertaken.[25] The lysimeter study described here is therefore ideally suited to study both the carry-over of PFAS in various plants as well as in plant matrices (straw and grain) and, in parallel, potential translocation of the substances from soil to leachate. Studies of this kind have been successfully carried out for other substances such as the veterinary antibiotics tetracycline and sulfadiazine.[26−28] Moreover, these long-term experiments will allow the

Received: November 29, 2012
Revised: February 1, 2013
Accepted: February 4, 2013
Published: February 4, 2013

dx.doi.org/10.1021/jf305003h | J. Agric. Food Chem. 2013, 61, 1784−1793

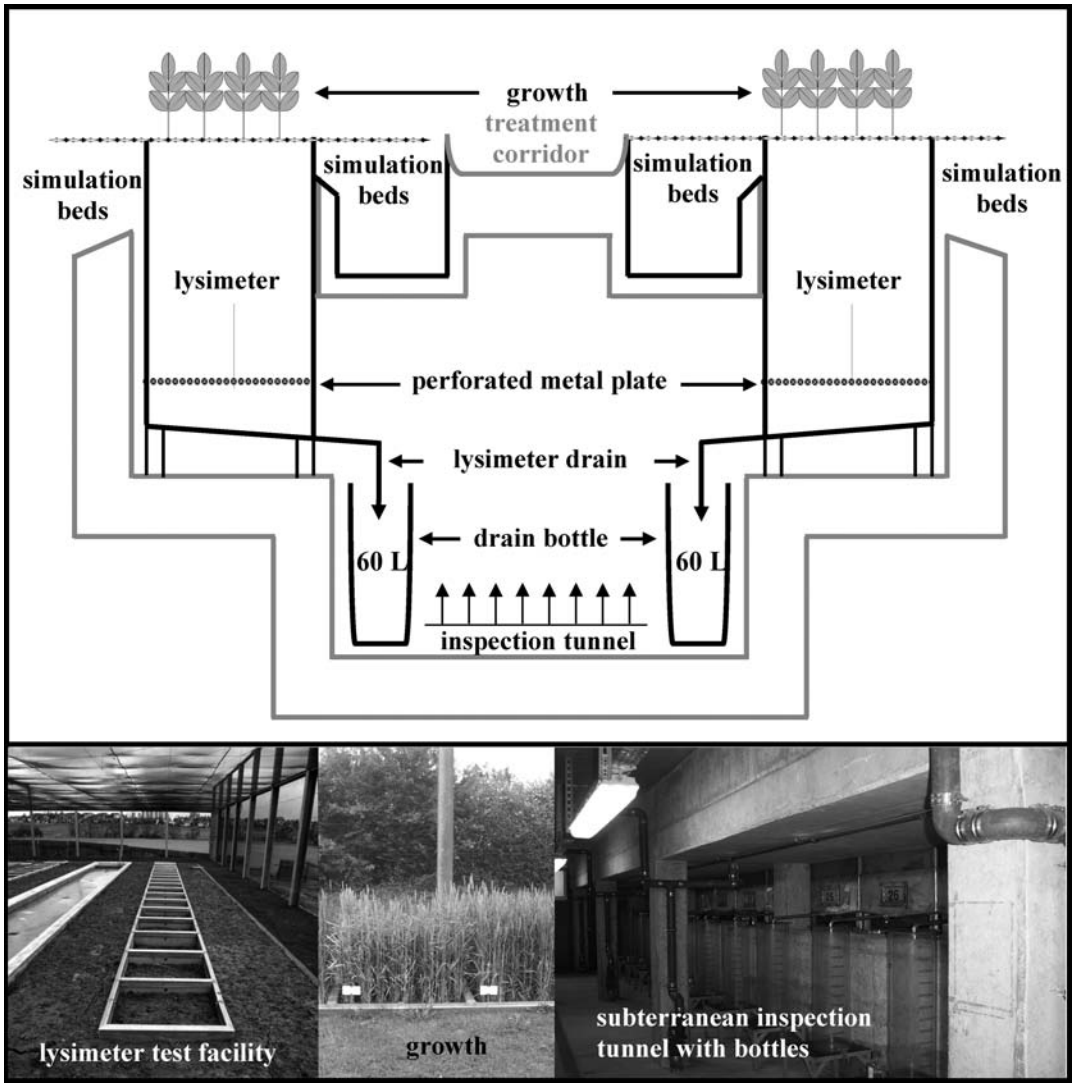

Figure 1. Schematic cross section and photographs of the lysimeter test facility.

Table 1. Crop Sequence in the Experimental Period from 2007 to 2011 Showing Grain and Straw Yields plus Leachate Volume

	2007	2008	2009	2010	2011
grain and straw yield					
plant species	winter wheat	winter rye	canola	winter wheat	winter barley
grain yield (kg/m^2)	0.48	1.08	0.38	0.79	0.62
straw yield (kg/m^2)	0.80	0.83	0.77	0.81	0.88
leachate volume (L/m^2)					
January	–[a]	46.5	0	17.5	11.2
February	–	20.9	0	60.2	19.9
March	–	40.3	0	25.8	4.65
April	begin	0	0	8.58	0
May	0[b]	0	0	0	0
June	0	0	0	0	0
July	0	0	0	0	0
August	0	0	0	0	0
September	0	0	0	0	0
October	4.1[c]	0	0	0	0
November	51.2	0	0	0	0
December	62.6	0	0	0	0

[a]Volume of lysimeter water not reported (prior start of experiments). [b]The number "0" in the table indicates that in these particular months no leachate accumulated. [c]The first lysimeter leachate eluted in October 2007, 6 months after the soil had been spiked in April of the same year.

dx.doi.org/10.1021/jf305003h | J. Agric. Food Chem. 2013, 61, 1784−1793

analysis and statistical validation of temporal trends in regard to PFAS translocation in plants and in leachate. To the best of our knowledge no long-term soil studies of PFASs have been previously carried out or published. The principal focus of the experiments described here was the uptake and elution behavior of perfluorooctanoic acid (PFOA) and PFOS. In addition, however, results are also presented regarding short-chain (PFBA, PFBS, PFPeA, PFHxA, PFHxS, and PFHpA) contaminants that are present in technical mixtures of PFOA and PFOS. The results may allow further conclusions to be drawn concerning elution and uptake behavior of PFASs in relation to chain length and functional groups.

