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Uptake and distribution inside the plants

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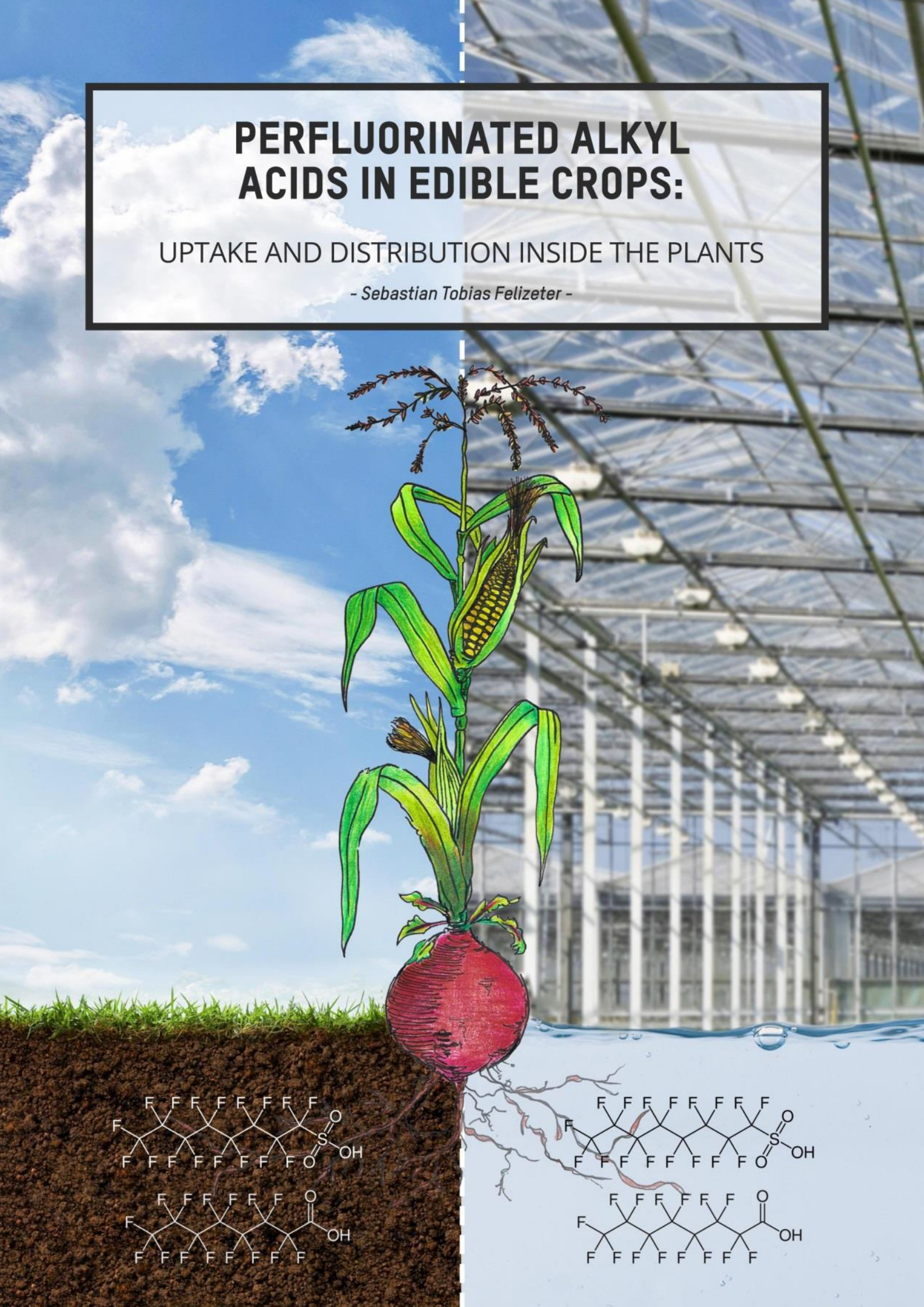
UPTAKE AND DISTRIBUTION INSIDE THE PLANTS

- Sebastian Tobias Felizeter -



Perfluorinated alkyl acids in edible crops: Uptake and distribution inside the plants

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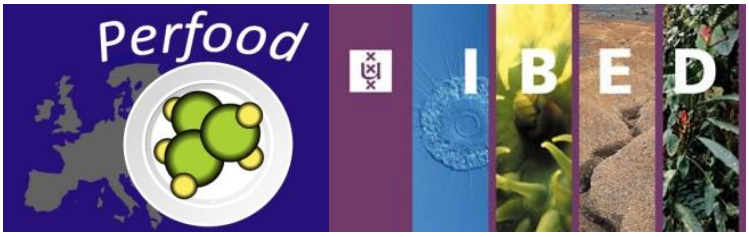

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Chapter 1

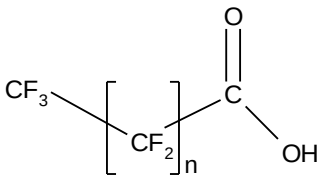
GENERAL INTRODUCTION

Food is essential for human and animal life. Food supplies us with nutrients, energy, everything we need to keep our body functions going. But in an industrialized modern world we also take up synthetic chemicals with our food, chemicals that can cause cancer or other severe health effects. We do this involuntarily. We don't have a choice, because these chemicals are everywhere in the environment. But the more we know about these chemicals, how they get into the environment and then into the food chain, the more we can take action to minimize the risk of exposure. The chemicals studied in this thesis, namely perfluoroalkyl acids (PFAAs), have been produced since the 1950s. Since then, the amount produced annually increased significantly from a couple of hundred tons to several thousand tons in the 2000s. In the early 2000s this group of chemicals started to attract scientific interest after having been found to be persistent in the environment and soon they were detected ubiquitously on a global scale, including in human blood and breast milk. Food was identified as a source for human exposure, but it remained unknown how PFAAs get into the food chain in the first place. The PERFOOD project (PERFluorinated Organics in Our Diet, see below), of which the work in this thesis is a part, was designed to answer this question and to assess the origin of poly- and perfluoroalkyl substances (PFAS) in our diet and the diet's contribution to the total human exposure to PFAS.

Perfluoroalkyl substances

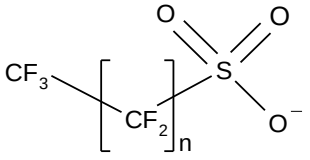
The group of perfluorinated alkyl substances (PFAS) consists of a wide range of different chemicals. In this thesis the nomenclature proposed by Buck et al. is used (Buck et al. 2011). The work of this thesis focusses on a subgroup of PFAS, the perfluoroalkyl acids (PFAAs), which in turn consists among others of the subgroups of perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs), often referred to as perfluorinated sulfonates, as they are in the ionized form at environmentally relevant pH-levels. PFAAs have a perfluorinated carbon chain of variable length, which is hydrophobic, and a hydrophilic functional group. The structures of PFCAs and PFSAs are shown in Figure 1.

(A) Perfluorinated carboxylic acids (PFCAs)



n = 2-12

(B) Perfluorinated sulfonates (PFSAs)



n = 3,5,7

Figure 1: Structures of PFCAs (A) and PFSAs (B)

PFCAs and PFSAs are the most important groups of PFAS as they are the final, stable end products of the degradation process of other PFAS groups, e.g., fluortelomer alcohols

(FTOHs) (Dinglasan et al. 2004; Ellis et al. 2004; Prevedouros et al. 2006), and they are usually the PFAS with the highest concentrations in the environment (Giesy et al. 2002; de Voogt et al. 2006a; Ahrens et al. 2009a; Eschauzier et al. 2012b). A full list of the compounds analyzed in this thesis, including their acronyms and molecular structures is given in Table 1.

Table 1: List of the compounds analyzed in the preset thesis, their abbreviations and molecular formula

Abbreviation	Compound	Molecular Formula
PFBA	Perfluoro-n-butanoic acid	$CF_3(CF_2)_2COOH$
PFPeA	Perfluoro-n-pentanoic acid	$CF_3(CF_2)_3COOH$
PFHxA	Perfluoro-n-hexanoic acid	$CF_3(CF_2)_4COOH$
PFHpA	Perfluoro-n-heptanoic acid	$CF_3(CF_2)_5COOH$
PFOA	Perfluoro-n-octanoic acid	$CF_3(CF_2)_6COOH$
PFNA	Perfluoro-n-nonanoic acid	$CF_3(CF_2)_7COOH$
PFDA	Perfluoro-n-decanoic acid	$CF_3(CF_2)_8COOH$
PFUnA	Perfluoro-n-undecanoic acid	$CF_3(CF_2)_9COOH$
PFDoDA	Perfluoro-n-dodecanoic acid	$CF_3(CF_2)_{10}COOH$
PFTrDA	Perfluoro-n-tridecanoic acid	$CF_3(CF_2)_{11}COOH$
PFTeDA	Perfluoro-n-tetradecanoic acid	$CF_3(CF_2)_{12}COOH$
PFBS	Perfluorobutane sulfonate	$CF_3(CF_2)_3SO_3$
PFHxS	Perlfuorohexane sulfonate	$CF_3(CF_2)_5SO_3$
PFOS	Perfluorooctane sulfonate	$CF_3(CF_2)_7SO_3$

Physico-chemical properties of PFAAs

PFAAs have unique physio-chemical properties due to the extremely strong covalent C-F bond. Fluorine is the element with the highest electronegativity, needing only one electron in the outer shell to fulfill the octet rule for atoms. Therefore, the C-F bond is highly polarized, giving it a large dipole moment, making the C-F bond the strongest bond in organic chemistry ($\Delta H \approx 407$ kJ/mol). In a fully fluorinated carbon chain the carbon atoms are also effectively shielded by the fluorine atoms from external influences (O'Hagan 2008). Because of their electronegativity, the fluorine atoms in such a completely fluorinated carbon chain repel each other in such a way that a helical, and relatively rigid chain results (Liu et al. 2009). As a consequence PFAAs are resistant to acid, alkaline, oxidative, reductive, photolytic and microbial degradation and cannot be digested by animals (Kissa 2001a). PFAAs are also thermally stable and can be heated up to 400°C without any significant decomposition (Kauck et al. 1951; Ellis et al. 2002). These properties are also retained in organic solvents (Key et al. 1997; Kissa 2001a; Ellis et al. 2002) and are very useful for

industrial products and processes, but they are also the reason for the persistence and the global distribution of PFAAs in the environment (Prescher et al. 1985; Key et al. 1997; Key et al. 1998).

PFAAs are fairly well soluble in water and have low vapor pressures. The water solubility is usually in the mg to g per L range. As PFAAs are surfactants with a hydrophobic chain and a hydrophilic head group, PFAAs with carbon chain lengths of 4 or longer form micelles when present in water at high enough concentrations. The reported critical micelle concentration (CMC) for PFOS and PFOA is approximately 4 g/L.

PFAAs are relatively strong acids, with acid dissociation constants (pKa) of less than 1.6 for all PFCAs and less than 0.3 for all PFSAs (Vierke et al. 2013). pKa values are important parameters for risk assessment or uptake models to determine the behaviour of a compound in the environment (Goss 2008a). The longer the perfluorinated carbon chain, the more acidic is the compound, due to the negative inductive effect of the fluorine atoms. PFAAs are almost fully deprotonated at environmentally relevant pH-levels, which is important for the environmental fate as the ionic form contributes to the water solubility of the compounds.

The octanol-water partitioning coefficient (Kow) is another important chemical parameter for the determination of the environmental fate of a chemical compound. The Kow shows if a compound is hydrophilic or lipophilic. While lipophilic compounds move to the octanol phase, hydrophilic compounds move to the water phase. The octanol phase is a model for organic material in sediments or tissue in organisms. The determination of hydrophobicity parameters for PFAAs is difficult because of the surfactant-like behaviour of PFAAs. The hydrophobic tail moves to the octanol phase, while the ionic head goes to the water phase. Thus, PFAAs move to the interface of the two phases. Consequently, Kow values of PFAAs cannot be determined in the classical way. Thus, most published hydrophobicity parameters of PFAAs were determined by computer models using other chemical properties to calculate Kow values. De Voogt et al. presented an alternative hydrophobicity parameter for PFAAs, which was measured on an HPLC system with a C18 column as a proxy for the octanol, showing that the longer the fluorinated carbon chain is, the more lipophilic the PFAA is (de Voogt et al. 2012). This means that longer chain compounds tend to enrich in sediment and biota, while shorter chain compounds stay in the water phase. This also plays a significant role in the bioavailability of the compounds. For instance, in agricultural ecosystems when the compounds are strongly adsorbed to soil, they won't be available for plant uptake, while compounds that are less lipophilic might be more available for uptake by plants.

Production

PFAS do not occur naturally in the environment. There are two production pathways for PFAS, electrochemical fluorination (ECF) and fluorotelomerization. ECF was invented in the early 1940's by Joseph Simons of the 3M Company (Simons 1950; Banks 1994), while fluorotelomerization was developed by Haszeldine in 1949 and has been used from 1969 onward by the DuPont Company (Kissa 2001a; Hekster et al. 2003).

ECF operates with organic substances which are dissolved or dispersed in liquid hydrogen fluoride. An electric current is passed through this solution, whereby hydrogen is evolved at the cathode and the organic substance is completely fluorinated:

(1) $C_nH_{2n+1}COCl + (2n+2)HF \rightarrow C_nF_{2n+1}COF + HCl$ + by-products

(2) $C_nH_{2n+1}SO_2Cl + (2n+2)HF \rightarrow C_nF_{2n+1}SO_2F + HCl$ + by-products (Kissa 2001a)

In this way perfluorocarbonyl and perfluorosulfonyl fluorides (POSF) are produced that can be converted into multiple PFAS. Hydrolysis leads to PFCAs and PFSAs respectively. The usage of carboxylic acids and alkane sulfonic acids to gain perfluoroalkanoic acid fluoride and perfluorosulfonyl fluoride (POSF), respectively, is obsolete, because the yields are lower and water is formed as a by-product, which forms explosive oxygen difluoride and causes oxidative degradation (Kissa 2001a). The yields of the ECF production process vary from about 10% to 80% depending on the chain length of the original substances, which means that a large amount of by-products is formed in this process (Kissa 2001a; Schultz et al. 2003). ECF was used for the major part of production of PFCAs, but was discontinued in 2002 by 3M in North America. However, the ECF is still used by other manufacturers in other parts of the world (e.g., China, (Lim et al. 2011)).

The telomerization process is a form of polymerization, which mainly yields even chain length PFAS. First the telogen, pentafluoroethyl iodide, is synthesized out of tetrafluoroethylene, pentafluoro- iodide and iodine:

(3) $5CF_2=CF_2 + IF_5 + 2I_2 \rightarrow 5C_2F_5I$

In the next step of the process tetrafluoroethylene reacts with pentafluoroethyl iodide which yields perfluoroalkyl iodides with an even-number of carbon atoms:

(4) $C_2F_5I + nC_2F_4 \rightarrow C_2F_5(C_2F_4)_nI$

Perfluoroalkyl iodide is converted with ethylene to perfluoroalkylethyl iodide which can be converted to the corresponding alcohols, thiols and sulfonyl chlorides, which are intermediates for fluorinated surfactants.

The total global annual emissions of PFAS show a steady increase between 1951 and 2002 (sum: 1790–14220 tonnes) with the highest annual emissions from the mid-1990s to ca. 2001. Due to the phase-out of the ECF production starting in 2000 (see below), there was a sudden and sharp decrease of ca. 40% of the annual emissions. Afterwards the annual emissions increased again between 2002 and 2012 (sum: 820–7180 tonnes) (Wang et al. 2014). The annual production volume of PFOS for example rose from approx. 200t in 1979 to approx. 3000t in 2000 (Fielding 1979; Holloway 2000). Overall PFAS production increased by 220% from 1988 to 1997 (Prevedouros et al. 2006; Paul et al. 2009). 3M estimated a global production of POSF of 3665 t in the year 2000 with 3250 t in the US alone (USEPA 2000). Due to the persistence and toxic effects (see below) of PFOS, and after increased concentrations of PFOS were detected in blood samples of the general population and fluorine production workers, 3M began to phase out the production of some compounds like POSF along with the ECF production in 2000 (3M 1999; 3M 2000; USEPA 2000). In 2006 the European

Parliament decided to restrict the use of PFOS to limited applications and in 2009 PFOS, its salts and POSF were listed by the Stockholm Convention on Persistent Organic Pollutants in the annex B, which states that "Parties must take measures to **restrict** the production and use of the chemicals listed under Annex B in light of any applicable acceptable purposes and/or specific exemptions listed in the Annex". PFOA, its salts and PFOA-related compounds are still under review to be listed under the Convention, but PFOA and Ammonium pentadecafluorooctanoate (APFO) have been listed as substances of very high concern by the European Chemicals Agency (ECHA 2013). Since there is still a high industrial demand for PFAAs for many purposes, where no alternatives are available yet, the production with the telomerization process has increased and production has also changed to short-chained compounds with an even number of carbon atoms or to not fully fluorinated compounds. These compounds are supposed to be less bioaccumulative and toxic (Martin et al. 2003b; de Voogt et al. 2006c).

Usage

PFAS have unique properties which make them very useful for various products. Among the characteristics of PFAS are high surface activity and weak intermolecular interactions (Kissa 2001a; Lehmler 2005). The ability to repel both water and oil has made PFAS preferred substances in surface coatings for paper, carpets, furniture and textiles. They also find uses in firefighting foams, insecticides, antistatic agents, anti-mist films, biomaterials and cleaners (Key et al. 1997; Kissa 2001a).

In the year 2000 3M reported that 41% of its American production of POSF-based products was used for coatings on paper and packaging products (33% in the European Union), 37% was used for impregnation of textiles, leather and carpets (49% in the EU), 10% went into ingredients in industrial surfactants, additives and coatings (15% in the EU), and 3% was incorporated into aqueous firefighting foams (AFFF) (Schultz et al. 2003).

Brand names like Teflon®, Gore-Tex®, Scotchgard®, Stainmaster® and Silverstone® are known to use PFAS either in their products or in the production process.

Environmental fate

PFAAs have been found ubiquitously in the environment, from remote areas of the poles to highly populated areas. Sources of PFAAs to the environment can be direct or indirect. Direct pathways include the (involuntary) discharge of PFAS from production facilities via wastewater treatment plants (WWTP) or through the atmosphere, and the leaching of PFAAs from products where PFAAs are incorporated (e.g., AFFF or papers and textiles). Indirect sources are precursor compounds that degrade in the environment to PFAAs. Long range transport in the environment can mainly happen via two pathways, through flowing water and through atmospheric transport. Wastewater treatment plants are discussed to be a major source of PFAS to the environment (Becker et al. 2008; Bossi et al. 2008; Ahrens et al. 2009b; Filipovic et al. 2013). Because PFAS are persistent they don't get degraded in WWTPs, which means that they pass through the WWTP and end up in the effluent or in the sludge. When they are released with the effluent, they distribute in the environment via

rivers and oceanic currents (McLachlan et al. 2007). Sludge from WWTPs often ends up in landfill sites, where PFAS can leach out into the environment (Busch et al. 2010; Huset et al. 2011; Eschauzier et al. 2013; Frömel et al. 2016; Eriksson et al. 2017), or it is sold as fertilizer to farmers due to the high nutrient content of the sludge (Lindstrom et al. 2011; Sepulvado et al. 2011). In the latter case PFAS are directly applied on fields where the PFAS can either be taken up by the crops or leach into the groundwater and end up eventually in surface waters.

Atmospheric transport is more relevant for precursor compounds, like FTOHs, because these compounds are volatile and can be released directly into the atmosphere, whereas PFAAs are less volatile and usually released to water. Precursors can be transported for several thousands of kilometers with the wind before they degrade to PFAAs, because the half-lives of these compounds are between 20 and 164 days (Ellis et al. 2003; Ellis et al. 2004; Paragot et al. 2020).

Toxicology and Human Exposure

PFOS and PFOA are highly bioaccumulative and extremely persistent, which can lead to higher exposure, which in turn can lead to adverse effects on organisms. The toxicity of chemicals is characterized by the lethal dose (LD50), the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). So far only the compounds PFOA and PFOS are fairly well investigated, since these are the most abundant PFAAs found in the environment. The lethal dose for rats, for example, is 189 mg/kg for PFOA and 251 mg/kg for PFOS (Olsen et al. 1983; Renner 2001). The NOAEL and LOAEL of PFOS for the daily food intake of rats are 0.1 mg/kg and 0.4 mg/kg respectively (USEPA 2000; Seacat et al. 2003; Lau et al. 2007). In more recent studies it has been shown that even at low PFOA exposure doses body burdens of humans increase (see e.g., (Post et al. 2012)) and immunology of children is affected (Grandjean et al. 2015).

Bioaccumulation refers to the accumulation of a chemical in an organism in general, while bioconcentration refers to the accumulation from the water phase alone and biomagnification to the accumulation via the food chain. For PFOS a bioaccumulation factor (BAF) of 6,300 – 125,000 for fish (Moody et al. 2002a), a bioconcentration factor (BCF) of approx. 1000 for benthic invertebrates and a biomagnification factor (BMF) for bald eagle (*Haliaeetus leucocephalus*) and American mink (*Mustela vison*) of approx. 10-20 were determined (Kannan et al. 2005). Biomagnification factors at lower trophic levels are between 1 and 10 (De Vos et al. 2008). Another study calculated a BCF of PFOS of approx. 9,000 for fish and a BMF for goosander (*Mergus merganser*) of approx. 9 (Sinclair et al. 2006b). For PFOA a much lower bioaccumulation was reported. The BCF of PFOA generally lies between 4 and 8 and the BMF is about 1, which means that no accumulation takes place along the food chain (Moody et al. 2002a; Martin et al. 2003b; Martin et al. 2003a; Tomy et al. 2004). Houde et al. on the other hand found a BMF in bottlenose dolphins (Tursiops truncatus) of approx. 2-13 (Houde et al. 2006). In general, PFSAs accumulate more than PFCAs with the same perfluoroalkyl chain length, whereas for both types of compounds bioaccumulation increases with increasing chain length (Martin et al. 2003b; Martin et al. 2003a; Martin et al. 2004).

The much higher risk from PFAAs comes from chronic effects rather than from acute toxicity. Due to their ubiquitous presence in the environment we are constantly exposed to PFAAs and because they have a high bioaccumulation potential we take them up in our bodies. Half-lives of PFAAs in humans have been found to increase with increasing chain length from 75h for PFBA (Chang et al. 2008) to a couple of years for e.g., PFOA or PFOS, with sulfonic acids having longer half-lives than carboxylic acids (Hekster et al. 2003; Olsen et al. 2007; Li et al. 2018a). PFOA and PFOS primarily partition to the liver and serum where they bind to albumin and other proteins (Han et al. 2003; Jones et al. 2003). Half-lives reported for other species, e.g., monkeys, are remarkably shorter than for humans (Lau et al. 2007).

Due to their persistence and ongoing emissions, concentrations of PFAAs were increasing in the environment and wildlife up to the early 2000s (Muir et al. 2019). For instance, increasing trends of PFAS concentrations in seal liver (Bossi et al. 2005), guillemot eggs (Holmström et al. 2005), polar bear liver (Smithwick et al. 2006) and seabirds (Butt et al. 2007) have been observed. However, recently some increasing trends have also been reported in the years after 2010 in arctic wildlife (Muir et al. 2019).

It has been shown that the human body burden of PFAAs, reflected in the average serum concentrations found worldwide, stems from exposure to PFAAs and exposure to precursor compounds which can be metabolized into PFAAs (D'eon and Mabury, 2011a and 2011b; Vestergren and Cousins, 2009). In a comprehensive review by Post et al. (2012) average background values between 2 and 8 ng/mL serum in the industrialized world were reported. For occupationally exposed humans concentrations above 100 ng/mL have been reported. In 2006 the US Environmental Protection Agency (USEPA) invited the eight major fluoropolymer and telomer manufacturers to join in a global stewardship program with the two goals to reduce the emissions of PFOA by 95% from a year 2000 baseline until 2010 and a complete elimination until 2015. After the PFOS phase out and the voluntary reduction of PFOA emissions by the major manufacturers in North America and Europe, human serum concentrations of PFOS and PFOA have decreased in Europe and North America, while concentrations of PFOS and PFOA increased in human blood in China due to a shift in production from western countries towards China, because of the ongoing huge demand inside and outside of the country (Chen et al. 2009; Liu et al. 2019a). A massive increase of emissions in China was estimated due to this shift (Xie et al. 2013; Li et al. 2015).

New short chain alternatives to PFOA and PFOS, mostly C4 and C6 PFAAs, also increased in human serum in the past years (Glynn et al. 2012) and became the predominant contaminant in some areas (Ahrens et al. 2016; Tan et al. 2018). Furthermore short chain PFAAs are highly mobile and have a higher potential for long-range transport than long-chain PFAAs due to their high aqueous solubility and relatively low adsorption (Vierke et al. 2014; Krop et al. 2021).

Possible exposure routes to humans are the diet, including drinking water, exposure from inhalation and skin contact (Vestergren et al. 2009; D'Hollander et al. 2010b). The diet was identified to be the main source for human exposure with the exception of workers in the fluoro-chemical industry, for whom direct exposure can be a major source, and toddlers, for whom exposure from house dust can play a significant role (Klenow et al. 2013).

Prior to the start of the PERFOOD project and the work in this thesis, seafood and fish were believed to be the main sources for exposure through the diet, since PFAAs were mainly found in surface and marine waters (Haug et al. 2010b).

The European Food Safety Agency (EFSA) proposed tolerable daily intake rates (TDI) for PFOS and PFOA in 2008 based on risk assessments (EFSA 2008; Johansson et al. 2009). The TDI value for PFOS was set to 0.15 µg/kg body weight per day and for PFOA 1.5 µg/kg body weight per day. In 2018 the EFSA established new much lower intake rates with 13 ng/kg body weight per week for PFOS and 6 ng/kg body weight (bw) per week for PFOA (EFSA 2018). They recently revised these TDIs again and established a new and much lower group tolerable weekly intake rate of 4.4 ng per kg bw per week for the extended group of PFOA, PFNA, PFHxS and PFOS (EFSA 2020). For other homologues of PFCAs or PFSAs still no tolerable intake rates exist.

The following effects were identified to be probably related to the exposure to PFOA: high cholesterol, thyroid disease, pregnancy induced hypertension, ulcerative colitis, and kidney and testicular cancer (Lopez-Espinosa et al. 2012; Barry et al. 2013; Darrow et al. 2013; Steenland et al. 2013; Vieira et al. 2013b). PFOA is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO). No other PFAAs have been evaluated by the IARC so far (Sunderland et al. 2019).

PFOA affects primarily the liver of an organism. Possible symptoms are liver enlargement, liver epithelium cell necrosis, low blood lipids, change of liver metabolism and elevated peroxisome proliferation, which can cause cancer of the liver in rats (Abdellatif et al. 1990). PFOA elevates the risk of cancer in general, so fluorine chemistry factory workers for example showed elevated rates of cancer of the prostate, bladder and liver (Gilliland et al. 1993; Alexander et al. 2003). Approx. 3400 kidney and testicular cancer cases of residents near a production site from DuPont in West Virginia, USA, were linked to the exposure to PFOA (Vieira et al. 2013a). PFOA is also capable of overcoming the blood-brain barrier, and it can cause an increased release of stress hormones in rats (Ylinen et al. 1990).

Several studies found associations between PFAS exposure and suppression of antibody response to different vaccinations, such as tetanus, diphtheria, rubella, mumps and influenza (Grandjean et al. 2012; Granum et al. 2013; Looker et al. 2014; Stein et al. 2016a; Stein et al. 2016b).

Quite a number of studies evaluated the association of PFAS exposure with metabolic effects and diseases, such as high cholesterol or diabetes. While some studies found positive relationships, overall the results are inconclusive (Sunderland et al. 2019). Furthermore, it was proven that several PFAS can inhibit cellular signal transmission (Hu et al. 2002) and PFCAs can have acute toxic effects on enzymes. In this connection, the toxicity of long-chain molecules was higher than of short-chain molecules (Mulkiewicz et al. 2007; Wilhelm et al. 2010).

Plant uptake of chemicals

Plant uptake of chemicals can occur in a variety of ways. Root uptake can happen via the vapor or the water phase of soil. The uptake of anthropogenic organic chemicals by plant roots has been shown to be a passive, diffusive process, with the exception of a few hormone-like chemicals (Bromilow et al. 1995). Chemicals can also be deposited via atmospheric deposition on the surface of the leaves (from where they can be transported deeper into the leaves). This atmospheric deposition can occur in gaseous form, by dry deposition of particles bearing the chemicals, or via precipitation (either as chemical dissolved in water or as chemical sorbed to particles scavenged by raindrops/snow). Another pathway is through the stomata of the leaves (for volatile chemicals in air). The difference between the dry gaseous deposition above from uptake via the stomata is that the former involves deposition from gas to the outer surfaces of the plant, the latter to the inner surfaces of the plant. Sorption by the vegetative parts of the plants when coming in direct contact with the chemicals (e.g., pesticides when they are sprayed on the plants or by soil splash) is yet another pathway. The difference between wet deposition of particles above from soil splash is the source of the particles (atmosphere versus soil) and the path of the water.

The most likely exposure of plants to PFAAs is through contaminated soil or irrigation water. Due to their good water solubility and low volatility, uptake of PFAAs will happen mainly through the water phase of the soil. Experiments with other organic chemicals in hydroponic solutions have shown that adsorption to the root surface involves equilibration of the chemical in the surrounding phase with the aqueous phase in the roots (diffusion from the surrounding water phase to the water phase inside the roots), and sorption onto lipophilic root solids on the root surface, including lipids in membranes and cell walls (Briggs et al. 1982). Once chemicals entered the root surface they have to pass through membranes (symplastic pathway) or move between the cell walls (apoplastic pathway) on their way into the deeper root tissue. The main barrier for chemicals on the way to the center of the root is the Casparian strip, a band of cell wall material deposited on the radial and transverse walls of the endodermis made of suberin and lignin. The Casparian strip stops the apoplastic movement of chemicals, forcing them to pass through the cell walls. While for nutrients there are special “gates” to move through, the cell wall is a hard to penetrate barrier for other chemicals. After passing through the Casparian strip the chemicals can be transported with the xylem sap through the xylem - a special tissue of plants to transport water and nutrients - from the roots to the vegetative parts of the plant. Transport from the leaves back to the roots or to fruits occurs with the phloem - another transport tissue of the plant, which transports photosynthates, like e.g., sugars. Both transport pathways lead to a distribution of the compounds in the whole plant.

How well chemical compounds are taken up by and distributed in the plants depends on the physico-chemical properties of the respective compounds. Early on it was suggested that there is an optimum of the polarity of a chemical for translocation (uptake from roots to shoots), with more polar or lipophilic compounds being less well translocated (Crowdy 1973; Briggs et al. 1982). Too polar or too lipophilic compounds would partition or interact more with the adjacent tissues, so that transport would be hindered. Briggs et al. showed in 1982

that the relationship between transpiration stream concentration factors (TSCFs, ratio of concentration in transpiration stream to concentration in external solution) and $\log K_{OW}$ of non-ionized pesticides followed a Gaussian bell curve with its peak at a $\log K_{OW}$ value around 2 (Briggs et al. 1982). Later it was found out that for highly soluble compounds with very low $\log K_{OW}$ values the bell shaped curve does not apply and instead the relationship follows a sigmoidal curve (Collins et al. 2006; Dettenmaier et al. 2009). Prior the start of the work of this thesis, it was unknown if PFAAs follow existing models for plant uptake due to their unique chemical properties. Due to the high electronegativity of the fluorine atoms, PFAAs might behave more like neutral compounds, even though the compounds carry a negative charge at environmentally relevant pH-levels. This is important, because lipophilic sorption to roots should be a negligible process for anions (Trapp 2000). Furthermore, the surfactant nature of PFAAs might cause them to behave different than other chemicals.

PERFOOD Project

The PERFOOD project (PERFluorinated Organics in Our Diet), in the framework of which the work for this thesis was conducted, was initiated in 2009 to boost the knowledge about PFAS in multiple ways. The objectives of the project were defined as follows:

"The aims of the present project are to develop robust and reliable analytical tools including reference materials for the determination of perfluorinated compounds (PFCs) in food items, and to use these to (i) qualify and quantify PFCs in our diet, employing a large European sampling campaign; (ii) understand how PFCs are transferred from the environment into dietary items, and (iii) quantify the possible contribution of food/beverage contact materials and food and water processing to the overall PFC levels in our diet. The newly gained knowledge will enable us to evaluate the possible routes, including their relative importance, of human exposure to PFCs via our diet, to assess the role of the technosphere in the contamination of our food, and to identify ways to reduce the PFC contamination of dietary articles."

Objectives of this thesis

The main objective of this thesis was to gain a better understanding of PFAA uptake from soil to food and feed crops. At the start of this work very little was known about the human exposure through the diet. While seafood and drinking water had already attracted the interest of the scientific community, the occurrence of PFAS in vegetables and other food items had been rather neglected up until then. A possible reason is that no methods were available to analyze PFAAs in complex matrices such as vegetables or food items with sufficiently low detection limits. Only a single publication was available in the peer-reviewed literature about the uptake of PFAAs by plants (Stahl et al. 2009). The main objective of this thesis was therefore to gain a deeper insight into the uptake of PFAS by plants under controlled and field conditions. In the first part of this thesis the uptake behaviour of various crops was investigated under controlled hydroponic conditions in a greenhouse, while the second part of the thesis focused on finding out if the results from the first part are

applicable under field conditions. For this purpose, an open air experiment with lysimeters was conducted where several crops were grown in spiked soil.

Research questions

The following main research questions were formulated for this thesis:

1. What are the PFAA uptake rates into the roots of crops?
2. To what extent are PFAAs transferred from the roots to the vegetative parts of the plants?
3. Do different plants behave similarly or are there differences in the uptake of PFAAs?
4. How do the properties of PFAA (e.g., hydrophobicity, chain length, etc.) influence the uptake and distribution in plants?
5. What mechanisms steer the uptake into plants?
6. Are the results from controlled greenhouse conditions applicable for field grown vegetables?
7. How does the soil influence the uptake?
8. Is uptake by plants a viable route for PFAAs to get into the human food chain?

Chapter 2 describes a hydroponic experiment with lettuce plants, where the plants were exposed to PFAAs via the nutrient solution. A broad range of exposure concentration was chosen. This experiment provided the first evidence of PFAA uptake by a crop via nutrient solution.

Chapter 3 builds upon the first experiment. Three more plant species were exposed to PFAAs in the same manner as in Chapter 1. Differences in the uptake between different plants, i.e., inter-species variability, are discussed. This chapter also describes in more detail how PFAAs are transported inside more complex plants.

Chapter 4 focusses on the behaviour and fate of PFAAs in soil during the field experiment with lysimeters. Here it is described how the leaching of the tested compounds is influenced by chain length and sorption capacity. Distribution coefficients (K_D) were determined in a lab experiment and with those a model was built to estimate the PFAA concentrations at the end of the experiment.

Chapter 5 further describes the role of soil in the field experiment conducted during the thesis. Here it is discussed to what extent the presence of soil influences the uptake and hence to what extent the results of the hydroponic greenhouse experiments with lettuce are applicable to field grown lettuce.

Chapter 6 discusses the uptake of 3 more plant species from the field experiment. The transport to different plant tissues is explored in more depth and differences between the plant species and similarities to the greenhouse experiment are shown.

In chapter 7 a synthesis of the work is presented.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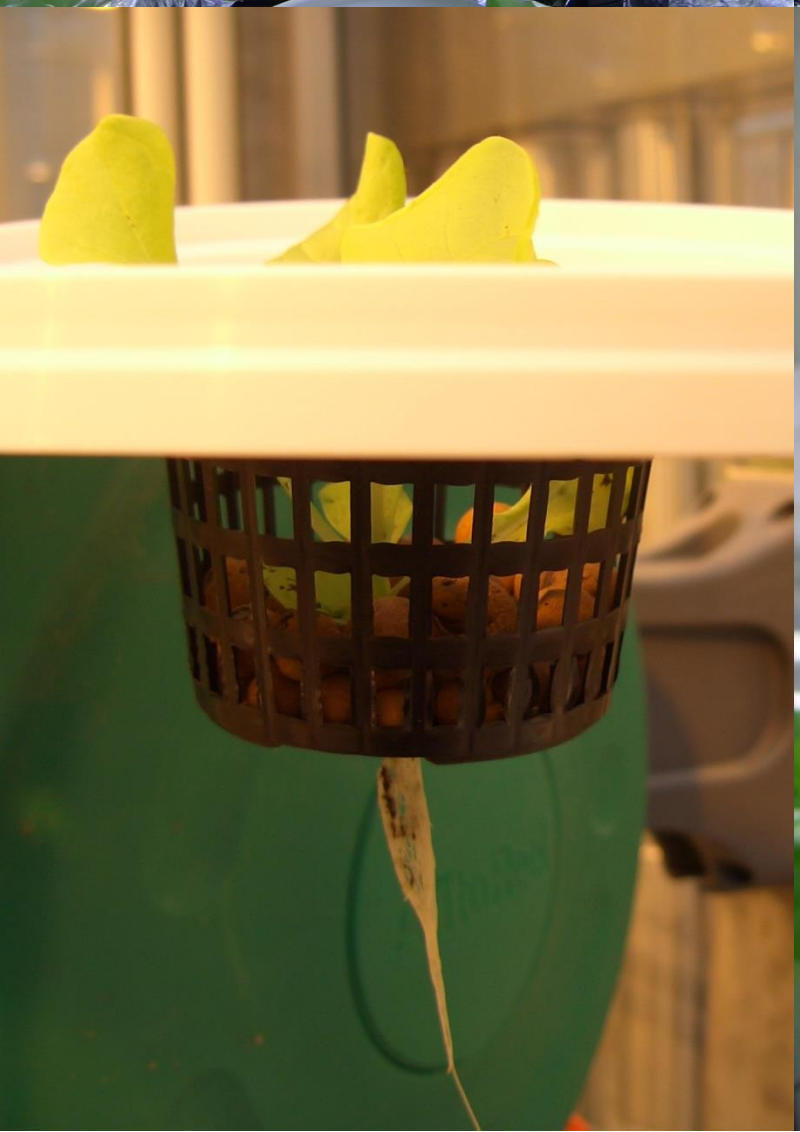

Chapter 2

UPTAKE OF PERFLUORINATED ALKYL ACIDS BY HYDROPONICALLY GROWN LETTUCE (*Lactuca sativa*)

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Abstract

An uptake study was carried out to assess the potential human exposure to perfluorinated alkyl acids (PFAAs) through the ingestion of vegetables. Lettuce (*Lactuca sativa*) was grown in PFAA-spiked nutrient solutions at four different concentrations, ranging from 10 ng/l to 10 µg/l. Eleven perfluorinated carboxylic acids (PFCAs) and three perfluorinated sulfonic acids (PFSAs) were analyzed by HPLC-MS/MS. At the end of the experiment, the major part of the total mass of each of the PFAAs (except the short-chain, C4-C7, PFCAs) taken up by plants appeared to be retained in the non-edible part, viz. the roots. Root concentration factors (RCF), foliage/root concentration factors (FRCF), and transpiration stream concentration factors (TSCF) were calculated. For the long chained PFAAs, RCF values were highest whereas FRCF were lowest. This indicates that uptake by roots is likely governed by sorption of PFAAs to lipid-rich root solids. Translocation from roots to shoots is restricted and highly depending on the hydrophobicity of the compounds. Although the TSCF show that longer-chain PFCAs (e.g., perfluorododecanoic acid) get better transferred from the nutrient solution to the foliage than shorter-chain PFCAs (e.g., perfluoroheptanoic acid), the major fraction of longer-chain PFCAs is found in roots due to additional adsorption from the spiked solution. Due to the strong electron-withdrawing effect of the fluorine atoms the role of the negative charge of the dissociated PFAAs is likely insignificant.

Introduction

Perfluorinated Alkyl Acids (PFAAs) have been detected in human blood (Guruge et al. 2005; Lau et al. 2007; D'Hollander et al. 2010a) and breast milk (Tao et al. 2008; Volkel et al. 2009; Karrman et al. 2010; Llorca et al. 2010). It is important therefore to understand the pathways of human exposure to PFAAs. PFAAs occur ubiquitously (Giesy et al. 2001; Prevedouros et al. 2006) and exposure pathways to the environment have attracted considerable scientific interest (Sinclair et al. 2006a; Ahrens et al. 2009b; Möller et al. 2009), but little quantitative assessment of exposure pathways has been undertaken.