MATERIALS AND METHODS

Lysimeter. The lysimeter test facility used in this study was constructed in 1992 and 1993 and consists of 32 stainless steel lysimeters, each with a square-shaped surface area of 1 m^2 and a depth of 1.5 m. The cells contain monolithic soil columns of the type "gray brown podzolic soils" from eolian silt deposits and have perforated metal bottom plates through which the leachate can pass. The system is not artificially irrigated, but is subjected only to natural rainfall. Glass drain bottles to collect leachate (each with a capacity of 60 L) are located in a subterranean inspection tunnel beneath the lysimeter columns. So-called simulation beds are located on either side of the two rows of lysimeters and are designed to reduce the insular effect within the test chambers. The complete facility is protected with a fine mesh screen to keep birds from eating the grain. Figure 1 shows a schematic cross section and photographs of the lysimeter test facility.

Vegetation Study. In an earlier study extensive experiments were performed using Mitscherlich pots within the framework of a joint project to study the carry-over of PFASs from soil to plants in 2007.[6] Additionally, aqueous solutions of PFOA and PFOS (technical mixtures of both) were applied with a target concentration of 25 mg/kg soil to four non-neighboring lysimeter soil plots in June 2007. To avoid disturbing the structure of the soil plot of the lysimeters, samples were not analyzed before application of the substances. Due to the relatively high doses applied in the experiments, however, any PFASs already present in the soil can be disregarded because analyses of previous soil samples have shown that background contamination in Hesse, Germany, is generally <10 μg/kg. The long-term experiments on the spiked soil plots of the lysimeters began with winter wheat in 2007. Table 1 shows the crop sequence in the ensuing 5 years as well as the most important parameters in regard to the results of the study (grain yield, straw yield, and leachate volume per year and month).

Chemicals. The technical mixtures of PFOA (chemical purity = 96%) and PFOS (chemical purity = 98%) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All standards for chemical analysis (perfluoro-*n*-butanoic acid, perfluoro-*n*-pentanoic acid, perfluoro-*n*-hexanoic acid, perfluoro-*n*-heptanoic acid, perfluoro-*n*-octanoic acid, perfluoro-*n*-nonanoic acid, perfluoro-*n*-decanoic acid, perfluoro-*n*-dodecanoic acid, potassium perfluoro-1-butanesulfonate, sodium-perfluoro-1-hexanesulfonate, and sodium-perfluoro-1-octanesulfonate (chemical purity each ≥ 98%)) as well as mass-labeled internal standards (perfluoro-*n*-[1,2,3,4-$^{13}C_4$]butanoic acid, perfluoro-*n*-[1,2-$^{13}C_2$]hexanoic acid, perfluoro-*n*-[1,2,3,4-$^{13}C_4$]octanoic acid, and sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate (chemical purity each ≥ 98%, isotopic purity ≥ 99%)) by Wellington Laboratories (Ontario, Canada) were obtained from Campro Scientific (Berlin, Germany). Acetonitrile, methanol, and water with a purity of ≥99.97% (passed through a filter with a pore size of 0.1 μm, filled into bottles under inert gas) as well as ammonium acetate, ACS reagent (passed through a filter with pore size of 0.1 μm before final crystallization) were obtained from Biosolve BV (Valkenswaard, The Netherlands). QuEChERS-Mix (QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe) (self-prepared) used for cleanup during sample preparation contained trisodium citrate dihydrate (15%) with a purity of ≥99.5%, magnesium sulfate (62%) with a purity of ≥97.0%, and disodium hydrogen citrate sesquihydrate (8%) with a purity of ≥99.0% from Sigma-Aldrich Chemie GmbH and sodium chloride (15%) with a purity of ≥99.5% obtained from Merck (Darmstadt, Germany). An aqueous solution of formic acid with a purity of ≥96.0% from Sigma-Aldrich was used for conditioning and washing solid phase extraction cartridges. Ammonium hydroxide, ACS reagent for eluting PFASs from cartridges, was obtained from Sigma-Aldrich. Unless otherwise noted all chemicals and solvents were obtained from Merck and from Sigma-Aldrich (Taufkirchen, Germany) of the quality "purum" or "suprapur".

Analytical Method for Plants. Every year the straw and grain were harvested separately from the four soil plots. Subsets from the individual samples from lysimeters were combined to the two samples "straw" and "grain". The combined growth samples were dried in a drying oven (Memmert, Schwabach, Germany) at 40 °C to constant weight, and an aliquot of 100 g was then ground and homogenized in a knife mill Grindomix GM 200 (Retsch, Haan, Germany). The subsequent sample preparation was performed according to the methods of Stahl et al.[6,29] Samples of 1 g of homogenized plant material were suspended with 50 μL of internal standard solution consisting of $^{13}C_4$-perfluorobutanoic acid, $^{13}C_2$-perfluorohexanoic acid, $^{13}C_4$-perfluorooctanoic acid, and $^{13}C_4$-perfluorooctanesulfonate with a concentration of 100 μg/L, in 2 mL of acetonitrile and 2 mL of ultrapure water in a centrifuge tube (VWR, Darmstadt, Germany) and were vigorously shaken for 30 s by hand. The tube was then tightly closed and shaken on a mechanical shaker (Heidolph, Schwabach, Germany) for 15 min. After the addition of 1.5 g of QuEChERS-Mix (see also Chemicals) (developed by Anastassiades[30] for the determination of pesticide residues in agricultural products), the samples were shaken again for 30 s by hand and centrifuged (centrifuge, Sigma, type 3k15, Osterode am Harz, Germany) for 5 min at 1800*g*. Addition of the salt mixture to the samples resulted in a well-defined phase separation. The organic supernatant was mixed with 2 mL of ultrapure water, and the target molecules were enriched by solid phase extraction. Oasis WAX (60 mg sorbent, 3 mL cartridge, Waters, Eschborn, Germany) that had been conditioned first with 2 mL of 0.1% formic acid in water (v/v) and then with 2 mL of methanol served as solid phase. Finally, the organic supernatant mixed with 2 mL of ultrapure water was applied to the column. It was then washed first with 2 mL of 0.1% formic acid in methanol and then with 2 mL of methanol. Elution of the target compounds was performed with 2 mL of 0.1% ammonia in methanol (v/v). The extracts were evaporated to dryness at 39 °C under nitrogen gas (biostep, sample concentrator, Jahnsdorf, Germany) and reconstituted with 250 μL of a methanol/water mixture (50:50 v/v) by means of a vortex mixer (VWR). The samples were then strained through a polyester filter (pore size = 0.45 μm, Macherey & Nagel, Düren, Germany) attached to a 2 mL disposable syringe and transferred to sample vials. Analysis was performed by HPLC−tandem mass spectrometry with electrospray negative ionization. Data were collected on an Alliance 2695 separation module coupled to a Quattro-Micro tandem mass spectrometer (Waters). Separation was performed on a binary gradient of methanol and ammonium acetate solution (2 mmol) using a Luna C18 HPLC column, 150 × 3 mm, 3 μm particle size (Phenomenex, Aschaffenburg, Germany). Chromatographic separation was performed on a gradient (0.3 mL/min) beginning with 55% A (25 mmol ammonium acetate in methanol) and 45% B (25 mmol ammonium acetate in water). The proportion of A increased to 95% in the first 4.0 min and was then increased to 98% from 4.0 to 6.6 min and then maintained at 98% from 6.6 to 10 min. Initial conditions were restored beginning at the 11 min. The injected volume was 20 μL.

Analytical Method for Water. The lysimeter water (leachate) of each lysimeter was collected through a valve in the bottom of the 60 L glass jars. The leachate collection bottles were completely emptied at each sampling period. The leachates of the four bottles were combined and homogenized with a stirrer in the laboratory. One liter aliquots were drawn off as laboratory samples. Approximately 10 mL of the homogenized aliquots was filtered through a 45 μm syringe polyester filter (Macherey & Nagel). The concentrations of the individual substances can vary greatly from sample to sample; however,

determination of concentration is necessary to determine temporal trends in changes of concentration and also to calculate the mass balance (see the mass balance study for PFOA and PFOS). Fifty microliters of the internal standard solution consisting of $^{13}C_4$-perfluorobutanoic acid, $^{13}C_2$-perfluorohexanoic acid, $^{13}C_4$-perfluorooctanoic acid, and $^{13}C_4$-perfluorooctanesulfonate with concentrations of 10 μg/L each was added to 0.25 mL of the filtered lysimeter water. To eliminate the possibility of cross-contamination and for quality assurance, a PFAS-free water sample was prepared in the same manner in triplicate. Analysis was performed by HPLC−tandem mass spectrometry with negative ionization. Data were collected on an Acquity UPLC (Waters) separations module coupled to an Acquity TQD tandem mass spectrometer (Waters). Chromatographic separation was performed on a gradient at 0.3 mL/min, beginning with 55% A (methanol) and 45% B (25 mmol of ammonium acetate in water). The proportion of A was gradually increased to 95% by 4 min and from 6.6 to 10 min was held constant at 98%. At 11 min the initial conditions were restored. A 10 μL aliquot of the sample was injected, and analytes were chromatographically separated using a Kinetex C18 HPLC column, 100 × 2.1 mm, 2.6 μm particle size (Phenomenex) at a flow rate of 0.4 mL/min.

Analytical Method for Technical Mixtures of PFOA and PFOS. By nature of their manufacture, technical mixtures of PFOA and PFOS are known to generally be contaminated with shorter chain homologues. Because these substances could be detected both in the growth samples and in the lysimeter water, the quantitative compositions of the technical mixtures used were analyzed. This was necessary because the safety specifications for PFOA and PFOS do not list any information on the concentration of possible contaminants and because the manufacturer did not respond to our request for this information. After the substances had been dissolved and diluted (1:1000) in reagent grade water, the samples were analyzed as described under Analytical Method for Water. Mass spectrometric measurement of the PFOA technical mixture showed that 3.5% consisted of PFBA, PFPeA, PFHxA, and PFHpA and that PFBS and PFHxS together made up approximately 1.5% of the PFOS technical mixture. As a result of matrix effects caused by the preponderance of PFOA (95%) and PFOS (which makes up as much as 98% of the total), it is not currently possible to determine exact amounts of the individual components with adequate statistical certainty.

Data Processing. MassLynx V4.0 and V4.1 (copyright 2008 Waters Inc.) were used for data processing.

Statistical Analysis. Statistical evaluation was performed using the statistical program package BMDP.[31] Statistical analysis of the temporal decrease in PFAS concentration in both grain and straw was performed on winter wheat, the only crop grown in two seasons (compare Table 1). Analysis was made by two-way analysis of variance with repeated measures using the program BMDP2 V. Global representation of the change in temporal course from 2007 to 2011, individually by compartment (straw and grain), was performed using the program BMDP6D. The BMDP3D program was used to determine rank correlation coefficients according to Spearman (r_s) to describe and test the global change in temporal course of PFAS concentration. The rank correlation coefficient was used because some values of PFAS concentrations were below their limit of quantification (LOQ). The BMDP3D program was also used to compare PFASs concentration in the compartments (straw and grain), individually according to year (using either the t test or the Wilcoxon-Mann−Whitney test, if there were data below the LOQ), to compare the species- specific uptake of PFASs. In all statistical tests the level of significance was set to $\alpha = 0.05$.