Possible exposure pathways for humans are the diet and respiration of airborne PFAAs or their precursors (Fromme et al. 2009). Of the former, drinking water (Ericson et al. 2009; Eschauzier et al. 2012b) and fish (Haug et al. 2010b) have been analyzed quite extensively, but also vegetables can contain considerable amounts of PFAAs (Haug et al. 2010a). If crops are grown on PFAA-contaminated soils, it is possible that PFAA are taken up by the plants. Sludge or biosolids from wastewater treatment plants (WWTPs) are widely used in agriculture due to their high nutrient levels, but they also can contain high levels of PFAA (Lindstrom et al. ; Schultz et al. 2006; Sinclair et al. 2006a; Loganathan et al. 2007) from household or industrial wastewater. In Germany, for example, sludge from WWTPs is not allowed to be brought on agricultural land when it contains PFAAs with a sum concentration over 100 µg/kg dry weight. High PFAA concentrations in the Moehne and Ruhr rivers in Germany were traced back to industrial waste that had been applied as soil improver to agricultural land (Skutlarek et al. 2006; Wilhelm et al. 2008). The contamination (up to 8.6 mg PFOS per kg dry soil and around 1 mg per kg PFOA) occurred several years before the

PFAA residues were discovered (Wilhelm et al. 2008). It is unknown how much PFAA was taken up by agricultural products grown on these sites.
Most studies on PFAAs focus only on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), even though some recent studies have shown that other PFAAs like perfluorobutane sulfonate (PFBS) or perfluorobutanoic acid (PFBA) might be more abundant in the environment (Ahrens et al. 2009a; Möller et al. 2010).

There is very little data published on uptake of PFAAs by crops or plants. Market basket studies have shown that some plant food items contain PFAAs (Gem 2006; Ericson et al. 2008; Haug et al. 2010a). Stahl et al. showed that PFOS and PFOA can be transferred from contaminated soils to crops (Stahl et al. 2009). They investigated the uptake of PFOS and PFOA by rye grass, grains and potatoes. The study focused on the concentrations in edible parts of the plant, but no mass balance or uptake factors were given. Accumulation took place much more in vegetative compartments (e.g., straw, leaves) of the plants than in storage organs (e.g., tubers, ears). Analysis of the peel of potato tubers indicated that sorption and/or diffusion processes of PFOS and PFOA from soil to tubers outweighed translocation of the compounds from plant to tubers, for example, via the phloem.

Lechner and Knapp investigated carryover of PFOS and PFOA from soil to carrots, potatoes and cucumbers (Lechner et al. 2011). They also found highest concentrations in vegetative parts of the plants. While they could not find a difference in concentrations between peeled carrots and peelings, they confirmed the findings of Stahl et al. regarding higher concentrations in potato peelings than in peeled tubers.

To our knowledge these are the only publications in the peer-reviewed literature that deal with systematic uptake of PFAAs by vegetables. In an article on uptake by grass species (Yoo et al. 2011) Yoo et al. observed linear relationships of log gras-accumulation factors with carbon chain length for C6 to C14 PFCAs. The uptake of other persistent organic pollutants like polycyclic aromatic hydrocarbons (Kipopoulou et al. 1999; Fismes et al. 2002) and polychlorinated dibenzo-p-dioxins and dibenzofurans (Hulster et al. 1993; Muller et al. 1994; Zhang et al. 2009) from soils by plants has been extensively studied. These studies have shown that root uptake and translocation of these chemicals is negligible, the one notable exception being plants of the family *Cucurbitaceae* (Huelster et al. 1994). The major vector from soil to foliage for these contaminants is transfer of soil particles to leaf surfaces. However, the transfer behaviour of PFAAs is likely to be different, as in contrast to these neutral, hydrophobic compounds, PFAAs are ionic and hydrophilic. pKa values of PFAAs are much lower than their nonfluorinated homologues and show that PFAAs are in their anionic form at environmentally relevant pH values (see Table S2 in Supporting Information (SI)) (Goss 2008b; Steinle-Darling et al. 2008b; Rayne et al. 2009b). Uptake studies of ionic pharmaceuticals showed that compounds with a carboxylic group exhibit higher relative uptake rates than compounds with other functional groups and that compounds with a lower molecular weight have a higher uptake potential (Calderon-Preciado et al. 2012). Root uptake was usually higher than translocation to the vegetative parts of the plants (Eggen et al. 2011). But, again, due to the multitude of different chemical structures within pharmaceuticals and the special characteristics of PFAAs, it is unlikely that the results can be

extrapolated to PFAAs. Lipophilic absorption would be a negligible uptake mechanism for anions (Trapp 2000), but due to the strong electron-withdrawing effect of fluorine atoms resulting in dislocation of the negative charge (Jing et al. 2009), PFAAs may behave more like neutral compounds.

In the present study we investigate the root uptake efficiency and distribution of PFAAs in lettuce. We chose lettuce as a leafy vegetable to evaluate the hypothesis that PFAAs are taken up and distributed with the plant's water system. This hypothesis implies, since water is taken up in the roots and translocated to the leaves where it evaporates, that PFAA accumulation would take place predominantly in the leaves of the plant. A set of 11 PFCAs and 3 PFSAs was selected to assess the differences in behaviour between PFAAs. In this study we use the terminology proposed by Buck et al. (Buck et al. 2011).

Materials and methods

Chemical reagents and lab materials

Isotope-labeled internal standards and non-labeled calibration standards of PFAAs were provided by Wellington Laboratories (Ontario, Canada). A list with abbreviations of the non-labeled and labeled standards is provided in Table S1 of the SI. PFBA, 98%, perfluoropentanoic acid (PFPeA), 97%, perfluoroheptanoic acid (PFHpA), 99%, PFOA, 96%, perfluorononanoic acid (PFNA), 97%, perfluorodecanoic acid (PFDA), 98%, perfluoroundecanoic acid (PFUnA), 95%, perfluorododecanoic acid (PFDoDA), 95%, perfluorotridecanoic acid (PFTrDA), 97%, perfluorotetradecanoic acid (PFTeDA), 97%, perfluorobutanesulfonic acid potassium salt (K-PFBS), ≥98%, and Perfluorohexanesulfonic acid potassium salt (K-PFHxS), ≥98%, Perfluorohexanoic acid (PFHxA), ≥97%, and PFOS-potassium salt (K-PFOS), ≥98% were all obtained from Sigma Aldrich (Zwijndrecht, Netherlands). Materials used for sample treatment and analysis included Florisil cartridges CUFLS 6cc (1000 mg) from United Chemical Technologies Inc. (Bristol, USA); Oasis WAX 3cc SPE cartridges (60 mg) from Waters (Wexford, Ireland); Acrodisc LC13 GHP Pall 0.2 µm filters from Pall Corporation (NY, USA); 50 and 15 mL polypropylene (PP) tubes with screw caps from Sarstedt (Nümbrecht, Germany); and Supelclean ENVI-Carb 120/140 from Supelco (Bellefonte, USA). Tetrabutylammoniumhydrogensulfate (TBA) and sodium hydrogencarbonate were purchased from Merck (Darmstadt, Germany), sodium carbonate and ammoniumhydroxide a.c.s. from Sigma Aldrich; Methanol and water (ULC/MS grade) and tert-butyl methyl ether (MTBE, HPLC grade) from Biosolve (Valkenswaard, Netherlands). 2.0 and 0.3 mL PP vials were purchased from VWR International (Amsterdam, Netherlands. The 10 L PP buckets were obtained from Harcotom (Purmerend, Netherlands).

Plant culture and exposure experiments

The experiments were conducted in a greenhouse (25°C, 14h Light). Lettuce plants (*Lactuca sativa, var. attraction*) were pre-grown for 2 weeks in soil. Next, the plants were removed from the soil, residual soil was washed off the roots with demineralized water, and plants were transferred to an experimental hydroponic system containing a PFAA-spiked nutrient solution. The hydroponic system was employed to avoid sorption of the dosed chemicals to soil and to help ensure that the spiked PFAAs were completely bioavailable. The plants were set in mesh pots, which were inserted in the lids of 10 L PP buckets. To support the plants

the mesh pots were filled with baked clay pebbles. The buckets were wrapped in aluminum foil to keep the root zone dark. A schematic drawing of the experimental set up is provided in SI Figure S1.

For nutrient supply a modified half-concentrated Hoaglands solution was used (for composition, see SI Table S3). Nutrient solutions were spiked with 100 µL/L of PFAA stock solutions to achieve a final nominal concentration of 10 ng/L, 100 ng/L, 1 µg/L or 10 µg/L of each compound. The buckets were filled with 8 L of the spiked nutrient solution, so that not the mesh pots, but only the roots of the plants were in contact with the nutrient solution. Six replicates for each concentration as well as 3 replicates of blank controls were grown. Additionally, 2 buckets with no plants were placed among the other plants to determine water evaporation. After 40 days of growth in the hydroponic system the plants were harvested, divided into foliage and roots, and stored at -20°C until extraction. During the exposure time, the spiked nutrient solution was exchanged twice to keep the PFAA's concentration at a constant level and to avoid microbial growth in the nutrient solution. Plant growth and transpiration was measured by weighing. During the 40 days of exposure the plants grew from less than 1 g to over 300 g total biomass on average (SI Figure S2). The average amount of transpired water was 10.96 ml/g biomass (SI Figure S3). Neither visible nor measurable differences in growth of individual plants were observed between the different spiking levels. No effects of the compounds on the plant health (discoloring, spots) were visible.

Extraction

Plant samples (roots or foliage) were washed with demineralized water, dried superficially and homogenized with a household blender (Braun Multiquick MX 2050). Extraction is based on the method published by Hansen et al. (Hansen et al. 2001) with modifications proposed by Vestergren et al. (Vestergren et al. 2012). Ten g of homogenate was weighed in a 50 mL PP tube and spiked with internal standards before adding 6 ml of 0.4 M NaOH. After vortex-mixing the samples were left in the fridge overnight. 4 mL of a 0.5 M TBA solution and 8 mL of a 0.25 M Na_2CO_3/$NaHCO_3$ buffer were added. After mixing, 10 mL of MTBE was added and the sample was vortex-mixed for 1 min and placed in an ultrasonic bath for 10 min. The samples were then centrifuged for 10 min at 3000 rpm. The supernatant was transferred to a 15 mL PP tube and concentrated under a gentle nitrogen stream. The extraction was repeated 2x with 5 mL of MTBE and the supernatants were combined, evaporated to 1 mL, and passed through a Florisil SPE cartridge, conditioned with 10 ml methanol and 10 ml MTBE. The non-polar matrix was eluted with 10 ml of MTBE before extracting the analytes with 10 ml of MeOH/MTBE (30:70, v:v). After evaporation to 1 mL a clean-up step with ENVI-Carb following the Powley method (Powley et al. 2005) was performed for the foliage samples, since they still showed an intensive green color after the SPE clean-up.

Water samples were extracted with weak anion-exchange SPE cartridges. The SPE cartridges were subsequently conditioned with 3 ml 0.1% NH_4OH in methanol (v:v) and 3 ml water. 200 ml, 20 ml and 5 ml (for 10 and 100 ng/L, 1 µg/L and 10 µg/L, respectively, to stay inside the calibration line) of each test solution were spiked with internal standards and loaded on the SPE cartridges with a speed of about 1-2 drops per second. The cartridges were then washed

with methanol:water (40:60, v:v) and dried under vacuum before the PFAAs were eluted with two times 500 µl of 2% NH_4OH in Methanol (v:v).
The final extracts of both water and plant samples were filtered through an Acrodisc LC 13 GHP Pall (flushed with 1 mL of methanol) into a 2 mL PP vial (pre-rinsed with methanol) and stored at 4 °C until analysis.

Analysis

Prior to analysis the purified extracts and the calibration standards were diluted 1:1 with water. These 1:1 solutions were tested for analyte losses due to wall sorption in PP vials: no appreciable losses were observed. 20 µl of the mixture was injected into a HPLC (LC-20AD XR pump, SIL-20A autosampler and SCL-10A VP system controller, Shimadzu, Kyoto, Japan) connected to a tandem MS (4000 QTrap, Applied Biosystems, Toronto, Canada) via an electrospray ionization interface operating in negative ionization mode. Mass transitions used are given in Table S1 of the SI.

For separation an ACE 3 C18-300 column (ID 2.1 mm; length 150 mm, particle diameter: 3 µm, Advanced Chromatography Technologies, Aberdeen, Scotland) was used, held at a constant temperature of 30 °C. A pre-column (Pathfinder 300 PS-C_{18} column, ID 4.6 mm; length 50 mm; particle diameter 3.5 µm, Shimadzu, Duisburg, Germany) was placed before the injection valve to trap and delay the background of PFAAs from the HPLC system. All parts containing PTFE were, if possible, replaced by stainless steel or PP parts to reduce contamination of samples.

Separation was achieved using gradient elution with two mobile-phase constituents, A (40:60 methanol:water) and B (95:5 methanol:water; both with 2 mM ammonium acetate). Prior to injection of each sample the system was allowed to equilibrate for 8 min with the initial mobile phase composition (60% A) at a flow of 0.2 mL/min. After injection the solvent composition linearly changed to 100% B at 10 min, then held isocratic until 20 min. The solvent composition returned to initial conditions after 20 min until 22 min.
Peak integration and processing of the raw data was done with Analyst 1.5 (Applied Biosystems). More details of the instrumental analysis are given in the SI.

Quality assurance and control

All samples were extracted and injected in duplicate. A twelve-point calibration line was used for quantification. The fitted lines had R^2 values of at least 0.99 for all analytes, no weighting was applied. Compounds with no direct internal standard were corrected with the closest available IS. This correction can lead to possible bias, but this is believed to be minimal and the best available method anyway. Concentrations of analytes were only used and reported when the following criteria were met:

I. The nominal concentration and the measured concentrations in the calibration standards did not deviate more than 30% (which occurred only at the lower levels). II. The peak area of the analyte in a sample was in between the areas of the lowest calibration standard (defined by point I.) and the highest calibration standard. III. The response ratio of mass transitions 1 and 2 did not deviate more than ±30% from the same ratio determined for the most proximate calibration standards.

Recoveries were determined by comparing matrix-free direct injections of the same amount of spiked 13C internal standards with 13C spiked blank extracts and 13C spiked samples. Recoveries of the 13C standards in spiked blanks were between 66 and 81% (foliage procedure), 67-95% (roots) and 60-97% (water). Mean recoveries of the 9 different 13C labeled internal standards ranged from 63% to 72% for foliage samples, from 79% to 107% for root samples and from 58% to 103% for water samples. Since repetitive exhaustive extraction did not recover significant amounts of native compounds we can rule out the possibility that the uptake pattern we have observed is a result of variation of extraction efficacy. Further matrix effects examination was done by fortification of root and foliage extracts with internal standards immediately before injection into the HPLC-MS/MS system and comparing these with internal standards added to methanol. Foliage extracts caused slight signal-enhancement for most PFCAs (up to 27%), while only PFDA was slightly enhanced in root matrix (SI Figure S4). The values are similar to those reported by Vestergren et al. (Vestergren et al. 2012). We conclude that the recoveries are mainly lower due to losses during sample treatment. The difference between the recoveries of foliage and root extracts is caused by the additional clean-up step with carbon, which was applied to foliage, but not to root samples. The only exception is PFBA, for which recoveries were much lower in both foliage and roots (16% and 30%, respectively) but also in the spiked blanks (24 and 38%). No recoveries for PFBA have been previously reported for this or a similar method. Since the recoveries were low, but consistent we decided to include results of PFBA.

Limits of quantification (LoQs) and detection (LoDs) (SI Table S4) were calculated on the basis of method blanks (n=4) (when present in blanks) as follows: the average concentration in the blanks plus 10x or 3x the standard deviation of the blank concentration, respectively. When no analyte was present in blanks, LoQ and LoD were calculated from the lowest validated calibration standard: amount injected back-calculated to an extract of 1 mL and divided by the average sample quantities extracted, yields the LoQ. The LoD is 30% of the LoQ, calculated in this manner. Method blanks were prepared using the extraction procedure for the samples.

Background concentrations were taken from plants growing in non-spiked nutrient solution (n=3), and used to correct mean PFAA concentrations found in spiked experiments in roots and foliage. If the resulting concentration was below the LoQ, the value was neglected. Background concentrations for foliage above the LoQ were observed for PFBA, PFHpA, PFOA and PFDA to PFTrDA as well as branched PFOS. In roots, background concentrations were present above LoQ for all compounds except PFBA and PFPeA. The resulting concentrations after correction were below LoQ for PFHxA, PFHpA, PFBS and PFHxS. For the other compounds background concentrations accounted for on average about 30% of the measured concentration in the lowest spiked concentration. Background concentrations measured in the unspiked nutrient solutions were also subtracted from concentrations found in the spiked nutrient solutions, but the influence was negligible for all compounds except PFBA, where the background concentration was responsible for 30% of the measured concentration in the lowest spiked level.

Branched isomers were detected for various PFAAs, but quantification of branched isomers was only possible for PFOS, being the only compound containing branched isomers in the standards used for calibration. For PFOS, where the branched isomers made up 21.2% of the total PFOS in the standard, no preference in uptake of either linear or branched isomers was observed. All concentrations given in this article are sum-concentrations of all PFOS isomers, and uptake factors were calculated from sum-concentrations.

Uptake factors calculated from the data were evaluated for outliers using box-plots with SigmaPlot (Systat Software, Inc.). Outliers and values below LoQ were not included in data interpretation.

Results and discussion

Concentrations in nutrient solution

The average of concentrations measured in the solution was used for the calculation. Mean recoveries of the spiked concentrations were good for the shorter-chained compounds, but drop for long-chained PFAAs to below 30% of nominal concentrations (SI Table S5). Reth et al. reported that actual and nominal concentrations of PFAAs in a spiked aqueous solution can differ quite significantly, especially for long-chained PFAAs (Reth et al. 2011). For PFDoDA and PFTeDA they could not detect any appreciable concentration in the solution after spiking. The concentration range Reth et al. worked with was in the lower ng/L range, similar to our lowest spiked concentration. The findings of Reth et al. were confirmed in our study. Reth et al. explained the low recoveries by either losses to surfaces of the system or enrichment at the air-water-interface. In the latter case the PFAAs would be still available for uptake by the plant roots. However, only a very small part of the plant roots were in contact with the air-water-interface. Hence, the actual concentrations measured in the nutrient solution and not the nominal concentrations were used for uptake factor calculations.

Roots

Examining the PFCA concentrations in roots as a function of perfluorocarbon chain length, a decrease from PFBA to PFHxA was observed, followed by a rapid increase with increasing chain length to PFUnA, after which the concentrations decreased with PFTrDA having lower concentrations than PFDoDA and PFTeDA. The concentrations of PFSAs in roots increased with increasing chain length (SI Table S6).

Isotherms. Root concentrations of long-chained PFAAs follow a non-linear Freundlich or Langmuir isotherm, whereas the short-chained PFCAs (PFBA to PFHxA) and PFBS showed a linear regression (SI Figure S5). The Freundlich coefficients were between 1.4 and 1.9.

A possible explanation for the non-linearity is adsorption of compounds to the root surface. This hypothesis is supported by the fact that generally adsorption of PFAA gets stronger with increasing chain length (Higgins et al. 2006). Briggs et al. state that the uptake by roots of rather lipophilic chemicals is dominated by physical sorption (Briggs et al. 1982) although they did not discriminate between adsorption and absorption.

Another explanation for non-linear root concentration isotherms could be formation of micelles at the highest concentration spiked. The reported critical micelle concentrations (CMC) of PFAAs, however, are in the mg/L to g/L range (de Voogt et al. 2006a) which is at least a factor of about 1000 higher than the highest nominal spiked concentration used in the present study (10 µg/L used against e.g., ~7 mg/L CMC for PFTeDA, which has the lowest reported CMC) (Bhhatarai et al. 2011). Even the sum of the nominal concentrations of all PFAAs used in the present study is still 50 times below this CMC. Thus, we assumed that formation of micelles did not affect the actual concentrations.