Validation of Methods for Testing Plant Samples. Calibration solutions were prepared with substance concentrations between 0 and 100 μg/L (0, 20, 50, and 100 μg/L). The limits of quantification (LOQ; signal-to-noise ratio of 3:1) were determined for the plant material specifically according to matrix. Our prerequisite internal specification required that the correlation coefficient for daily calibration for each compound had to be between 0.991 and 0.999. If this internal quality target was not achieved, a new calibration was undertaken and, if necessary, new standard solutions were prepared. Samples that were found to contain higher concentrations than the highest calibration standard were diluted until the concentrations were within the calibration range. Samples with high PFAS concentrations were diluted with blanks. These then contained the same concentrations of internal standards as the other samples and were treated analogously.

Quality assurance was carried out on two samples of our standard quality control plant materials (carrots, wheat) using 10 replicates of each material. The concentrations of each of the compounds analyzed in each plant species were below the LOQ of 1 μg/kg. The samples were spiked with 10 μg/kg of each compound to be measured. The absolute recovery rate (mean) ranged between 85% for PFBS (carrots) and 112% for PFHxA (wheat). Coefficient of variation values between 1% (PFOA) and 9% (PFPeA, carrots) were determined (Hessian State Laboratory, 2011). Interlaboratory soil testing study was also undertaken as well as the validation series for solid matter of the German Institute for Standardization (German Institute for Standardization, DIN 38414-14 German standard methods for the examination of water, wastewater and sludge − Jointly determinable substances (group S) − Part 14: Determination of selected polyfluorinated compounds (PFC) in sewage sludge, compost and soil − Method using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS) after solid−liquid extraction), which was carried out not only for soil and sewage sludge but also for compost. To validate these methods especially for plant material, a PFAS-contaminated potato reference sample was homogenized and aliquoted, and samples were sent to 12 laboratories for analysis (see also Validation of Methods for Testing Plant Samples). The mean values from the interlaboratory comparison (all participating laboratories) were determined to be 94.1 μg/kg (PFBS), 69.4 μg/kg (PFOA), and 271 μg/kg (PFOS). The mean values from our own laboratory were 103 μg/kg with a coefficient of variation of 10% (PFBS), 87.0 μg/kg with a coefficient of variation of 2% (PFOA), and 249 μg/kg with a coefficient of variation of 3% (PFOS). The resultant Z scores for all three of the determinable (>1 μg/kg) components (PFBS, PFOA, and PFOS) were <1.5 and > −1.5.

Validation of Methods for Aqueous Matrices. Calibration solutions were prepared with substance concentrations between 0 and 100 μg/L (0, 1, 2, 5, 20, 50, and 100 μg/L). Prerequisite internal specifications required that the correlation coefficient for daily calibration for each compound had to be between 0.991 and 0.999. If this internal quality target was not achieved, a new calibration was undertaken. Samples that were found to contain concentrations higher than the highest calibration standard (100 μg/L) were diluted until the concentrations were within the calibration range (for details see Validation of Methods for Testing Plant Samples). The limit of quantification for each substance was determined to be 1 μg/L (signal-to-noise ratio 3:1). Means (n = 5) of the recovery rates (spiked analytical grade water, free of PFAS) were between 91% (PFOS) and 130% (PFPeA). Coefficient of variation values were determined to be between 2% (PFHxA) and 8% (PFOA). Procedural blanks were regularly tested (after every 5 samples) to eliminate the possibility of cross-contamination. To validate the methods especially for PFAS-contaminated water, however, PFAS-contaminated surface water was homogenized by stirring, aliquoted, and sent to 12 laboratories for testing. The resultant Z scores for our laboratory and for all of the determinable (>1 μg/kg) components PFBA, PFBS, PFHxA, PFHpA, and PFOA in the water samples were <2 and > −1.75.

RESULTS AND DISCUSSION

Growth Samples. Quadruple determinations were made from the combined and homogenized plant samples grown on the lysimeter soil plots. The results of PFOA and PFOS determinations of the growth samples are shown in Figure 2.

Temporal Trends in PFOA and PFOS Concentrations for the Years 2007−2011. To detect possible monotonic decreasing trends for all PFOA and PFOS data from the years 2007−2011 separately for grain and straw, the rank correlation

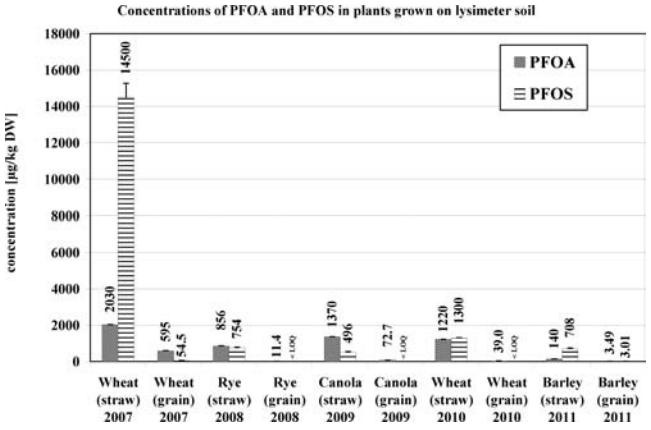

Figure 2. Arithmetic (n = 5) mean of concentrations of PFOA and PFOS in straw and grain from plants grown on lysimeter soil 2007−2011 showing standard error bars.

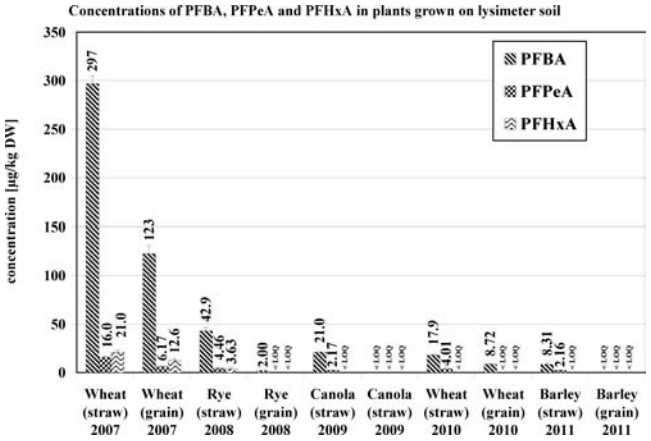

Figure 3. Arithmetic (n = 5) mean of concentrations of PFBA, PFPeA, and PFHxA in straw and grain from plants grown on lysimeter soil 2007−2011 showing standard error bars.