We conclude that the high concentrations of PFAAs observed in roots and the non-linearity of the isotherms are likely due to adsorption of long-chained compounds to lipophilic root solids, such as lipids/proteins in membranes and cell walls, rather than actual intake into the roots.

Root concentration factor (RCF). RCFs were calculated for each of the PFAAs (Figure 1). The RCF is defined as the ratio of the concentration of a compound in roots divided by its concentration in solution (Trapp 2000):

$$\mathrm{RCF} = \frac{\text{Concentration in root (ng g}^{-1})}{\text{Concentration in nutrient solution (ng ml}^{-1})}$$

RCFs should be independent of the concentration in solution, provided that the uptake isotherms are linear. Non-linear uptake isotherms were observed for all PFAAs, except PFBA, PFPeA, PFHxA and PFBS (Figures S5 in SI), but when the highest spiked concentration was excluded the isotherms became linear. A *t* test confirmed in these cases the significant difference ($p<0.05$) between the RCF calculated from the highest spiked concentration (average of 6 plants) and the RCF calculated from the other concentrations (average of 18 plants). Only the RCF values from the linear part of the isotherms are included in Figure 1. The error bars denote the standard error of the RCF for all plants (n=18 or 24) from all spiking levels in the linear range of the isotherms.

RCFs for PFCAs decrease with increasing chain length from PFBA to PFHxA, then increase markedly between PFHxA and PFUnA, and finally are quite similar for PFUnA through PFTeDA. The concentrations observed in roots can be influenced by uptake into root tissue and by sorption to root surface. Furthermore, chemicals taken up by roots can be transferred from roots to foliage. We hypothesize that uptake into root tissue is increasingly inhibited with increasing carbon chain length, e.g., due to increasing molecule size, while adsorption becomes stronger with increasing carbon-chain length. With this model, root uptake would be the dominant accumulation process for PFBA and PFPeA, while adsorption would be dominant for PFHpA → PFTeDA.

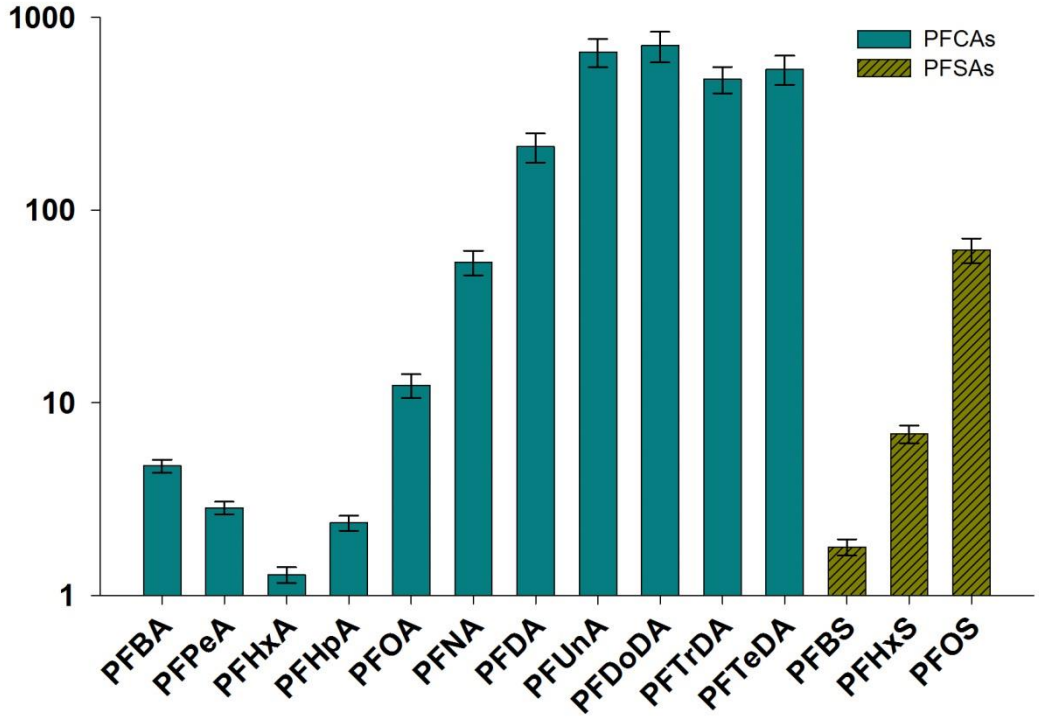

Figure 1: Root concentration factors (RCF) calculated from concentrations of PFAAs in roots divided by their concentrations in the spiked nutrient solution, logarithmic scale. Individual RCFs were calculated for each plant from the linear portion of the sorption isotherm (see text). Mean and standard error are shown (n between 14 and 21).

Water and therefore chemicals solved in the water can enter the plant by two pathways, the symplastic and the apoplastic. While the chemicals on the symplastic path move from cell to cell across cell membranes or via plasmodesmata, they move through the cell walls and/or intercellular spaces on the apoplastic path (Sperry et al. 2002). The combination of a compound's solubility in water and in the lipid-rich cell membrane determines its movement into roots and subsequent translocation to shoots (Collins et al. 2006). The apoplastic path is interrupted at the anticlinal walls of the endodermis by the Casparian strip, which consists of hydrophobic suberin and lignin and is impermeable to water and chemicals (Sperry et al. 2002). Thus the water and the chemicals are forced through the endodermic symplast, which could be a barrier for PFAAs. The cells outside of the Casparian strip are likely to be in equilibrium with the external solution, so if there was no sorption to lipophilic root solids, one would expect a RCF of <1 due to the high water content of cells and the fact that cells inside the Casparian strip are not in full equilibrium with the external solution, as was proposed by Briggs et al. (Briggs et al. 1982). In the present study none of the compounds had a RCF <1. While the increase of the RCF with increasing chain length can be explained by sorption to the root surface, it does not explain the RCFs of PFBA and PFPeA. We hypothesize that PFBA and PFPeA are able to pass through or bypass the Casparian strip better than the other compounds and then accumulate in the vascular tissue in connection with the foliage. This hypothesis is supported by foliage to root concentration factors (FRCF)

values >1 and the highest transpiration stream concentration factor (TSCF) values of all measured compounds for PFBA and PFPeA (see below).

Foliage

The highest concentrations in the foliage were observed for PFBA followed by PFPeA. Foliage concentrations of PFCAs decreased further with chain length until PFHpA, and then increased again with increasing chain length, achieving a maximum for PFDA. No trend was observed for the PFSAs (table S6 in SI).

Foliage to root concentration factor. The FRCF were calculated by dividing the foliage concentrations by the root concentrations (Figure 2):

$$\mathrm{FRCF} = \frac{\text{Concentration in foliage (ng g}^{-1})}{\text{Concentration in root (ng g}^{-1})}$$

FRCFs decrease exponentially with increasing chain length for both PFCAs and PFSAs. A similar relationship is seen for the distribution of chemical mass between leaves and roots; the fraction in leaves decreases with increasing chain length (see SI Figure S6).

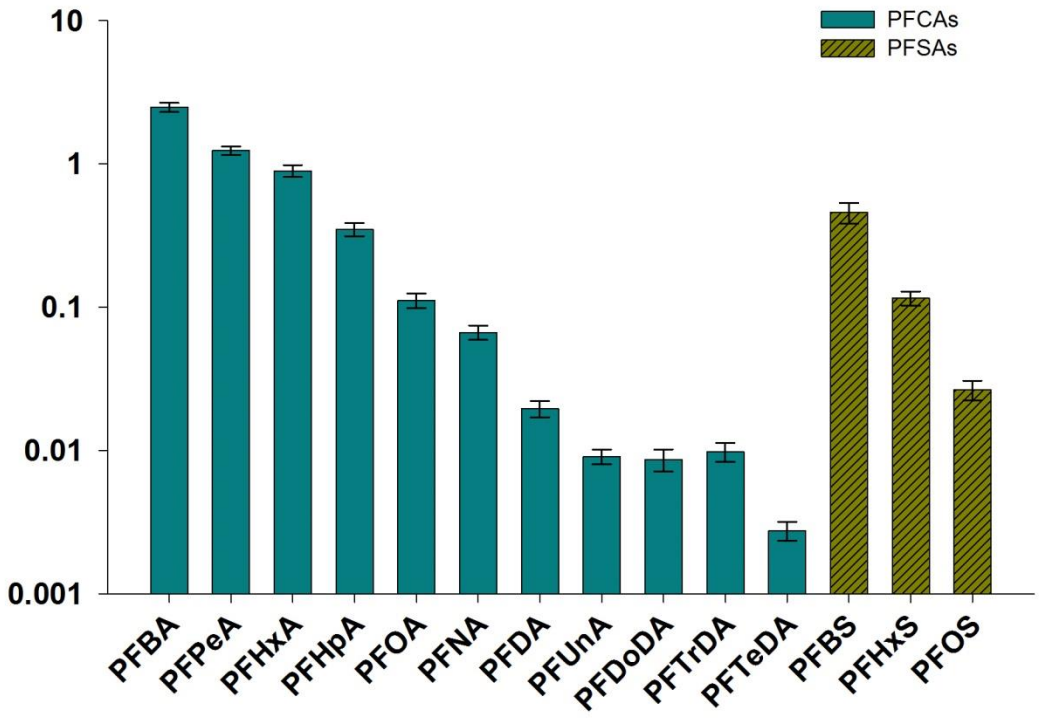

Figure 2: Foliage to root concentration factors (FRCF) of lettuce calculated from concentrations in leaves divided by concentrations in roots, logarithmic scale. FRCFs were calculated for each plant from all spiking levels. Mean and standard error are shown (n between 17 and 23).

Only PFBA and PFPeA had higher concentrations in leaves than in roots, leading to FRCF values greater than 1. One explanation for the decrease in FRCF with chain length is sorption of the chemicals to plant tissue while they are translocated through the plant.

Sorption of PFAAs from water to organic material is known to increase with chain length (Higgins et al. 2006); this could lead to a stronger retention and thereby reduced transport of the longer-chain chemicals. A second explanation was outlined above, namely that the ability of the PFAAs to cross the Casparian strip may decrease with chain length. Even though higher concentrations of the longer-chain PFAAs are transferred to roots (Fig. 1), the great majority of these chemicals may not cross the Casparian strip, and thus would not be available for translocation to foliage.

Transpiration stream concentration factor

The translocation of compounds from roots to foliage can be described by the TSCF, the chemical's concentration in the transpiration stream divided by its concentration in soil pore water (Briggs et al. 1982; Trapp 2000). We estimated the TSCF by dividing the mass of the chemical in foliage by the chemical concentration in the nutrient solution multiplied with the volume of water transpired by the plant:

$$\mathrm{TSCF} = \frac{\text{Concentration in foliage (ng g}^{-1}) * \text{foliage weight (g)}}{\text{Concentration in nutrient solution (ng ml}^{-1}) * \text{water transpired (ml)}}$$

Note that in estimating TSCF this way it is assumed that the chemical is not degraded in the plant, that chemical elimination from the plant is negligible (e.g., loss of the compounds from leaves to atmosphere or back to roots), and that compounds are only taken up through roots and not via other pathways like atmospheric deposition. Given the persistence and low concentrations of PFAAs in the foliage of control plants (no PFAAs added to the nutrient solution), these are reasonable assumptions. The TSCF values thus obtained were much less than 1 for all PFAAs except for PFBA (Figure 3). This means that transfer from the nutrient solution to leaves was inhibited.

The TSCFs of PFCAs decrease with increasing chain length from PFBA to PFHpA, then increase between PFHpA and PFDoDA, closing with a markedly lower value for PFTeDA. The TSCF of PFOS is significantly higher than that of PFBS and PFHxS. The TSCF results suggest that PFUnA, PFDoDA and PFTrDA are able to pass the Casparian strip more easily than the rest of the PFCAs with the exception of PFBA. This contradicts the interpretation offered in the RCF section, namely that the ability of the PFAAs to cross the Casparian strip decreases with chain length. The TSCF results suggests that uptake behaviour of the PFAAs is more complex, i.e.,, that there is no simple relationship between uptake efficiency into vascular tissue of the plant and PFAA chain length.

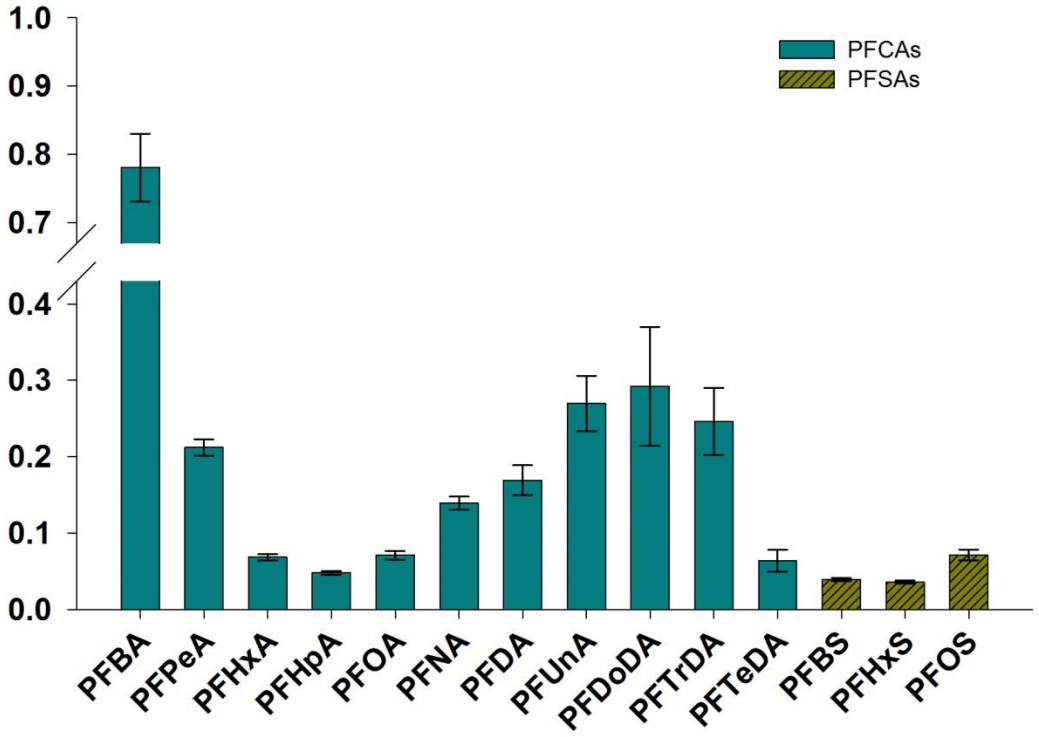

Figure 3: Transpiration stream concentration factors of PFAAs in lettuce calculated from concentrations in foliage multiplied with foliage weight divided by amount of water transpired (see text). Mean and standard error are displayed (n between 11 and 20).

Correlations with hydrophobicity

For non-ionic organic chemicals the degree of uptake via plant roots seems to be inversely related to their hydrophobicity, which is often approached by the octanol-water partition coefficient (K_{OW}) (McKone et al. 2007). The surfactant nature of perfluorinated alkyl acids makes it impossible to determine the K_{OW} with classical standard methods (Jing et al. 2009). Jing et al. (Jing et al. 2009) determined experimental logKow substitutes, log$P^{0\prime}$-values, of PFAAs by voltammetric methods. Of the compounds tested in the present study, 9 log$P^{0\prime}$-values were available. De Voogt et al. determined hydrophobicity values from capacity factors for several PFAAs using reversed-phase HPLC (de Voogt et al. 2012). The *k*0 value is the capacity factor at a (hypothetical) mobile phase consisting entirely of water, and is derived from (extrapolation of) capacity factors recorded at different isocratic mobile phase compositions. The log*k*0 values are highly correlated with and similar to calculated log K_{OW} values proposed by Arp et al.(Arp et al. 2006) (SI Table S7). Within a given substance group they are also well correlated with the length of the perfluorinated chain. Log*k*0 values are available for eleven compounds of the present study.

Figures 1-3 indicate that RCF, FRCF, and TSCF are correlated with chain length for part of the dataset, but not for the full range of chemicals. This indicates that RCF, FRCF and TSCF are also correlated with hydrophobicity (log*k*0) for part of the dataset, but not for the full set of chemicals. Nevertheless, plotting these experimental parameters against log*k*0 can provide new insight by indicating whether hydrophobicity explains differences between PFCAs and

PFSAs. Figures 4A and 4B show that the linear regression of RCF and FRCF against log*k*0 almost fall on the same line for the PFCAs and the PFSAs. Also the differences in TSCF between PFCAs and PFSAs are negligible (Figure 4C). This indicates that differences in hydrophobicity explain a large portion of the chemical group specific differences in RCF and FRCF.

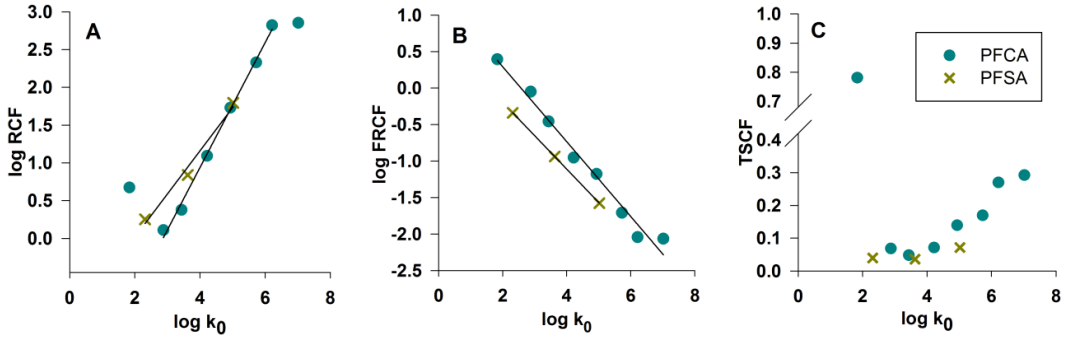

Figure 4: Correlations between log k0 and (A) log RCF; (B) log FRCF and (C) TSCF. Regression for PFCA in Figure 4A based on linear range only. Dots denote PFCAs, crosses PFSAs (legend in 4C valid for 4A and B).

For non-ionic chemicals the TSCF-LogK_{ow} relationship has been reported by Briggs et al. to follow a Gaussian bell curve with the peak at log K_{OW} values around 2-3 (Briggs et al. 1982; Collins et al. 2006). Collins et al. also state that for highly soluble compounds with very low log K_{OW} values, the bell shaped curve may not apply and they might approach unity (Collins et al. 2006). This was confirmed by Dettenmaier et al., who reported a sigmoidal relationship (Dettenmaier et al. 2009). For PFAAs neither a bell shaped nor a sigmoidal correlation was found between TSCF and log*k*0 (Figure 4C). In fact, PFAAs with a log*k*0 value around 2-3 showed the lowest TSCF values. This indicates that the plant uptake of PFAAs cannot be described using existing models, with the exception of PFBA, which could fit in a sigmoidal correlation as described above.

Implications for human exposure

The results show that lettuce grown in highly contaminated nutrient solution does not accumulate concentrations of the PFAAs in the edible plant parts to levels that pose a risk to human health. The European Food Safety Authority established tolerable daily intake values (TDIs) for PFOA (1500 ng/kg body weight) and PFOS (150 ng/kg body weight) (EFSA 2008). To exceed the TDI for PFOS and PFOA a person of 70 kg body weight would need to eat about 900 g and 9000 g, respectively, of the lettuce grown in the highest concentration of 10 µg/L. No TDI values for other PFAAs currently exist. The highest concentrations in the edible part were observed for the short chained PFAAs, for which a lower toxicity is reported than for PFOA (Renner 2006).

The higher concentrations found in the present study in roots of lettuce indicate that root vegetables, like carrots or radishes, might pose a higher risk for human exposure than leaf or fruit vegetables. Lechner et al. (Lechner et al. 2011) and Yoo et al. (Yoo et al. 2011) determined transfer factors (TF) for different plant compartments they analyzed, as well as from data published by Stahl et al. (Stahl et al. 2009). TF are calculated as the mean

concentrations in the plant compartment divided by the mean concentration in soil. The use of soil and sorption of PFAAs to soil will have a large impact on transfer factors, especially for the long-chained compounds which exhibit strong sorption, which would yield a lower bioavailable concentration. Therefore it is not possible to compare values derived from a hydroponic study with values from an experiment using soil. Also the comparison of two soil related studies is difficult, since different soil properties will largely affect the TF. Furthermore, the values by Yoo et al. and Stahl et al. are based on the dry weight concentrations. However, accumulation factors by Yoo et al. decrease steadily with increasing chain length, while we found a maximum for PFUnA. Lechner et al. calculated TF on wet weight bases and found average values for PFOA of 0.4, 0.53 and 0.88 and for PFOS of 0.36, 0.38 and 0.17 for the vegetative compartment of potatoes, carrots and cucumbers, respectively. The transfer factors for PFOA and PFOS from our foliage data (1.1 and 1.2, respectively) are higher than those of Lechner et al. This can be possibly explained by the different experimental set-up of using hydroponics instead of soil.