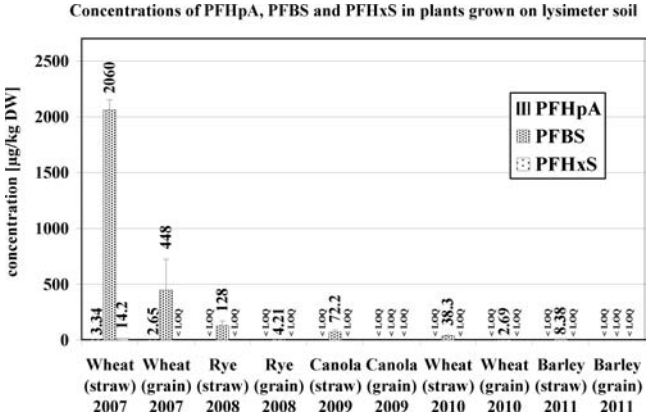

Figure 4. Arithmetic (n = 5) mean of concentrations of PFHpA, PFBS, and PFHxS in straw and grain from plants grown on lysimeter soil 2007−2011 showing standard error bars.

coefficient according to Spearman was calculated and tested for deviation from zero. Statistically significant decreasing trends were found for PFOA in both straw (r_s = −0.668; p < 0.05) and grain (r_s = −0.648; p < 0.05) as well as for PFOS in straw (r_s = −0.420; p < 0.05), whereas a significant decreasing trend for PFOS in grain (r_s = −0.067; p = 0.757) was not detected. This is the result of the fact that in the years 2008, 2009, and 2010 no PFOS concentrations above the limit of quantification (1.0 µg/kg) were found; however, a value above the LOQ was seen in 2011. This may be due to the type of plant (barley), which possibly takes up PFOS preferentially. This is, however, purely speculative and cannot be demonstrated with statistical certainty at this stage in these long-term studies. With regard to the differences in concentration of PFOA and PFOS in grain and straw, our results from experiments using Mitscherlich jars in 2007,[6] in which we showed that the concentration in straw is always significantly higher than that in grain, are confirmed by the lysimeter experiments.

Temporal Trends in PFBA, PFBS, PFPeA, PFHxA, PFHxS, and PFHpA Concentrations for the Years 2007−2011. To test for possible monotonic decreasing trends, the rank correlation coefficient test according to Spearman was performed on all data for PFBS, PFPeA, PFHxA, and PFHpA from 2007 to 2011, separately for straw and grain. Statistical evaluation of the data (see Figures 3 and 4) reveals significant decreasing trends for PFBA in straw (r_s = −0.981; p < 0.05) and grain (r_s = −0.4278; p < 0.05) as well as for PFBS in straw (r_s = −0.961; p < 0.05) and grain (r_s = −0.787; p < 0.05), for PFPeA in straw (r_s = −0.750; p < 0.05) and grain (r_s = −0.568; p < 0.05), for PFHxA in straw (r_s = −0.801; p < 0.05) and grain (r_s = −0.631; p < 0.05), and for PFHpA in straw (r_s = −0.613; p < 0.05) and grain (r_s = −0.656; p < 0.05).

Statistical Analysis of the Influence of Growth Year on Wheat. Because the experiments were performed under consideration of agricultural aspects such as use of several types of fertilizer and avoidance of monocultures, 6- or 3-year crop rotation was performed using diverse grains. For this reason only one type of grain (wheat) was grown twice within the time frame of the experiments. Thus, it is possible to statistically test wheat for the individual influence of year and species of plant. This is not possible for the other types of grain because they were each planted only once within the experimental time frame, and thus the year and species may have an effect on the PFAS concentration. Calculation of the rank correlation coefficient according to Spearman showed that the concentration of all of the substances tested (as the sum of the concentrations in straw and grain) in wheat grown in 2010 was significantly (p < 0.05 for all) lower than in 2007. Statistical examination of the influences of year and compartment (for wheat) by two-way analysis of variance reveals a significant interaction (p < 0.05 for all of the substances tested), implying that the reductions in concentration in straw and grain for the years observed are not identical.

Pairwise Comparison of the Concentrations in Straw and Grain for Each Year. In a study on the carry-over of PFOA and PFOS from soil to plants, Stahl et al.[6] showed that uptake and storage are clearly more intensive in the vegetative compartment (straw) of the plants than the transfer within the plant to the storage organs (grain). These results were also verified statistically. The results presented in the present study were also subjected to analysis for significant differences in the mean values for grain and straw (n = 5 per sample) using the t test. Standard deviation cannot be calculated for results <LOQ so that the Wilcoxon-Mann−Whitney U test was applied as a nonparametric procedure for such results. The respective significances calculated for the paired values (standard deviation) of straw and grain per parameter are shown in Table 2.

Table 2. Results of the Statistical Mean Value Comparison for the Paired Samples, Straw and Grain, per Substance (p Values Given)

year	PFBA	PFBS	PFPeA	PFHxA	PFHpA	PFOA	PFOS
2007 (wheat)	<0.05[a]	<0.05[a]	<0.05[a]	<0.05[a]	0.8616[a]	<0.05[a]	<0.05[a]
2008 (rye)	<0.05[a]	<0.05[a]	<0.05[a]	0.0539[b]	1.0000[b]	<0.05[a]	<0.05[b]
2009 (canola)	<0.05[b]	<0.05[b]	<0.05[b]	0.4926[a]	1.0000[b]	<0.05[a]	<0.05[b]
2010 (wheat)	<0.05[a]	<0.05[a]	<0.05[b]	1.0000[b]	1.0000[b]	<0.05[a]	<0.05[a]
2011 (barley)	<0.05[a]	<0.05[b]	<0.05[b]	1.0000[b]	1.0000[b]	<0.05[a]	<0.05[a]

[a]Comparison of mean values via t test. [b]Comparison of median values via Wilcoxon−Mann−Whitney U test.

Table 3. Calculated Significance for a Combination of Years (Mean) for Straw and Grain per Substance

annual comparison[a]	PFBA	PFBS	PFPeA	PFHxA	PFHpA	PFOA	PFOS
			Straw				
2009/2011	ns[b]	ns	ns	ns	ns	ns	<0.05
2009/2010	ns	ns	ns	ns	ns	ns	<0.05
2008/2010	ns	ns	ns	ns	ns	<0.05	<0.05
2008/2009	ns	ns	ns	ns	ns	<0.05	ns
			Grain				
2010/2011	ns	ns	ns	ns	ns	ns	<0.05
2009/2011	<0.05	ns	ns	ns	ns	ns	<0.05
2008/2011	ns	ns	ns	ns	ns	ns	<0.05
2009/2010	<0.05	<0.05	ns	ns	ns	ns	ns
2008/2010	<0.05	ns	ns	ns	ns	<0.05	ns
2008/2009	ns	ns	ns	ns	ns	<0.05	ns

[a]For annual comparison different years were always compared to one another; the earlier year is listed first. [b]ns, not significant.

As can be seen in Table 2 the differences in concentration in straw and grain for all of the years are significant for PFBA, PFBS, PFPeA, PFOA, and PFOS, confirming the results of Stahl et al.[6] The situation is different for the two substances PFHxA and PFHpA. A statistically significant difference was determined for PFHxA only in 2007, which may indicate that for the years 2008 and 2009 storage of this substance in vegetative organs (straw) occurs in a manner similar to the transfer within the plant to the storage organs (into grain). Concentrations of PFHxA above the limit of quantification were not detected in straw or grain in 2009, 2010, or 2011 so that it was not possible to perform statistical analysis on the differences. Concentrations of PFHxA and PFHpA above the limit of quantification were not detected in straw and grain from 2008 to 2011. Despite this, however, these substances will continue to be tested in straw and grain in the forthcoming years.

Annual Comparisons of Concentrations According to Compartment. Differences were examined for all possible combinations of years (n = 10) using the Wilcoxon−Mann−Whitney U test to determine whether the mean concentration was higher in the following year than it was in the previous year (one-sided test). This allows an indirect comparison of the different grains because the grains planted in later years show greater storage than those from earlier years. This may indicate differences in the various species of plants. For the sake of clarity only combinations of years for which increasing concentrations were detected are shown in Table 3. An entry of the significant differences in Table 3 ($p < 0.05$) indicates that there was an increase in concentration in the respective compartment in comparison to the previous year or one of the previous years.

No significant differences were determined for the substances PFPeA, PFHxA, and PFHpA. Significant differences were found for PFBA, PFBS, PFOA, and PFOS and for certain combinations of years (see Table 3). The statistically significant downward trending of the individual substances seen in the rank correlation tests according to Spearman (Table 3; values $p < 0.05$) may be an indication that the species of plant has an influence on the uptake of the individual substances. At present no comparable studies have been performed to examine the behavior of PFASs in long-term experiments in outdoor lysimeters. To the best of our knowledge, only three studies exist on the carry-over of PFASs in plants. Lechner and Knapp[7] examined the carry-over of PFOA and PFOS from soil spiked with PFASs-contaminated sewage sludge to carrots (*Daucus carota* ssp. *sativus*), potatoes (*Solanum tuberosum*), and cucumbers (*Cucumis sativus*). In the study of Felizeter et al.[8] the root uptake efficiency and distribution of PFASs in lettuce was investigated with a hydroponic system in a greenhouse experiment. Lettuce was chosen as a leafy vegetable to evaluate the hypothesis that PFASs are taken up and distributed with the plant's water system. This hypothesis implies that PFAS accumulation would take place predominately in the leaves of the plant because water is taken up in the roots and translocated to the leaves, where it evaporates. The third study was performed by our group[6] on the carry-over of PFOA and PFOS from spiked soil to spring wheat, oats, maize, potatoes, and ryegrass. Whereas in our study[6] we carried out systematic concentration-dependent experiments on five cultivated plants (PFOA and PFOS concentrations from 0.25 to 50 mg/kg soil), the experiments performed by Lechner and Knapp[7] were done using mixtures of soil and sewage sludge with various concentrations of PFOA (from 0.276 ± 0.022 mg/kg for potatoes to 0.805 ± 0.063 mg/kg for cucumbers) and PFOS (from 0.010 ± 0.003 mg/kg for carrots to 0.556 ± 0.065 mg/kg for cucumbers). Because of the different dosages and compounds used by Lechner and Knapp[7] and the greenhouse experiment with a hydroponic system used by Felizeter et al.,[8] these results cannot be directly compared to our lysimeter

experiments. It is, however, evident just as the case with Stahl et al.[6] that uptake and storage occur much more intensively in the vegetative compartments of the plants than transfer within the plant to the storage organs.

Lysimeter Leachate. The results of the lysimeter leachate testing per sample month are shown in Table 4. In October 2007, after the soil had been spiked, the first leachate passed through the soil plot of the lysimeter and could be collected. Throughout the course of the study, samples could be obtained only at those times in which leachate could be collected as a result of natural precipitation because the lysimeter was not artificially irrigated.

Table 4. PFAS Concentrations in Lysimeter Leachate per Sample

date	PFOA (μg/L)	PFOS (μg/L)	PFBS (μg/L)	PFHxA (μg/L)	PFHxS (μg/L)	PFHpA (μg/L)
Oct 2007	1206	2.7	2584	1.5	2.6	1
Nov 2007	5013	2.6	3240	3.2	13.7	7.7
Dec 2007	10421	4	4093	4.3	33.6	21.3
Jan 2008	20351	8.4	5053	6	56.8	28.3
Feb 2008	39176	3	5271	7	87.6	36
March 2008	58339	4.1	6627	10.7	108	42.3
Jan 2010	45285	139	2133	<LOQ	<LOQ	<LOQ
Feb 2010	51560	255	1466	<LOQ	<LOQ	<LOQ
March 2010	36060	260	1101	<LOQ	<LOQ	<LOQ
April 2010	35375	285	779	<LOQ	<LOQ	<LOQ
Jan 2011	19695	499	239	<LOQ	4.3	5.3
Feb 2011	33958	545	537	<LOQ	8.6	13.0
March 2011	38478	622	499	<LOQ	9.1	12.5

Due to the often substantial differences in concentrations and to provide clarity, it must be noted in the following diagrams that the concentrations are plotted logarithmically. Figure 5 shows the concentration gradient of PFOA and PFOS over time.