Our results show that PFAAs are taken up with water and are translocated with the plants transpiration system as hypothesized. However, the hypothesis that the compounds would accumulate predominantly in the foliage was refuted for most of the compounds. The results show that translocation from nutrient solution via roots to shoots was restricted, whereby this is apparently a complex function of the PFAA chain length / hydrophobicity.

Hydroponic greenhouse experiments have a number of advantages including strong plant growth due to stable supplies of nutrients and sunlight and well defined experimental conditions. However, while some vegetables are indeed commercially grown with hydroponic solutions, most crops are grown in soil on fields. Hence, the results of this experiment have to be confirmed for field grown lettuce.

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Supporting Information

Description of analytical method used.

The analytical methodology was according to the methods described by Eschauzier et al. (2010) (Eschauzier et al. 2010). The measurements were conducted in the scheduled MRM-mode (see Table S1). Briefly, instrumental settings included:

Ion Transfer Voltage:	-2000 V
Interface Temperature:	450^{o}C
Curtain gas:	10L min^{-1}
Collision gas:	6L min^{-1}
Collision Energy:	-10V for PFPeA to PFOA, -15V for PFBA, -25V for PFNA to PFTeDA and -70V for the PFSAs

The concentrations of calibration standards ranged from 0.005 (Calibration level 1) to 200 ng/ml (Calibration level 12). Peaks consisted of at least 24 scans and the smoothing width was 9 points.

Table S1: List of abbreviations of used analytes and ^{13}C-labelled standards including their measured mass transitions and expected retention times.

Abbreviation	Compound	Transition 1	Transition 2	Quantification by internal Standard	Molecular Formula	Retention time [min]
PFBA	Perfluoro-n-butanoic acid	213 → 169	-	$^{13}C_4$ PFBA	$CF_3(CF_2)_2COOH$	2.6
PFPeA	Perfluoro-n-pentanoic acid	263 → 219	-	$^{13}C_2$ PFHxA	$CF_3(CF_2)_3COOH$	3.4
PFHxA	Perfluoro-n-hexanoic acid	313 → 269	313 → 119	$^{13}C_2$ PFHxA	$CF_3(CF_2)_4COOH$	4.8
PFHpA	Perfluoro-n-heptanoic acid	363 → 319	363 → 169	$^{13}C_8$ PFOA	$CF_3(CF_2)_5COOH$	7.6
PFOA	Perfluoro-n-octanoic acid	413 → 369	413 → 169	$^{13}C_8$ PFOA	$CF_3(CF_2)_6COOH$	9.1
PFNA	Perfluoro-n-nonanoic acid	463 → 419	463 → 219	$^{13}C_9$ PFNA	$CF_3(CF_2)_7COOH$	10.6
PFDA	Perfluoro-n-decanoic acid	513 → 469	513 → 269	$^{13}C_6$ PFDA	$CF_3(CF_2)_8COOH$	11.8
PFUnA	Perfluoro-n-undecanoic acid	563 → 519	563 → 269	$^{13}C_7$ PFUnA	$CF_3(CF_2)_9COOH$	12.6
PFDoDA	Perfluoro-n-dodecanoic acid	613 → 569	613 → 319	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{10}COOH$	13.5
PFTrDA	Perfluoro-n-tridecanoic acid	663 → 619	663 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{11}COOH$	14.1
PFTeDA	Perfluoro-n-tetradecanoic acid	713 → 669	713 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{12}COOH$	14.7
PFBS	Perfluorobutane sulfonate	299 → 80	299 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_3SO_3$	3.4
PFHxS	Perlfuorohexane sulfonate	399 → 80	399 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_5SO_3$	7.6
PFOS	Perfluorooctane sulfonate	499 → 80	499 → 99	$^{13}C_8$ PFOS	$CF_3(CF_2)_7SO_3$	10.6
$^{13}C_4$ PFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	217 → 172	-			2.6
$^{13}C_2$ PFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	315 → 270	315 → 119			4.8
$^{13}C_8$ PFOA	Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	421 → 376	421 → 172			9.1
$^{13}C_9$ PFNA	Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]nonanoic acid	472 → 427	472 → 223			10.6
$^{13}C_6$ PFDA	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	519 → 474	519 → 219			11.8
$^{13}C_7$ PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	570 → 525	570 → 270			12.6
$^{13}C_2$ PFDoDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	615 → 570	615 → 369			13.5
$^{18}O_2$ PFHxS	Perfluoro-1-hexane[$^{18}O_2$]sulfonate	403 → 84	403 → 103			7.6
$^{13}C_8$ PFOS	Perfluoro-1-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanesulfonate	507 → 80	507 → 99			10.6

Table S2: pKa values of PFAAs reported by Rayne et al. (Rayne et al. 2009b) and Steinle-Darling et al. (Steinle-Darling et al. 2008a)

	Rayne *et al.*	Steinle-Darling *et al.*
PFBA	0.1	
PFPeA	-0.1	-0.10
PFHxA	-0.1	-0.16
PFHpA		-0.19
PFOA	-0.1	-0.20
PFNA		-0.21
PFDA	-0.1	-0.21
PFUnA	-0.1	-0.21
PFDoDA	-0.1	-0.21
PFTrDA		
PFTeDA		-0.21
PFBS		0.14
PFHxS		0.14
PFOS		0.14

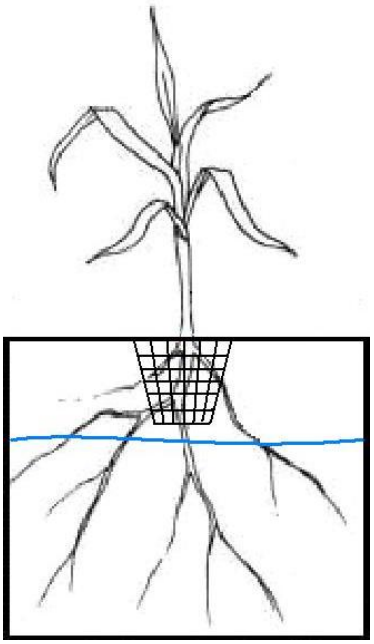


Figure S1: Schematic drawing of the used hydroponic system. The plant is put in a mesh pot, which is inserted in the lid of the bucket. Only the roots are in contact with the spiked nutrient solution.

Table S3: Chemical composition of the used half-concentrated Hoaglands nutrient solution and the composition of the stock solutions used to achieve the final concentrations.

Component	Conc. Stock Solution	mL Stock Solution/1L	final conc. in nutrient solution	
KNO_3	202g/L	1.25	N	105 ppm
$Ca(NO_3)_2 \times 4H_2O$	472g/L	1.25	K	117.5 ppm
NH_4NO_3	32g/L	1.25	Ca	100 ppm
$MgSO_4 \times 7H_2O$	493g/L	0.5	Mg	24 ppm
KH_2PO_4 (pH to 6.0 with 3M KOH)	136g/L	0.25	S	32 ppm
			P	15.5 ppm
Iron (Fe-EDTA sodium salt)	7.342g/L	0.5	Fe	0,56 ppm
Minors:		0.5		
H_3BO_3	2.86g/L		B	0.25 ppm
$MnCl_2 \times 4H_2O$	1.81g/L		Mn	0.25 ppm
$ZnSO_4 \times 7H_2O$	0.22g/L		Zn	0.025 ppm
$CuSO_4$	0.051g/L		Cu	0.01 ppm
$H_3MoO_4 \times H_2O$	0.09g/L		Mo	0.005 ppm

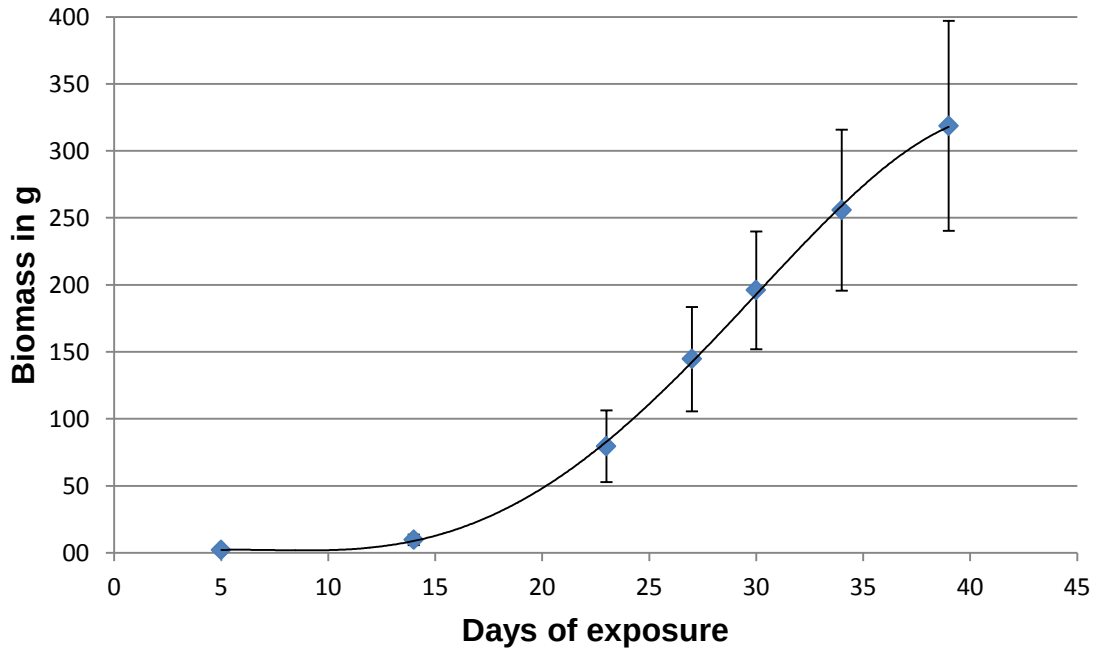


Figure S2: Biomass development during exposure time.

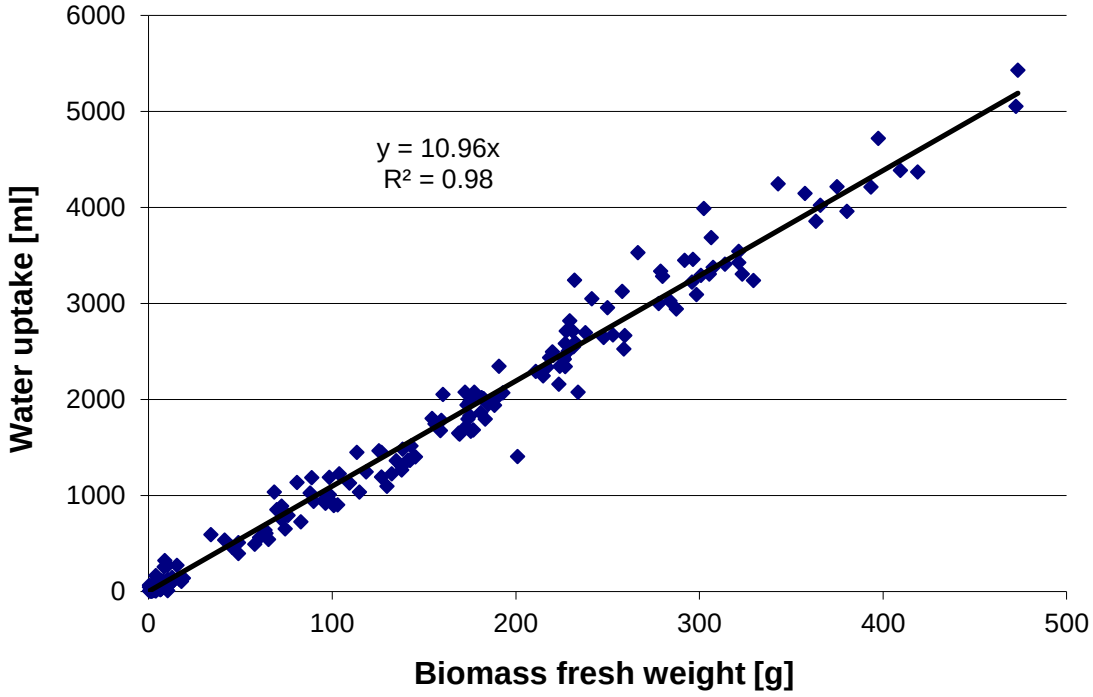


Figure S3: Correlation of water uptake in ml to biomass in g during exposure time.

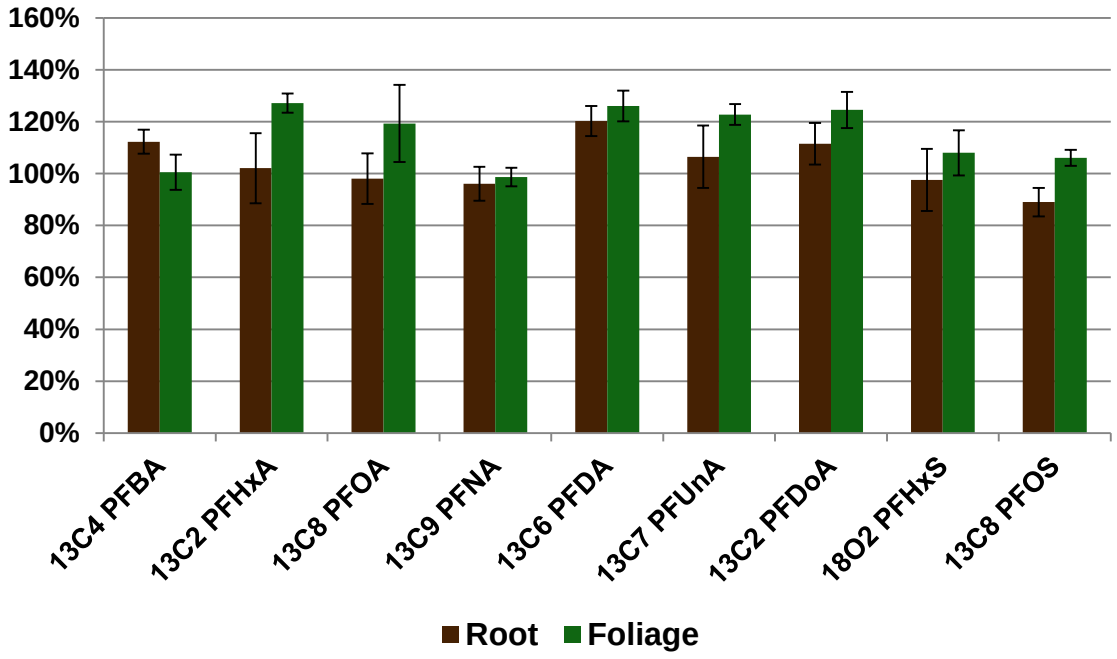


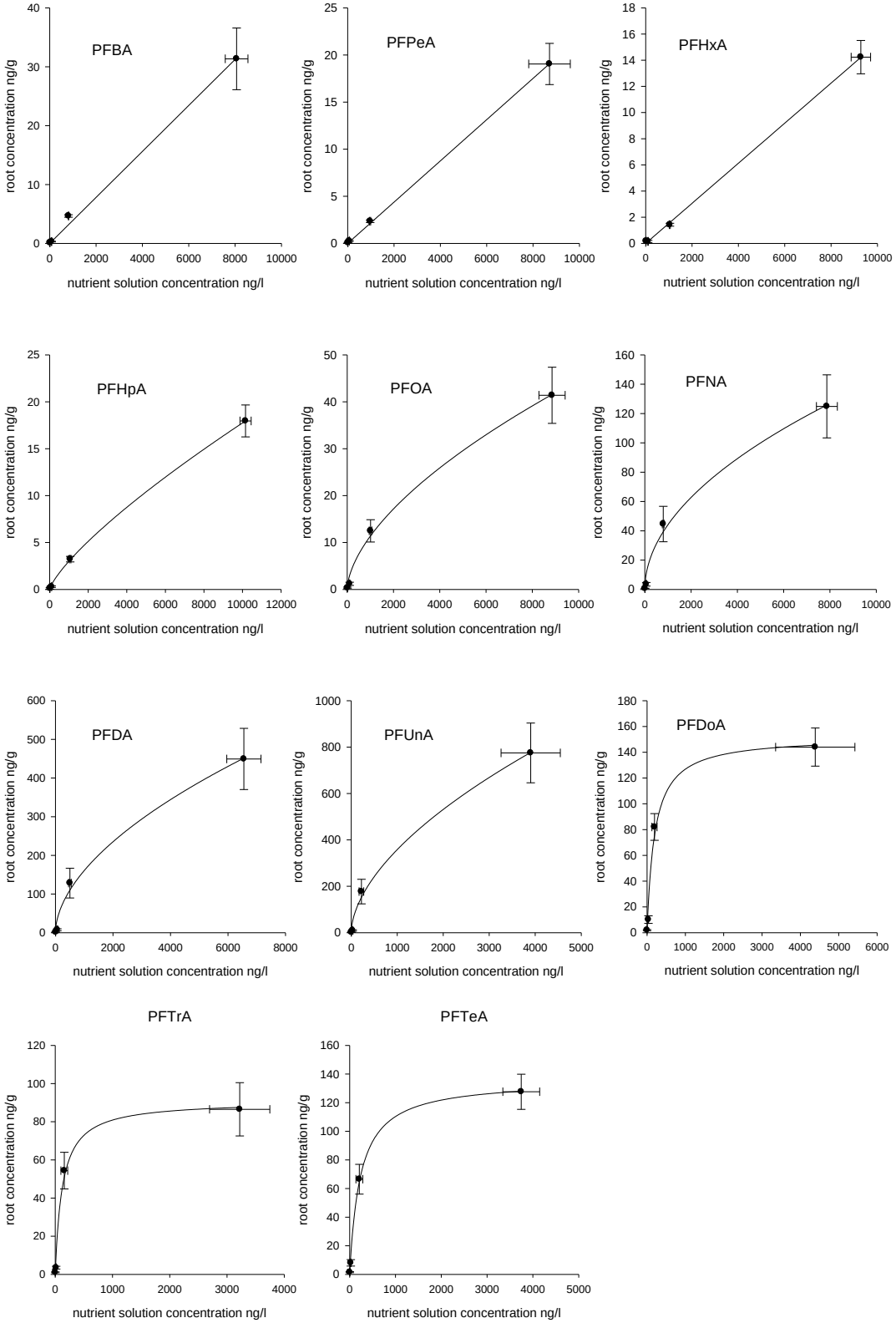
Figure S4: Ionization enhancement and/or suppression for the internal standards added to purified root and foliage extracts. Matrix effects are expressed as percentage in relation to signal area response of solvent-based, matrix free, internal standard solution (100% = no matrix effect). Error bars denote standard deviation (n=5).

Table S4: Limits of quantitation (LoQ) and detection (LoD) in lettuce foliage and root samples, as well as in nutrient solution. Values in ng/g fresh weight and ng/l.

	Foliage		Roots		Water	
	LoQ	**LoD**	**LoQ**	**LoD**	**LoQ**	**LoD**
PFBA	0,056	0,027	0,113	0,054	0,70	0,21
PFPeA	0,006	0,003	0,013	0,006	0,70	0,21
PFHxA	0,026	0,011	0,052	0,021	0,70	0,21
PFHpA	0,013	0,006	0,027	0,012	0,70	0,21
PFOA	0,016	0,008	0,031	0,016	0,70	0,21
PFNA	0,023	0,009	0,045	0,018	0,70	0,21
PFDA	0,023	0,010	0,045	0,020	0,17	0,05
PFUnA	0,018	0,007	0,036	0,015	0,17	0,05
PFDoDA	0,018	0,007	0,037	0,015	0,17	0,05
PFTrDA	0,015	0,006	0,031	0,013	0,17	0,05
PFTeDA	0,016	0,007	0,033	0,013	0,17	0,05
L-PFBS	0,023	0,008	0,045	0,017	0,17	0,05
L-PFHxS	0,028	0,009	0,055	0,019	0,17	0,05
L-PFOS	0,032	0,011	0,064	0,022	0,50	0,15
br-PFOS	0,002	0,001	0,004	0,001	0,13	0,04

Table S5: Recoveries of the spiked concentration of PFAAs in the nutrient solution in percentage of the nominal concentration

	Present study	*(Reth et al. 2011)*
PFBA	97	-
PFPeA	84	-
PFHxA	103	101
PFHpA	105	90
PFOA	96	85
PFNA	79	91
PFDA	65	59
PFUnA	44	22
PFDoDA	42	<7
PFTrDA	28	-
PFTeDA	32	<15
PFBS	74	-
PFHxS	94	83
PFOS	69	57


PFBA
root concentration ng/g
nutrient solution concentration ng/l
PFPeA
root concentration ng/g
nutrient solution concentration ng/l
PFHxA
root concentration ng/g
nutrient solution concentration ng/l
PFHpA
root concentration ng/g
nutrient solution concentration ng/l
PFOA
root concentration ng/g
nutrient solution concentration ng/l
PFNA
root concentration ng/g
nutrient solution concentration ng/l
PFDA
root concentration ng/g
nutrient solution concentration ng/l
PFUnA
root concentration ng/g
nutrient solution concentration ng/l
PFDoA
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PFTeA
root concentration ng/g
nutrient solution concentration ng/l

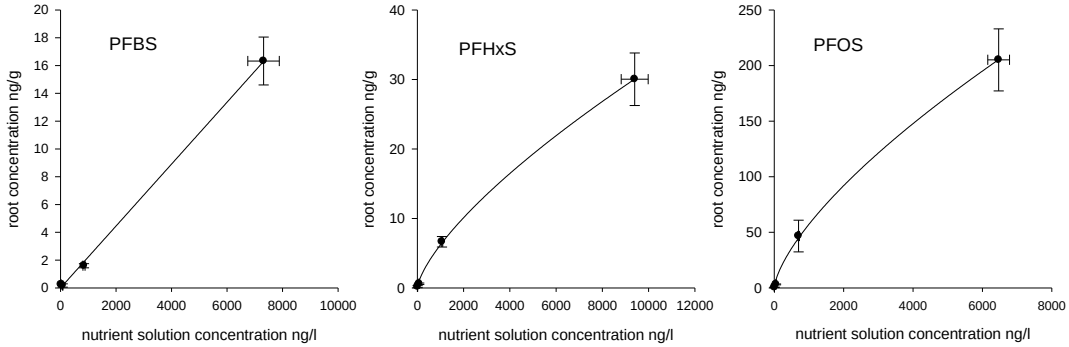


Figure S5: Root concentration isotherms of all PFAA used in the study. Error bars denote standard error. Linear regression line for PFBA, PFPeA, PFHxA and PFBS, Freundlich regression line for PFHpA, PFOA, PFNA, PFDA, PFUnA, PFHxS and PFOS, Langmuir regression line for PFDoDA, PFTrDA and PFTeDA.

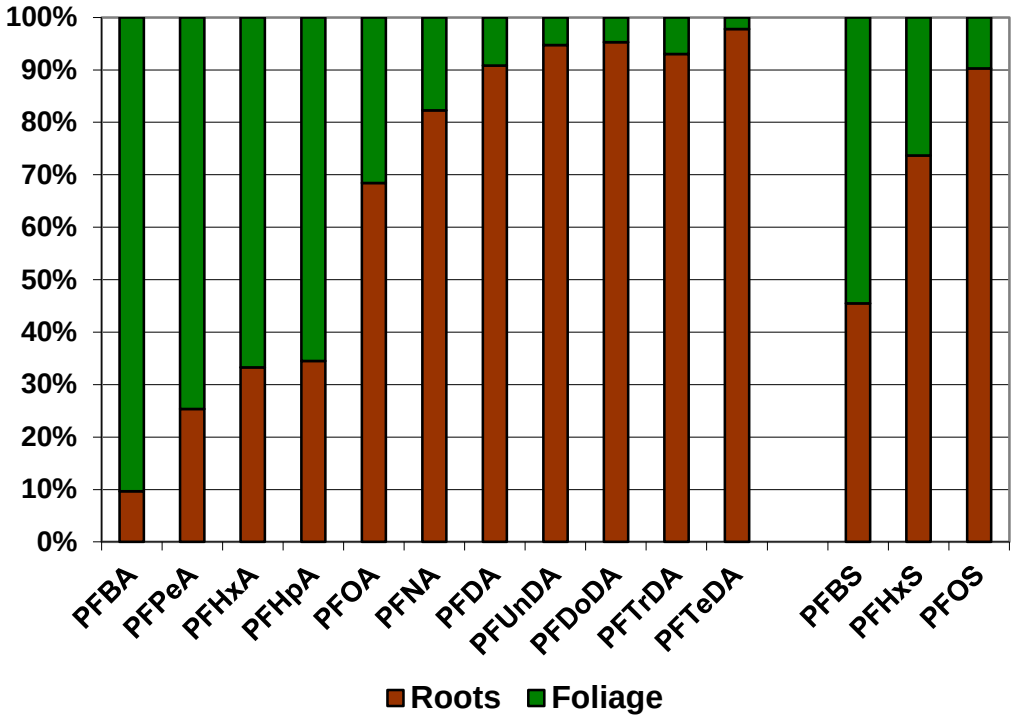


Figure S6: Mass distribution of PFAAs in foliage and roots in percent of the whole amount in the plant.

Table S6: Average concentrations found in the nutrient solution (ng/L), roots and foliage (ng/g) with standard deviation

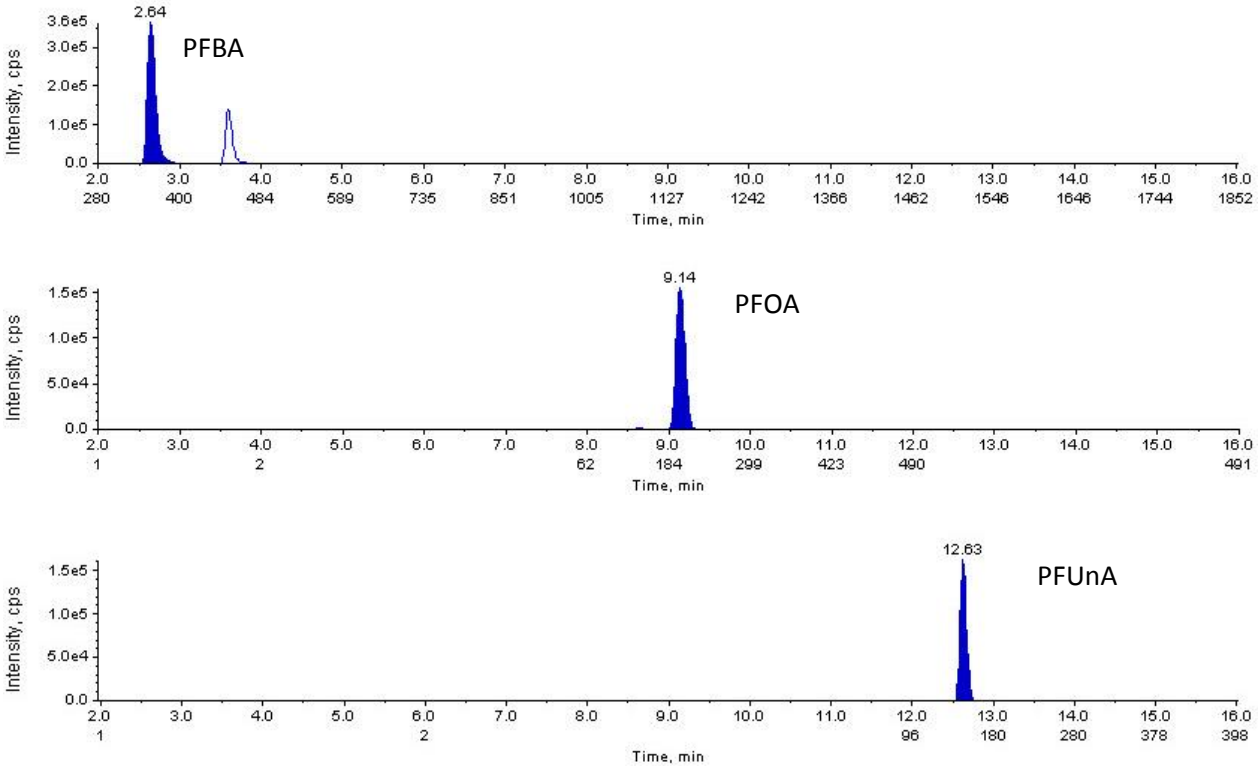
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
Nutrient solution								
Control	3.8 ± 1.6	<0.7	<0.7	<0.7	<0.7	<0.7	<0.17	0.18 ± 0.14
10 ng/L	12.8 ± 1.3	11.0 ± 0.8	15.1 ± 0.8	14.2 ± 1.1	13.4 ± 4.1	8.5 ± 0.8	5.8 ± 1.4	2.5 ± 0.8
100 ng/L	84 ± 19	71.7 ± 3.2	104 ± 25	105 ± 17	107 ± 32	64 ± 15	65 ± 30	32 ± 15
1000 ng/L	815 ± 20	969 ± 53	1042 ± 60	1067 ± 95	1010 ± 136	795 ± 96	504 ± 115	223 ± 126
10 µg/L	8069 ± 1198	8710 ± 2193	9284 ± 1025	10158 ± 709	8853 ± 1386	7866 ± 1103	6553 ± 1459	3901 ± 1579
Root								
Control	<0.113	<0.013	0.10 ± 0.08	0.17 ± 0.14	0.22 ± 0.19	0.3 ± 0.25	0.55 ± 0.39	0.53 ± 0.35
10 ng/L	<0.113	0.08 ± 0.06	0.14 ± 0.12	0.17 ± 0.10	0.50 ± 0.36	0.95 ± 0.40	2.1 ± 0.9	2.4 ± 0.9
100 ng/L	0.48 ± 0.14	0.26 ± 0.13	0.30 ± 0.21	0.47 ± 0.19	1.41 ± 0.83	3.8 ± 3.0	8.2 ± 6.0	10 ± 7
1000 ng/L	4.83 ± 0.51	2.35 ± 0.32	1.57 ± 0.27	3.4 ± 0.7	12.7 ± 5.8	45 ± 30	129 ± 94	178 ± 130
10 µg/L	31.5 ± 12.8	19.0 ± 5.4	14.4 ± 3.1	18.1 ± 4.2	41.6 ± 14.7	125 ± 53	450 ± 194	775 ± 316
Foliage								
Control	0.12 ± 0.01	<0.006	<0.026	0.03 ± 0.01	0.017 ± 0.004	<0.023	0.03 ± 0.01	0.03 ± 0.01
10 ng/L	0.24 ± 0.02	0.06 ± 0.03	<0.026	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.04 ± 0.02
100 ng/L	0.82 ± 0.17	0.24 ± 0.08	0.10 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	0.12 ± 0.03	0.13 ± 0.04	0.09 ± 0.03
1000 ng/L	11.7 ± 2.4	3.36 ± 0.38	1.04 ± 0.21	1.02 ± 0.38	1.55 ± 0.61	1.93 ± 0.75	2.0 ± 0.7	1.3 ± 0.4
10 µg/L	84 ± 22	27.5 ± 6.7	10.3 ± 1.9	9.4 ± 1.6	13.9 ± 1.9	17.9 ± 3.5	20 ± 5	12 ± 4

Table S6: Continued

	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	lin-PFOS	Br-PFOS
Nutrient solution							
Control	0.24 ± 0.11	0.31 ± 0.25	<0.17	0.33 ± 0.13	<0.17	<0.5	<0.13
10 ng/L	2.1 ± 0.5	2.1 ± 0.8	3.2 ± 1.2	15.8 ± 2.6	10.9 ± 0.5	5.5 ± 0.8	2.6 ± 0.4
100 ng/L	35 ± 15	17 ± 11	24 ± 13	80 ± 24	79 ± 16	35 ± 8	16.5 ± 3.5
1000 ng/L	195 ± 167	159 ± 144	212 ± 183	845 ± 95	1062 ± 85	474 ± 40	228 ± 23
10 µg/L	4388 ± 2531	3222 ± 1288	3746 ± 984	7315 ± 1393	9399 ± 1426	4397 ± 538	2072 ± 241
Root							
Control	0.55 ± 0.40	0.40 ± 0.35	0.36 ± 0.36	0.13 ± 0.11	0.16 ± 0.13	0.24 ± 0.19	0.08 ± 0.06
10 ng/L	2.5 ± 1.1	1.5 ± 0.6	1.9 ± 0.9	0.13 ± 0.15	0.23 ± 0.16	0.61 ± 0.33	0.23 ± 0.23
100 ng/L	11 ± 7	3.9 ± 1.7	8.4 ± 5.5	0.35 ±0.29	0.81 ± 0.27	2.8 ± 0.7	0.71 ± 0.21
1000 ng/L	83 ± 25	55 ± 24	67 ± 25	1.78 ± 0.36	6.9 ± 1.9	37 ± 27	10.4 ± 7.6
10 µg/L	145 ± 36	87 ± 34	128 ± 30	16.5 ± 4.2	30 ± 9	162 ± 57	43 ± 15
Foliage							
Control	0.02 ± 0.01	0.035 ± 0.015	<0.016	<0.023	<0.028	<0.032	0.004 ± 0.001
10 ng/L	0.03 ± 0.02	0.032 ± 0.011	0.015 ± 0.004	0.03 ± 0.02	0.014 ± 0.003	0.016 ± 0.008	0.005 ± 0.002
100 ng/L	0.06 ± 0.02	0.048 ± 0.018	0.024 ± 0.009	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.013 ± 0.004
1000 ng/L	0.77 ± 0.21	0.39 ± 0.08	0.11 ± 0.03	0.54 ± 0.12	0.55 ± 0.14	0.50 ± 0.31	0.14 ± 0.09
10 µg/L	5.0 ± 1.6	1.27 ± 0.36	0.30 ± 0.12	4.5 ± 1.3	5.8 ± 2.0	9.6 ± 4.2	2.2 ± 1.0

Table S7: Comparison of measured log k_0 values (de Voogt et al. 2012) with log K_{OW} values by two different models by Arp et al.(Arp et al. 2006), Kelly et al.(Kelly et al. 2009) and Wang et al.(Wang et al. 2011), and log $P^{0'}$ values by Jing et al. (Jing et al. 2009).

	log k_0	Calculated log K_{OW}				log $P^{0'}$
method	HPLC	COSMO-therm		Sparc		Voltammetry
ref	de Voogt	Arp	Wang	Arp	Kelly	Jing
PFBA	1.83	-	2.82	-	-	-0.68
PFHxA	2.88	3.26	3.42	3.12	-	0.54
PFHpA	3.44	3.82	4.06	3.82	2.8	1.15
PFOA	4.22	4.3	4.67	4.59	3.6	1.76
PFNA	4.93	4.84	5.30	5.45	4.5	2.37
PFDA	5.73	5.3	6.50	6.38	5.4	2.98
PFUnA	6.22	5.76	7.15	7.4	6.4	3.59
PFDoDA	7.02	-	7.77	-	7.1	4.20
PFBS	2.32	-	3.90	-	-	-
PFHxS	3.63	-	5.17	-	-	-
PFOS	5.02	5.25	6.43	5.26	4.3	2.57



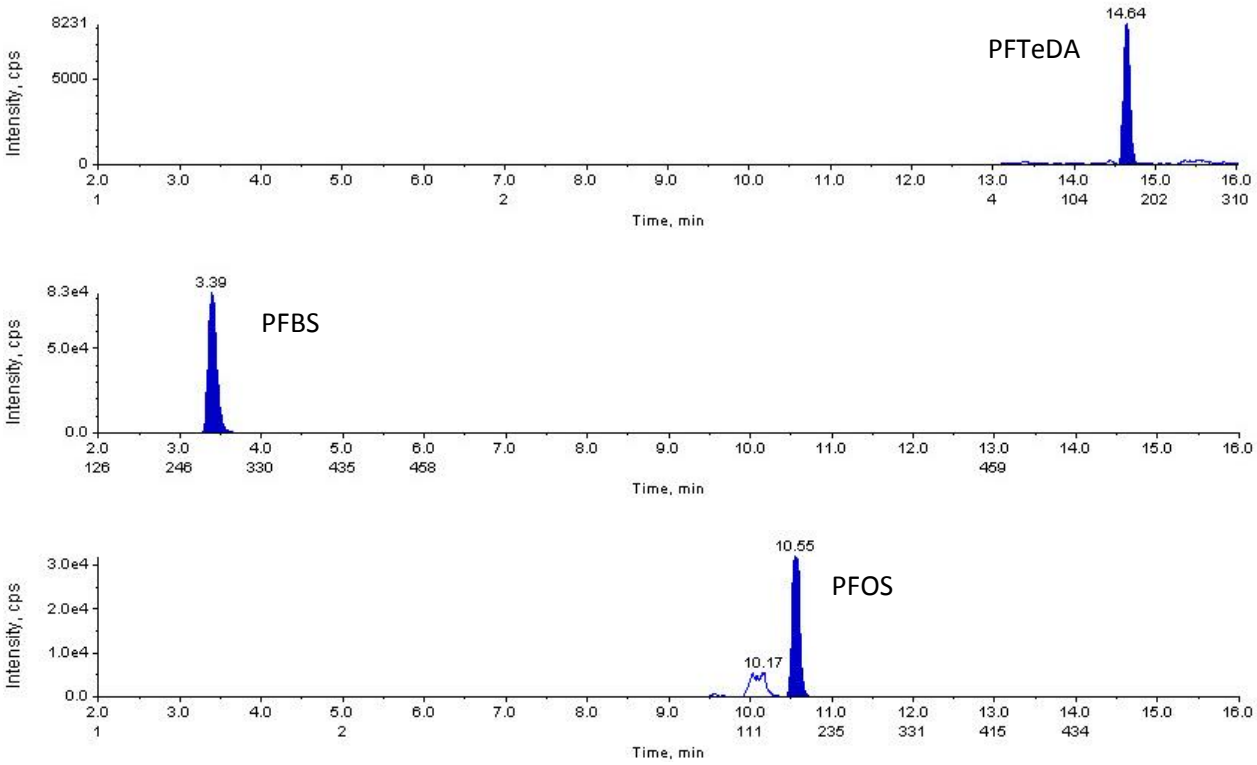


Figure S7: Example chromatograms with manual integration of peaks of PFBA, PFOA, PFUnA, PFTeDA, PFBS and PFOS

Chapter 3

ROOT UPTAKE AND TRANSLOCATION OF PERFLUORINATED ALKYL ACIDS BY THREE HYDROPONICALLY GROWN CROPS

Felizeter, S., McLachlan, M.S., De Voogt, P.

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Abstract

Tomato, cabbage and zucchini plants were grown hydroponically in a greenhouse. They were exposed to 14 perfluorinated alkyl acids (PFAAs) at four different concentrations via the nutrient solution. At maturity the plants were harvested, and the roots, stems, leaves, twigs (where applicable), and edible parts (tomatoes, cabbage head, zucchinis) were analyzed separately. Uptake and transfer factors were calculated for all plant parts to assess PFAA translocation and distribution within the plants. Root concentration factors were highest for long-chain PFAAs (>C11) in all three plant species, but these chemicals were not found in the edible parts. All other PFAAs were present in all above-ground plant parts, with transpiration stream concentration factors (TSCFs) of 0.05-0.25. These PFAAs are taken up with the transpiration stream and accumulate primarily in the leaves. Although some systematic differences were observed, overall their uptake from nutrient solution to roots and their further distribution within the plants were similar between plant species and among PFAAs.