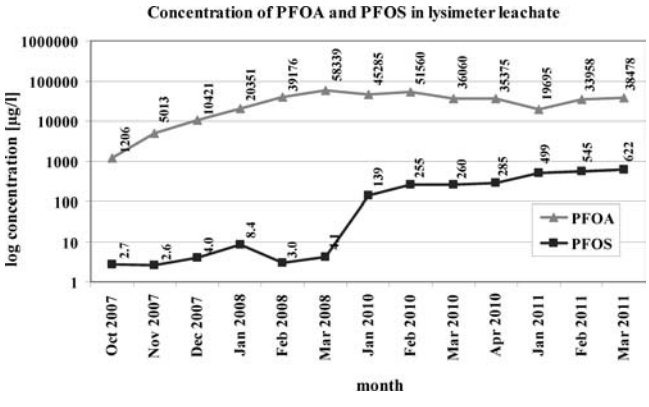

Figure 5. Concentrations of PFOA and PFOS in lysimeter leachate 2007–2011.

The concentration of PFOA in lysimeter leachate increased linearly (r^2 = 0.913) between October 2007 and March 2008 and then remained at a high level until March 2011. The concentration of PFOS remained almost constant from October 2007 to March 2008 and then rose in a nearly linear manner (r^2 = 0.955) to a value of 622 μg/L leachate in March 2011. These results indicate that PFOA is transported rapidly by water passing through the soil, whereas PFOS travels much more slowly and was found in increasing amounts in leachate only48 months after the soil had been spiked. Gellrich et al.[16] reached similar conclusions in their systematic laboratory-scale experiments on percolation of PFASs through soil columns. In that study PFOA was detected in the leachate after approximately 4 months, whereas PFOS could not be detected at levels above the LOQ (1 ng/L) after 32 months.

PFBS, PFHxA, PFHxS, and PFHpA as Contaminants of PFOA and PFOS. As can be seen in Figure 6 the leachate concentration of PFBS rose linearly from October 2007 to March 2008 (r^2 = 0.977) and decreased from March 2008 to March 2011, but like PFOA (Figure 4), compared to the other contaminants of the technical mixtures, at a high concentration (>200 μg/L). In February (537 μg/L) and March 2011 (499 μg/L) concentrations began to rise once again.

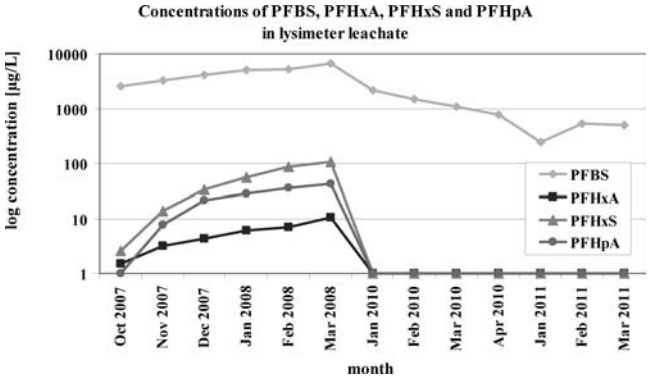

Figure 6. Concentrations of PFBS, PFHxA, PFHxS, and PFHpA in lysimeter leachate 2007–2011.

The temporal changes in concentrations for the substances PFHxA, PFHxS, and PFHpA were similar to those observed for PFBS. From October 2007 to March 2008 linear (PFHxA, r^2 = 0.952; PFHxS, r^2 = 0.983; PFHpA, r^2 = 0.986) increases in concentration were observed in the leachate. From January 2010 (the seventh sample) onward, PFHxA, PFHxS, and PFHpA could no longer be detected in concentrations above the LOQ (1 μg/L). These results (Figures 5 and 6) suggest that the passage of shorter chain perfluorocarbonic acids (chain length from C4 to C8) and shorter chain perfluorosulfonic acids (chain length from C4 to C6) through the soil is more rapid than for PFOS. This also confirms the results of laboratory-scale leaching experiments obtained by Gellrich et al.[16] Due to the constraints of the lysimeter construction, it is possible to perform these studies on only one type of soil. The soil used in the lysimeter plots ("gray brown podzolic soil" from eolian silt deposits) is well characterized and represents a typical agricultural soil in Germany. This is a medium to highly silty soil with somewhat higher clay content in the subsoil surrounded by a strongly silty clay substrate. Chemical analysis shows a humic upper layer that is well supplied with nutrients.

Table 5. Calculation of the Absolute Amounts of PFOA and PFOS Taken up by Plants Based on the Harvested Biomass (Grain and Straw) and the Measured Concentrations of the Substances

	2007	2008	2009	2010	2011	sum
substance	winter wheat	winter rye	grain canola	winter wheat	winter barley	
yield grain (kg)	0.48	1.08	0.38	0.79	0.62	3.35[a]
yield straw (kg)	0.80	0.83	0.77	0.81	0.88	4.09[b]
concentration of PFOA in grain (μg/kg)	595	11.4	72.7	39.0	3.50	–[c]
concentration of PFOA in straw (μg/kg)	2030	856	1370	1220	140	–[c]
concentration of PFOS in grain (μg/kg)	54.5	<LOQ	<LOQ	<LOQ	3.00	–[c]
concentration of PFOS in straw (μg/kg)	14500	754	496	1300	708	–[c]
absolute amount of PFOA in grain (μg)	286	12.3	27.6	30.8	2.17	359[d,e]
absolute amount of PFOA in straw (μg)	1624	710	1055	988	123	4500[f]
absolute amount of PFOS in grain (μg)	26.2	0	0	0	1.86	28[g,h]
absolute amount of PFOS in straw (μg)	11600	626	382	1053	623	14284[i]