Keywords: *PFAA, root uptake, translocation, crops, hydroponic, plants, PFOA, PFOS*

Mass distribution of PFAAs in Cabbage

Compound	Roots	Stem	Head	Leaf
PFBA	6%	4%	22%	67%
PFPeA	10%	3%	33%	53%
PFHxA	19%	3%	17%	62%
PFHpA	24%	1%	1%	74%
PFOA	39%	1%	1%	59%
PFNA	63%	1%	0%	35%
PFDA	79%	2%	0%	19%
PFUnA	91%	2%	0%	6%
PFDoA	97%	2%	0%	2%
PFTrA	99%	0%	0%	0%
PFTeA	100%	0%	0%	0%
PFBS	20%	1%	1%	78%
PFHxS	38%	1%	1%	61%
Br-PFOS	64%	2%	0%	34%
L-PFOS	82%	1%	0%	16%

Abstract Art

Introduction

Perfluorinated Alkyl Acids (PFAAs) have been used for decades in a variety of industrial and commercial products, such as coatings for textiles and papers or firefighting foams, due to their water- and oil-repellent properties combined with their stability (Kissa 2001b; de Voogt et al. 2006b). However, some of these properties also contribute to making PFAAs problematic environmental contaminants. They are not known to degrade in the environment (Liu et al. 2013) and thus can be found ubiquitously in, for instance, surface water and wildlife (Giesy et al. 2001; Giesy et al. 2002; Prevedouros et al. 2006). PFAAs have been detected in human blood and breast milk (Volkel et al. 2008; Karrman et al. 2010; Antignac et al. 2013; Barbarossa et al. 2013), which is of concern because some PFAAs have been proven or are suspected to have adverse effects on human and animal health (Lau et al. 2007; Domingo 2012; Saikat et al. 2013). Little is known so far about how humans are exposed. Exposure risk assessment identified the human diet to be one of the major sources of PFAAs in the human body (Fromme et al. 2009; D'Hollander et al. 2010a; Noorlander et al. 2011; Domingo et al. 2012). Although a considerable amount of data is available on PFAA concentrations in and sources to drinking water (Skutlarek et al. 2006; Ericson et al. 2009; Eschauzier et al. 2012b) and aquatic organisms (Moody et al. 2002b; Haug et al. 2010b; Houde et al. 2011), little research has been done so far on crops.

Food analysis studies (Gem 2006; Ericson et al. 2008; Noorlander et al. 2011) and a screening study of vegetables in Europe by Herzke et al. (Herzke et al. 2013) have shown that crops are contaminated with PFAAs, whereby concentrations in different vegetable subgroups (e.g., leafy vegetables or bulb vegetables) were observed to be similar. In a recent dietary exposure study by Klenow et al. (Klenow et al. 2013) vegetables were identified to be the most important food categories for exposure to PFHxA and PFOA, with up to 69% of the total exposure coming from vegetables, depending on the location. Furthermore, studies with cattle have shown that PFAAs can be taken up by cattle from feed, thus leading to a secondary exposure pathway from contaminated crops to humans via dairy products and meat (Kowalczyk et al. 2013; van Asselt et al. 2013). One possible source of PFAAs in crops is contaminated soil. Stahl et al. grew several crops (mainly cereals) in soil spiked with perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Stahl et al. 2009). They found a concentration-dependent uptake into plant tissues and higher concentrations in the straw of the plants than in the storage organs (e.g., tubers, ears). Roots and leaves were not analyzed in their study. Lechner and Knapp, who investigated the carryover of PFOS and PFOA from soil to carrots, potatoes and cucumbers, also found higher concentrations in the vegetative parts of the plant than in the storage organs. (Lechner et al. 2011). They also confirmed the sorption to the potato tuber peel that Stahl et al. reported. Yoo et al. investigated the uptake of PFAAs in grass growing on soil that had been amended with biosolids containing PFAAs, and found an exponential decrease of grass/soil accumulation factors with increasing carbon chain length (Yoo et al. 2011). Two other soil-related studies have been published by Stahl et al. (Stahl et al. 2013) and Wen et al. (Wen et al. 2014). Both studies focus on cereals. Stahl et al. investigated the leaching of the compounds in their lysimeter study over 5 years with repeated growing of cereal plants. However, no transfer factors and no data on soil properties were presented in their study. Wen et al. grew wheat on biosolid-amended fields and calculated transfer factors (TFs). They found the highest TFs for the roots for all compounds and decreasing TFs with increasing carbon chain length. In a previous study we investigated the root uptake and translocation of PFAAs to foliage in hydroponically grown lettuce

(Felizeter et al. 2012). PFAAs were retained mostly in the roots, with the exception of the short-chain compounds. Root/nutrient solution concentration factors were highest for the long-chain PFAAs, whereas foliage/root concentration factors were lowest, and it was concluded that sorption to root surface tissue was the major uptake mechanism for the long-chain PFAAs. Recently some more mechanistic uptake studies have been published, investigating the influence of abiotic parameters, such as pH-values or temperature (Zhao et al. 2013; Krippner et al. 2014), and investigating the influence of metabolic inhibitors on the uptake (Wen et al. 2013). These studies have been conducted with maize and wheat plants. Although these studies provide some initial insights into plant uptake of PFAAs from soil, there is still limited mechanistic understanding of the uptake, how it is influenced by the properties of the PFAAs and the characteristics of the plants, and how the PFAAs are distributed between different plant tissues.

The goal of the present study was to investigate the uptake of PFAAs and their distribution in different plant parts. To that end three crops were grown hydroponically in a greenhouse. A hydroponic system was used so that the water uptake could be readily monitored and the bioavailable concentrations of the PFAAs in the root zone could be directly measured. Further advantages are the controlled supply of nutrients, which together with the controlled light conditions ensures an optimal growth. A limitation of hydroponic studies is that they do not include the effect of soil on modulating chemical availability. A further drawback is that the nutrient solution has to be renewed regularly, which can lead to variability in exposure concentration for surface active chemicals like PFAAs.

The crops were chosen to represent a variety of physiology. Tomato is a fruit bearing crop of economic importance. Zucchini is another fruit bearing crop belonging to the family of Cucurbitaceae, which has been shown to have a unique capability to transfer some hydrophobic organic contaminants from roots to shoots (Huelster et al. 1994; Gent et al. 2007). Cabbage is an important crop of which part of the leaves (the cabbage head) is eaten, and leaves were hypothesized to be a plant tissue that would amass high concentrations of short-chain PFAAs. A set of 11 perfluorinated carboxylic acids (PFCAs; carbon chain length from C4 to C14) and 3 perfluorinated alkyl sulfonates (PFSAs; C4, C6, and C8) was selected to provide a broad foundation for assessing the influence of chemical properties on PFAA uptake and distribution. When harvested, the plants were divided into roots, stems, leaves, and edible parts (tomatoes, zucchinis, and cabbage heads) to explore PFAA distribution within the plant. In tomato and zucchini, the stem tissue was further divided into the main stem (called “stem”) and the tissue connecting the main stem to the leaves (“twigs”) to provide more spatial resolution in the study to the transport of the PFAAs from the roots to the above-ground plant parts.

Materials and methods

Chemical reagents and laboratory materials. All chemicals used in this study were of the highest quality and purity available. The abbreviations, suppliers and purities of the chemicals can be found in Table S1 of the Supporting Information (SI).

Materials used for extraction and clean-up of the samples included Florisil SPE cartridges (1000 mg, 6 mL) from Applied Separations (Allentown, PA, USA); Oasis WAX 3cc SPE cartridges (60 mg) from Waters (Wexford, Ireland) and Supelclean ENVI-Carb 120/140 from Supelco (Bellefonte, USA).

Polypropylene (PP) tubes (50 and 15 mL) with screw caps were purchased from Sarstedt (Nümbrecht, Germany); PP vials (2.0 and 0.3 mL) were purchased from VWR International (Amsterdam, Netherlands). Acrodisc LC13 GHP Pall 0.2 µm filters were obtained from Pall Corp. (Port Washington, NY, USA). The 10 L PP buckets were acquired from Harcotom (Purmerend, Netherlands).

Plant culture and exposure experiments. The uptake study was performed in a greenhouse (14 h of light). The plants were pregrown in soil until the seed leaves (cotyledons) were fully developed (BBCH stage 10). The soil was carefully washed off before the plants were transferred to the hydroponic system, where only the roots of the plants were exposed to the nutrient solution. The system is described in more detail in our previous study (Felizeter et al. 2012). Tomato (*Solanum lycopersicum* var. *moneymaker*) plants were grown in hydroponic solutions with nominal concentrations of 10, 100, 1000 and 10,000 ng/L for each PFAA. Because the highest spiked concentration for tomatoes resulted in very high concentrations in plant parts, the zucchini (*Cucurbita pepo* var. *black beauty*) and cabbage (*Brassica oleracea* convar. *capitata* var. *alba*) plants were exposed to 10, 100, 500 and 1000 ng/L. For each plant a 10 L bucket was filled with 8 L of Hoaglands nutrient solution (see Table S2 in the SI for composition) and spiked with 100 µL/L of the respective PFAA stock solution. Six replicates per concentration were used for cabbage and tomato, while only four replicates could be used for Zucchini due to their size. Two plants of each species were grown in unspiked nutrient solution as blank controls, and two pots without plants were used for evaporation measurements.

The plants were randomly distributed in the greenhouse room, and plant growth and water uptake were determined by weighing. The zucchini and tomato plants eventually grew too big to be moved, so no new randomization of the plant distribution was possible from then on. Furthermore, the tomato plants grew too big to be supported by the available equipment and had to be cut back from time to time. Secondary shoots were continuously removed as well. Thus, for tomato only the lower plant parts were analyzed. Pruning of tomato side shoots is a common practice and was done as soon as the secondary shoots were noticeable. The trimming of the top part of the plants was necessary and done for all plants at the same height, so that individual differences between plants were minimized. In total, the cutoff biomass for each plant was much less than the total biomass of the plants at the end of the experiment. We do not expect that the pruning and trimming had an influence on the uptake of PFAAs.

During the experiment the nutrient solution needed to be renewed several times due to the water uptake of the plants. This was done by replacing the buckets with new buckets containing freshly prepared spiked nutrient solution. Differences between the uptake of water and the uptake of PFAAs meant that the PFAA concentrations changed somewhat during the experiment. With decreasing water levels in the buckets, the PFAA concentrations in the solutions can significantly increase. We describe below how we dealt with these complications. On average the tomato plants took up 46.7 L of the nutrient solution, the cabbage plants 24.6 L, and the zucchini plants 41.4 L.

Tomato and zucchini fruits were harvested when they were ripe. Other plant parts were collected for analysis only after the experiment was stopped. Cabbage plants were divided into roots, stem, leaves and head. Tomato and zucchini plants were divided into roots, stem, twigs, leaves and fruits. Table S3 in the SI lists start and end dates of the growth experiment as well as dates of renewal of the nutrient solution and harvest dates of tomato and zucchini fruits. Samples were stored at -20°C until extraction. No differences in plant growth were observed between the different spiking levels, and

there were no visible effects of the compounds on the plant health (discoloring, spots). Not all of the cabbages survived until the end of the experiment, but at least three plants of each concentration survived. In total, 7 of 26 cabbage plants were lost. Cabbage survival was independent of the spiked concentration, and fatalities were most likely caused by the temperature in the greenhouse. The optimal temperature range for cabbage is between 15 and 20°C. Temperatures above 25°C, which were experienced for several days during the cabbage growth phase, can lead to inhibited growth and dropping of the outer leaves, which was observed for all cabbage plants in the experiment.

Extraction. After the plant samples had been washed with demineralized water, the material was dried superficially and homogenized with a household blender (Braun Multiquick MX 2050). The extraction method used is based on the method of Vestergren et al. (Vestergren et al. 2012), which is a modification of the method published by Hansen et al (Hansen et al. 2001). Briefly, 10 g of the homogenate was weighed in a 50 mL PP tube and spiked with mass-labeled surrogate standards. After 5 mL of 0.4M NaOH solution was added and vortex-mixed, the samples were left in the fridge over night to allow the internal standards to distribute in the plant matrix. Next, 4 mL of 0.5M TBA solution and 5 mL of a carbonate buffer (0.25M Na_2CO_3/$NaHCO_3$) were added to the samples and thoroughly mixed. After adding 10 mL MTBE and vortex-mixing for 1 min the samples were sonicated for 10 min. Phase separation was achieved by centrifuging for 10 min at 3000 rpm. The MTBE phase was transferred to a new 50 mL PP tube and the extraction repeated two times. The extracts were combined and concentrated to approximately 2 mL using a Rapidvap (Labconco Corp., Kansas City, MO, USA). Florisil SPE-cartridges were prepared with 1 g sodium sulfate on top and conditioned with 10 mL MeOH and 10 mL MTBE before they were loaded with the extract. The elution of the non-polar matrix was done with 10 mL MTBE before the target compounds were washed off the cartridge with 10 mL MeOH/MTBE (30:70, v:v). This extract was again evaporated with the Rapidvap to 1 mL final volume. An additional clean-up step following the Powley method with ENVI-Carb was added when the final extract was still strongly colored (Powley et al. 2005).

Nutrient solution samples were extracted with Oasis WAX-SPE cartridges, except the samples from the 10 µg/L variant for tomato, which were directly injected. Between 20 and 150 mL of sample, depending on the nominal concentration, were spiked with internal standards and passed through the cartridges, which had been conditioned with 2 mL 0.1% NH_4OH in MeOH (v:v) and 3 mL H_2O. The loading speed was set to not exceed 2 drops per second. After washing the loaded cartridges with MeOH:H_2O (40:60, v:v), they were dried under vacuum before the PFAAs were eluted with two times 500 µL of 2% NH_4OH in MeOH (v:v).

All final extracts were passed through an Acrodisc LC 13 GHP Pall nylon filter into 2 mL PP vials and stored at 4 °C until analysis.

Analysis. The analysis was performed on an HPLC system (LC-20AD XR pump, SIL-20A autosampler, and SCL-10A VP system controller, Shimadzu, Kyoto, Japan) coupled with a tandem mass spectrometer (4000 QTrap, Applied Biosystems, Toronto, Canada). The HPLC was equipped with a precolumn (Pathfinder 300 PS-C_{18} column, i.d. 4.6 mm; length 50 mm; particle diameter 3 µm; Shimadzu, Duisburg, Germany) prior to the injection valve to remove potential background contamination. An ACE 3 C18-300 column (i.d. 2.1 mm; length 150 mm; particle diameter 3 µm; Advanced Chromatography Technologies, Aberdeen, Scotland) was used for separation and

maintained at 30°C. The mass spectrometer was equipped with an electrospray ionization interface, operating in the negative ionization mode, and was run in the scheduled MRM-mode.

The purified extracts were diluted 1:1 with H_2O prior to analysis to match the initial composition of the mobile phase of the HPLC. A volume of 20 µL was injected. The mobile phase consisted of two eluents, A (40:60 MeOH:H_2O, v:v) and B (95:5 MeOH:H_2O; v:v; both with 2 mM ammonium acetate). The gradient used for separation and the mass transitions as well as other mass spectrometer settings can be found in the Supporting Information. Raw data was processed with Analyst software 1.5 (Applied Biosystems).

Quality assurance and control. Repeated extraction of a sample showed that the standard deviation of the concentrations between extractions was less than 10% for all analytes (n=5, see Table S5 in the SI). Consequently, the samples were extracted once and injected in duplicate.

The concentrations were quantified using a 12-point calibration with fitted correlation lines that had an R^2 value of >0.99 for all analytes (no weighting was applied).

Our laboratory also participated successfully in various interlab studies (see, e.g., (Weiss et al. 2013)). For further information on quality assurance and quality control measures, see also our previous study (Felizeter et al. 2012).

In this study the average recovery of the internal standards was between 48% (PFBA) and 97% (PFDoDA). Recoveries were determined by comparison with matrix extracts spiked with mass-labeled standards prior to injection. Interestingly, some matrices affected the signal of the compounds quite intensively. Cabbage head and zucchini roots, for example, reduced the PFBA signal by up to 80%. Signal enhancement on the other hand was at most 12%. See Table S6 and S7 in the Supporting Information for detailed information on recoveries and matrix effects for all compounds and all matrices.

Limits of quantification (LoQs) (Table S8 in the SI) were calculated on the basis of the lowest validated calibration standard (Signal to noise ratio ≥10). The LoQ was derived from the analyte mass injected scaled up to an extract volume of 1 ml and divided by the average extracted sample quantity for the matrix. Method blanks were prepared repeatedly with the same extraction procedure as the samples, but showed no quantifiable contamination. Solvent blanks were injected every ten injections to check for contamination from the LC system and for memory effects, but no contamination or memory effects were observed.

Background concentrations were taken from plants growing in non-spiked nutrient solution (n=2), and used to correct the PFAA concentrations found in spiked experiments by subtraction. Any resulting concentrations below the LoQ were neglected.
The non-branched (further referred to as 'linear PFOS (L-PFOS)') and branched isomers of PFOS were quantified separately, assuming equal response factors for branched and non-branched isomers. Branched isomers for other PFAAs were also observed but they were not quantified.

Data analysis. Uptake factors calculated from the data were evaluated for outliers using box-plots with SigmaPlot (Systat Software, Inc, Chicago, US.). Outliers and values below LoQ were not included

in data interpretation. Statistical analysis of the data was performed using IBM SPSS 21 (IBM, Armonk, US). One-way analysis of variance (ANOVA) in combination with the Tukey test was conducted to assess the significance of differences between spiking levels and plant species. *t* tests were performed to evaluate the differences between compounds. All statements regarding differences in this study are based on a significance level of $p<0.05$.

Results and discussion

Because there is no evidence for microbial, physical or any other degradation of PFAAs, we consider the breakdown of the PFAAs to have been negligible in all matrices analyzed.

PFAA concentrations in nutrient solution. With increasing water uptake the nutrient solution in the hydroponic system was depleted. When there was only a small volume left, high PFAA concentrations were measured that frequently exceeded the nominal spiked concentrations. There are two possible explanations. (I) As described in our previous study (Felizeter et al. 2012) and also shown by Reth et al. (Reth et al. 2011), PFAAs accumulate at the air-water interface. This has been observed to be particularly pronounced for long-chain PFAAs. When only a small volume of water was left in the bucket, it was not possible to avoid sampling some surface water. This would have led to sampling of PFAAs that had accumulated at the water-air interface, which may have resulted in elevated concentrations in the sample. (II) The plants may take up water more efficiently than they take up PFAAs. This would have resulted in an enrichment of the compounds in the water and thus to higher concentrations with time.

For the tomato, zucchini, and cabbage plants, the nutrient solution volumes at the time of sampling went down to a minimum of 113 mL. To calculate uptake factors, we determined average PFAA concentrations in the bulk solution that the plants had been exposed to during the entire period of exposure. To interpolate the concentrations in time and between pots, the following equations were used to calculate the nominal and bulk PFAA concentrations for each day:

$$Cnom_{(t)} = \frac{(Cnom_{(t-1)} * V_{(t-1)}) - \left((V_{(t-1)} - V_{(t)} - V_{evap}) * f * Cbulk_{(t-1)}\right)}{V_{(t)}} \quad [1]$$

$$Cbulk_{(t)} = \frac{Cnom_{(t)} * V_{(t)}}{(V_{(t)} + K * A * 1000000)} \quad [2]$$

f is the effectiveness of the plant's uptake of the PFAA with the nutrient solution, *K* is the interface to bulk solution partition coefficient (m), *V* is the volume of nutrient solution (m^3), and *A* is the area of the air-nutrient solution interface in the bucket (m^2). The average bulk concentrations in the spike control pots were used as the initial values of C_{bulk} and (together with the nominal concentrations and *A*) to calculate *K*. For each PFAA and plant species, *f* was then fitted against the amount of PFAA taken up by the plant (i.e.,, *f* was chosen such that the median quotient of the predicted and measured mass of the PFAA in the plant was equal to 1). For tomato, where the total amount of PFAA taken up was not measured, we estimated the total amount by using the concentrations for the lower plant parts also for the mid and top plant parts. The resulting values for *f* for tomato were similar to the ones for cabbage and zucchini. The water concentrations calculated for the sampling days were quite close to the measured water concentrations. The daily concentrations estimated in this manner were used to calculate average bulk concentrations weighted with the water volume

transpired by the plants. These bulk concentrations were employed to calculate plant tissue concentration factors (see below).