[a]Sum of the grain yields of the rotated crops 2007–2011. [b]Sum of the straw yields of the rotated crops 2007–2011. [c]Summation of the concentrations is not sensible. [d]Sum of the absolute amount of PFOA in grain of the rotated crops 2007–2011. [e]Rounded off (calculated value 358.97 μg). [f]Sum of the absolute amount of PFOA in straw of the rotated crops 2007–2011. [g]Sum of the absolute amount of PFOS in grain of the rotated crops 2007–2011. [h]Rounded off (calculated value 28.06 μg). [i]Sum of the absolute amount of PFOS in straw of the rotated crops 2007–2011.

Table 6. Calculation of the Amounts of PFOA and PFOS Removed from the Lysimeter Soil Plot through Leachate from 2007 to 2011

date	PFOA (mg/L)[a]	leachate volume[b] (L)	substance eluted[c] (mg)	PFOS (mg/L)[a]	leachate volume[b] (L)	substance eluted[c] (mg)
Oct 2007	1.21	4.1	5	0.003	4.1	0.011
Nov 2007	5.01	51.2	256	0.003	51.2	0.133
Dec 2007	10.4	62.6	653	0.004	62.6	0.251
Jan 2008	20.4	46.5	947	0.008	46.5	0.391
Feb 2008	39.2	20.9	819	0.003	20.9	0.063
March 2008	58.3	40.3	2351	0.004	40.3	0.165
Jan 2010	45.3	17.5	792	0.139	17.5	2.43
Feb 2010	51.6	60.2	3103	0.255	60.2	15.3
March 2010	36.1	25.8	930	0.260	25.8	6.71
April 2010	35.4	8.58	303	0.285	8.58	2.44
Jan 2011	19.7	11.2	221	0.499	11.2	5.59
Feb 2011	34.0	19.9	676	0.545	19.9	10.8
March 2011	38.5	4.65	179	0.622	4.65	2.89
sum			11235[d]			47.3[e]

[a]For the sake of clarity the measured PFAS concentrations in leachate are presented in mg/L. [b]Absolute volume collected in the drain bottles per sample. [c]The amount (mg) of the substance removed from the lysimeter soil plot by leachate as calculated from the measured concentration and the volume of leachate. [d]Total amount of PFOA (mg) removed from the lysimeter soil plot by loss to the leachate in 2007. [e]Total amount of PFOS (mg) removed from the lysimeter soil plot by loss to the leachate in 2007.

Magnesium content and pH value rise with increasing depth in a site-specific manner.

Mass Balance of PFOA and PFOS. *Growth Samples.* Because the yield of the grain and straw per yearly crop (kg) and the volume of leachate collected (L) are known, it is possible to calculate the mass balance of PFOA and PFOS transposed through carry-over and elution out of the lysimeter soil plot. In contrast, it is not possible to calculate the mass balance of the contaminants PFBS, PFPeA, PFHxA, and PFHpA, because the individual proportions could not be accurately measured due to the matrix effects as described (see Analytical Method for Technical Mixtures of PFOA and PFOS). As shown above (see Vegetation Study) 0.025 g of PFOA and PFOS was applied per kilogram lysimeter soil. This is equivalent to 360 g PFOA (96% purity) and 367.5 g PFOS (98% purity) per 1500 kg of lysimeter soil.

It can be seen in Table 5 that totals (grain and straw) of 4859 μg of PFOA and 14312 μg of PFOS were taken up by the plants and thus removed from the lysimeter soil plot. Thus, 0.001% of the applied PFOA and 0.004% of the applied PFOS were removed by plant carry-over.

Lysimeter Leachate Samples. The PFOA and PFOS concentrations and leachate volumes as well as the total amounts of the substance lost through leachate are shown in Table 6.

It is apparent from Table 6 that a total of 11235 mg of PFOA (equivalent to 3.12% of the total amount applied to the lysimeter) and a total of 47.3 mg of PFOS (equivalent to 0.013% of the total amount applied to the lysimeter) were removed from the lysimeter soil plot by the leachate.

Total Mass Balance. After 5 years, 96.88% of the PFOA and 99.98% of the PFOS originally applied to the soil were still in the lysimeter. These values were obtained by subtracting the rounded off amounts lost to plant uptake (PFOA, 0.001%; PFOS, 0.004%) and to leachate (PFOA, 3.12%; PFOS, 0.013%) from the total. It is possible that a negligible loss occurred due to wind erosion. The assessment made here verifies that PFOA is much more quickly transferred from the soil to groundwater

through precipitation than is PFOS. This may in turn mean that dependent upon the type of crop and the related root structure, PFOS is available to plant uptake for a longer period than PFOA. This hypothesis canbe tested only by continuing these experiments throughout the next years or decades. Nonetheless, the lysimeter experiments and results described here demonstrate that this method is well suited to study the carry-over of PFASs from soil to various plants and plant compartments (straw and grain) as well to examine in parallel the transfer of these substances from soil to groundwater.

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Funding

We express our gratitude to the Hessian Ministry for the Environment, Energy, Countryside and Consumer Protection for financial support.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are indebted to Johannes Heyn, Dierk Koch, and Carmen Bernhard from the Hessian State Institution of Agriculture for cultivating, harvesting, and drying the plants. Furthermore, we thank Georg Berthold from the Hessian State Office for the Environment and Geology for compiling the data and extend a special thank you to our technical staff, Rosa Maria Sobel, Maria Elisabeth Ebel, and our graduate student Vanessa Gellrich, for their efforts in sample preparation and analysis. We also thank Barbara Gamb for her assistance in the literature search and Katrin Teigeler for support and helpful suggestion regarding this paper.

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