Roots

The PFAA uptake in the roots was assessed using the root concentration factor (RCF). The RCF was defined as the ratio between the concentration of a compound in roots and its bulk concentration in the nutrient solution to which the roots were exposed (Trapp 2000):

$$\mathrm{RCF} = \frac{\text{Concentration in root (ng/g fresh weight (FW))}}{\text{Concentration in nutrient solution (ng/ml)}} \quad [3]$$

Over a broad concentration range, a linear relationship between exposure concentration and uptake of PFAAs was observed by Stahl et al. (Stahl et al. 2009) and in our previous study (Felizeter et al. 2012). We therefore averaged the RCFs from the different exposure concentrations. If the ANOVA-Tukey test showed that the RCF from a given exposure concentration was significantly different from all the other exposure concentrations, then this value was excluded from the calculation of the mean RCF. For tomato, PFDoDA, PFTrDA, and PFTeDA had significantly lower RCFs at the highest spiking level tested (10000 ng/L). This was not observed for cabbage and zucchini, which may be due to the lower maximum spiking level tested (1000 ng/L). We also observed lower RCF values for long-chain PFCAs (especially PFDoDA, PFTrDA and PFTeDA) in the 10000 ng/L spiking level in our lettuce study, where we concluded that the nonlinear uptake was caused by the nonlinear adsorption of the compounds to the root surface (Felizeter et al. 2012). For cabbage the lowest spiking concentration resulted in significantly higher RCFs for PFOA, PFNA, PFDA and Br-PFOS than for the other spiking concentrations. For zucchini the RCF for PFDA was significantly higher for the lowest spiking level than for other spiking levels. We have no explanation for these observations.

The RCFs for the PFCAs generally increased markedly with increasing chain length between PFBA and PFUnA and were quite similar for PFUnA through PFTeDA (Figure 1). The RCFs for the PFSAs generally increased with increasing chain length for all species. Different measures of hydrophobicity also increase with increasing chain length (SI Table S14), suggesting that the magnitude of root uptake may be determined by the hydrophobicity of the PFAA. However, for PFCAs with C > 11 the RCFs do not increase further, and hydrophobicity factors are not available for PFTrDA and PFTeDA, so it cannot be said with certainty that the root uptake is related to the hydrophobicity alone. The relationship between RCF and PFAA chain length is very similar to the relationship observed for lettuce in our previous study. The most pronounced difference is that lettuce showed a minimum RCF for PFHxA, whereas the three species studied here did not.

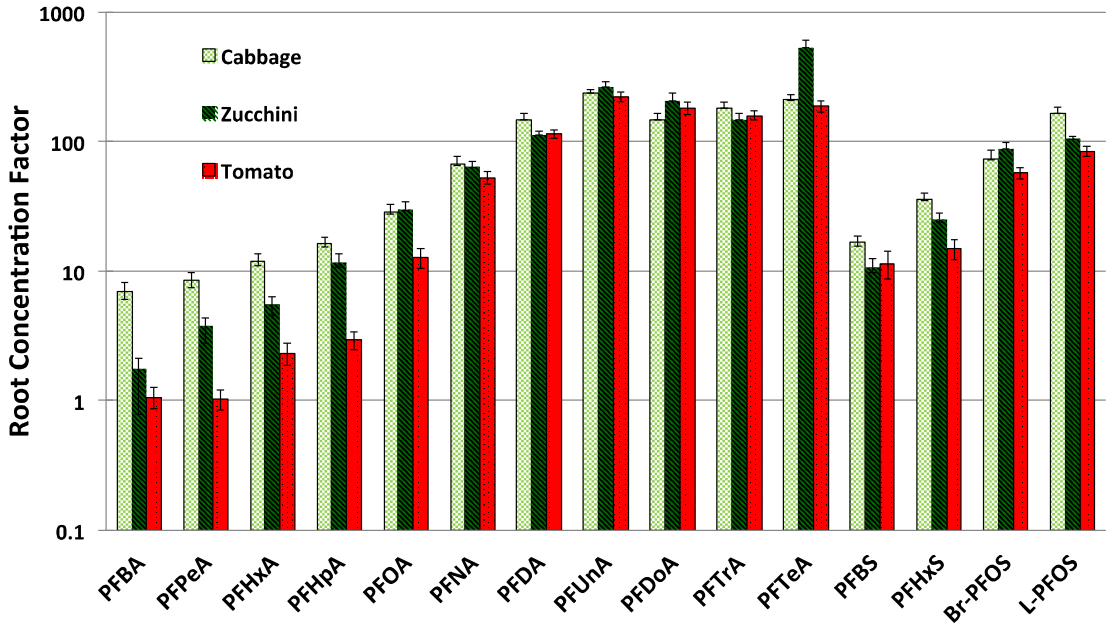

Figure 1: Root concentration factors (RCFs) for cabbage, zucchini and tomato [(ng·g^{-1})/(ng·ml^{-1})]. The mean RCFs from each spiking level were averaged, whereby outliers were excluded (see text). Note the logarithmic scale. The error bars denote the standard error.

For the lettuce study we discussed two mechanisms for root uptake: sorption to the tissue between the root surface and the Casparian strip and uptake across the Casparian strip into vascular root tissue. We concluded that for lettuce sorption to root surface tissue is the dominant process for root uptake of the long-chain PFCAs, whereas for the short-chain PFCAs and PFSAs uptake across the Casparian strip into the vascular root tissue might be equally or more relevant (Felizeter et al. 2012). Further support for this explanation can be found in the results presented below, which show that the stem concentration factors (SCFs) of PFDA are more than an order of magnitude less than the RCFs, even though PFDA has leaf concentration factors that are comparable to the short-chain PFCAs. Should uptake across the Casparian strip into vascular root tissue be the primary root uptake mechanism, one would expect the RCF and SCF to be similar if the vascular tissue in the roots and stem had a similar sorption capacity for PFDA. The fact that the SCF is much smaller suggests that the sorption capacity of the root vascular tissue must be much greater than the sorption capacity of the stem vascular tissue, or that sorption to the tissue between the root surface and the Casparian strip is an important root uptake mechanism for PFDA.

Above-ground plant parts.

The distribution of PFAAs in the plant was assessed using various concentration factors. Concentration factors for each plant part (stem, leaves, twigs for tomato and zucchini, and edible parts) relative to the nutrient solution were calculated in the same way as the RCF and named analogously stem concentration factor (SCF), leaf concentration factor (LCF), twig concentration factor (TCF) and edible part concentration factor (ECF). Additionally, concentration factors between plant parts (e.g., edible part/stem) were calculated to obtain insight into the translocation of the compounds within the plants.

Stem. All species had SCFs >1 for most of the compounds with the exception of PFBA, PFTrDA and PFTeDA for zucchini and PFHpA, PFTrDA and PFBS for cabbage. The SCF for PFTeDA for cabbage could

not be calculated because the concentrations in the stem were below the LoQ (Figure 2). SCF values > 1 denote an accumulation in the stem. The SCF values were in general much lower than the RCF values.

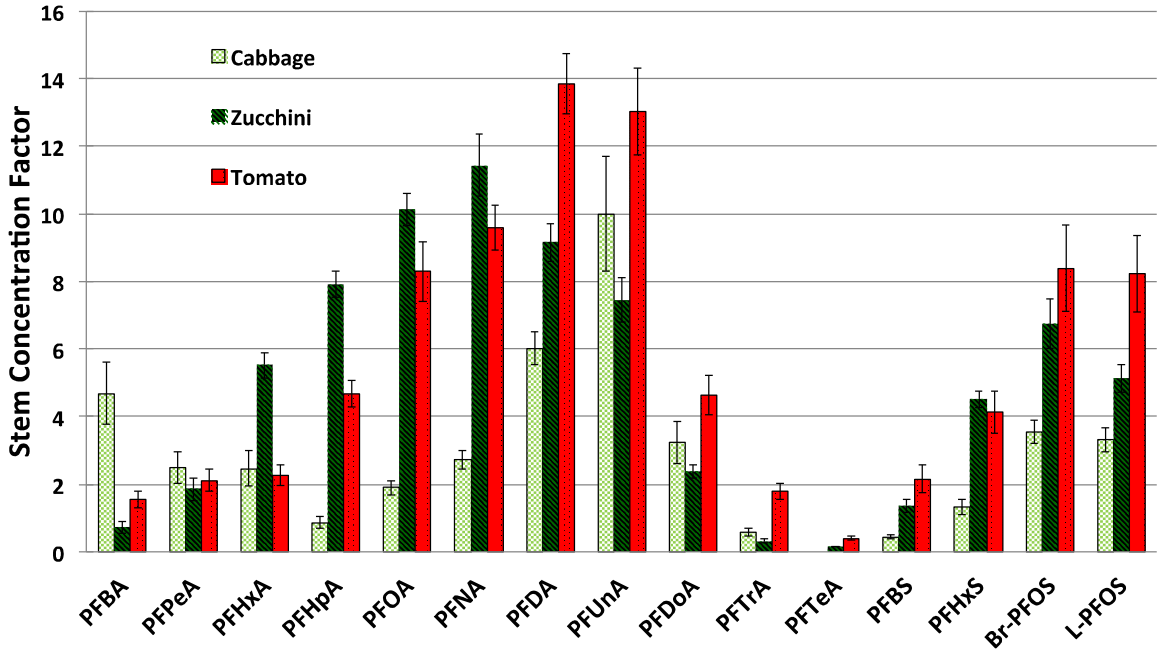

Figure 2: Stem concentration factors (SCFs) for cabbage, zucchini and tomato [[(ng·g-1)/(ng·ml-1)]. The mean SCFs from each spiking level were averaged. The error bars denote the standard error.

The magnitude of the SCF is expected to depend largely on (I) the amount of PFAA being delivered by the transpiration stream from the roots and (II) the balance between retention of the PFAA in stem tissue versus further translocation with the transpiration stream to twigs and leaves. Lower SCFs were observed for the short-chain PFAAs (Figure 2). An explanation could be that the short-chain PFAAs do not partition as strongly into the stem tissue as their longer chain analogues. This could result in them not being retained in the stem tissue to the same extent, but rather being translocated further into other plant tissues. The higher SCF values of the C8-C11 PFAAs on the other hand can likely be explained by comparatively stronger sorption of the compounds on the stem tissue, resulting in stronger retention in the stem tissue and less translocation to twigs and leaves. An increase in organic matter/water partition coefficients with increasing PFAA chain length has been reported, e.g.,, for sediment (Higgins et al. 2006). Also, experimentally determined hydrophobicity factors as well as modeled K_{OW} values have been observed to increase with increasing chain length (Arp et al. 2006; de Voogt et al. 2012) (SI Table S14). Therefore, it is not surprising that longer chain compounds are more retained in the stem tissue than short-chain compounds.

All three plant species showed a sharp decrease of the SCF for PFCAs with a chain length longer than C11. This is an indication that the translocation of these chemicals through the roots is considerably less efficient. One explanation could be restrictions on the ability of long-chain PFCAs to cross the Casparian strip. Another could be stronger partitioning of the long-chain PFCAs out of the xylem into the vascular tissue of the roots (see discussion under Roots above).
The low SCFs for the long-chain PFCAs also strengthen the argument for sorption to surface tissue being the dominant root uptake mechanism for these substances. Whereas the long-chain PFCAs had

very low SCFs, they had the highest RCFs. Consequently, only a very small portion of the long-chain PFCAs in the roots was translocated to the stem.

Twigs. Twigs are only present in tomato and zucchini, because the leaves of cabbage grow directly on the stem. The twigs of zucchini are actually elongated leaf stalks (petioles), but because of their size they were analyzed separately from the leaf. The relationship between TCF and PFAA chain length was quite similar to the pattern observed for SCF, with the exception of PFBA and PFBS for tomato, where TCFs were much higher than SCFs (SI Figure S2). Furthermore, the twig/stem concentration factors (SI Figure S3) were close to 1 for most of the PFAAs with the notable exceptions of PFBA (3.3 in zucchini and 4.0 in tomato) and PFBS (4.0 in tomato). The explanations given for the SCFs can also be applied here.

Leaves. The LCFs were >1 in all plant species for all PFAAs except the long-chain PFCAs (Figure 3). This shows that all three plant species translocate all PFAAs except the long-chain PFCAs to the leaves and accumulate them there. One remarkable feature of these results is the similarity in the LCF values in a given plant for all compounds except the PFCAs >C10. The LCF values vary by a factor of < 2 in both cabbage and zucchini. This indicates that the efficiency of these two plants at transferring the chemicals from nutrient solution to the leaves is relatively independent of the physical chemical properties within the property range bracketed by PFBA and PFDA/PFOS. Tomato shows a slightly different pattern with a maximum for PFOA and a pronounced minimum for PFPeA. The much lower LCF values for the PFCAs >C10 for all plants, despite their high RCF values (Figure 1), suggest that these substances are not transported effectively from the roots to the leaves. This was already indicated by the SCF and TCF results.

There were systematic differences in the LCFs between the plants; for zucchini and tomato they were on average 4.02 and 1.78 times higher, respectively, than for cabbage (with the exception of PFPeA in tomato). Factors that could influence the LCF include the amount of water transpired (higher transpiration would be expected to increase LCF) and leaf biomass (a high leaf biomass would tend to decrease LCF). However, no correlation could be found between LCFs and transpiration volumes, leaf biomass or the quotient of these two parameters. The LCF is the result of more complex interactions among different plant tissues. More mechanistic insight into this is provided later.

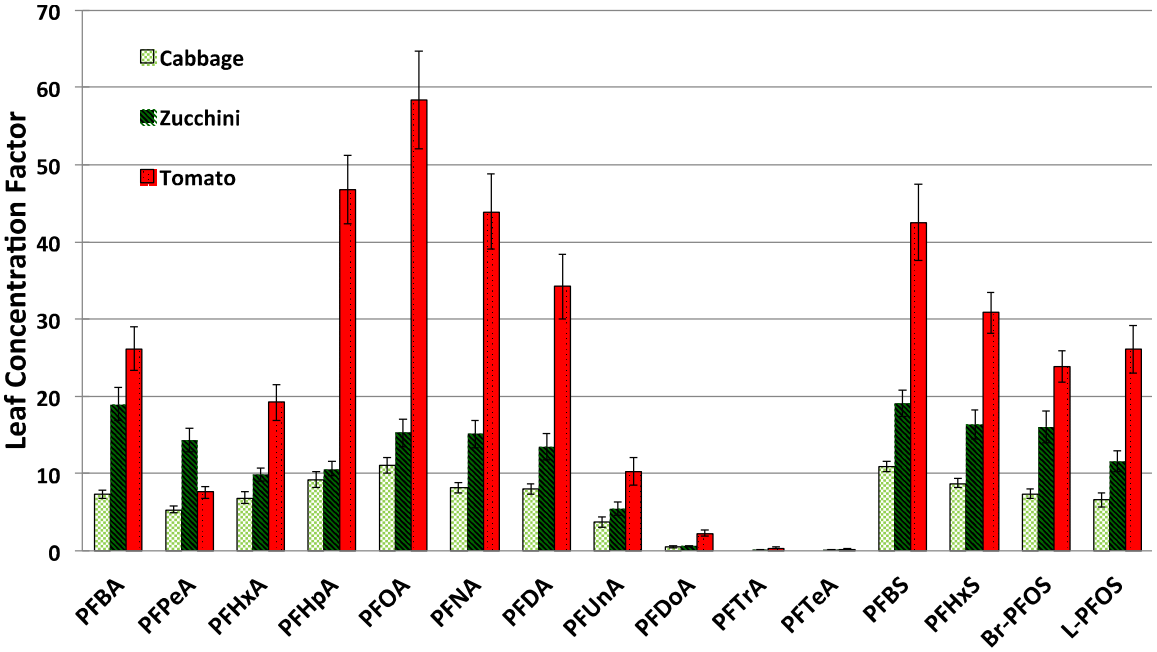

Figure 3: Leaf concentration factors (LCFs) for cabbage, zucchini and tomato [[(ng·g^{-1})/(ng·ml^{-1})]. The mean LCFs from each spiking level were averaged. The error bars denote the standard error.

Compared with the concentration factors of the other above-ground parts of the plants, the leaves show the highest concentration factors for most of the compounds. The mass distributions of the chemicals between the different plant tissues show that of the above-ground tissues, the leaves also store the largest mass of all of the PFAAs (with the exception of PFPeA in tomato, Table 1 and Tables S9/S10 in the SI). This can be explained by the transpiration occurring in the leaves. The PFAAs are translocated to the leaves in the transpiration stream; there the water transpires and the PFAAs remain as residues in the leaves where they accumulate.

Table 1: Mass distribution of PFAAs in different tissues of tomato plants, expressed as percent of the total amount of PFAA taken up by the plant. Values shown are averages from all plants.

	Roots	Stem	Twig	Leaf	Fruit
PFBA	3%	4%	10%	43%	40%
PFPeA	5%	8%	7%	20%	60%
PFHxA	12%	8%	9%	42%	30%
PFHpA	12%	8%	9%	67%	4%
PFOA	29%	7%	9%	53%	1%
PFNA	56%	5%	7%	32%	0%
PFDA	72%	5%	5%	17%	0%
PFUnA	88%	4%	4%	5%	0%
PFDoA	90%	5%	3%	2%	0%
PFTrA	96%	2%	1%	1%	0%
PFTeA	98%	1%	0%	1%	0%
PFBS	21%	4%	9%	65%	1%
PFHxS	38%	5%	7%	49%	0%
Br-PFOS	68%	6%	5%	21%	0%
L-PFOS	71%	5%	4%	19%	0%

Edible part. In the edible part (i.e., cabbage head, tomato and zucchini fruits), no concentrations were detected above the LOQ for the long-chain PFCAs (C12-C14), not even at the highest tested

exposure concentration (Table S11 in the SI). Consequently, no uptake factors could be calculated for these compounds.

The ECF (Figure 4) were highest for the short-chain PFCAs and decrease with increasing chain length. Zucchini fruits were far less contaminated than tomato fruits or cabbage heads. Thus, the exceptional ability of zucchini to accumulate some organic compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs) from soil (Huelster et al. 1994) or water (Gent et al. 2007; Zhang et al. 2009) was not observed for PFAAs.

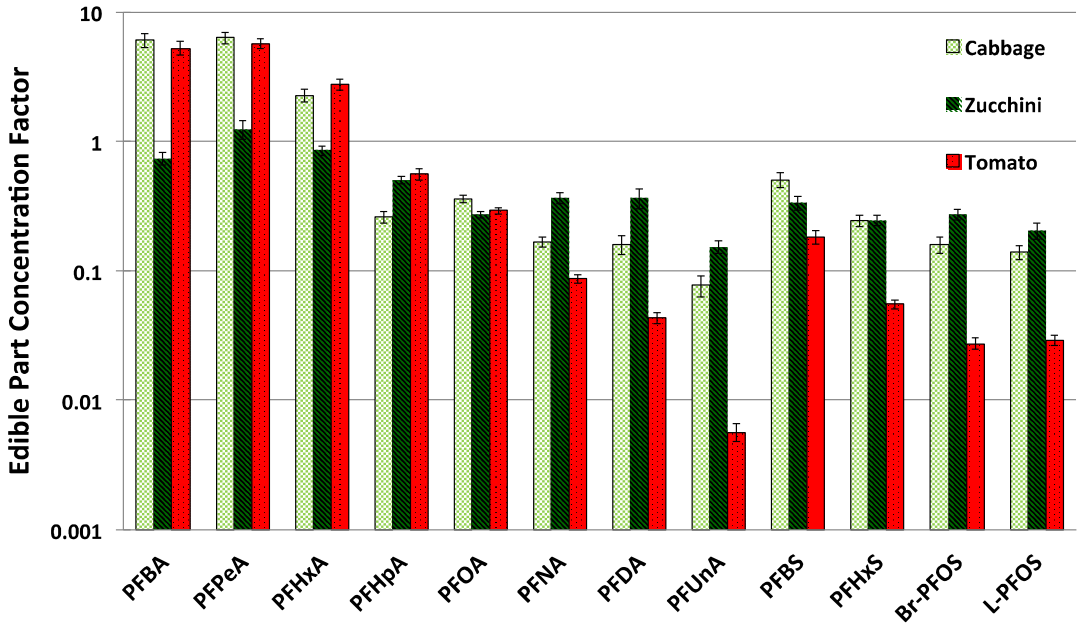

Figure 4: Edible part concentration factors (ECFs) for cabbage, zucchini and tomato [[(ng·g^{-1})/(ng·ml^{-1})]. The mean ECFs from each spiking level were averaged. The error bars denote the standard error.

The edible part/leaf transfer factors provide insight into the processes governing PFAA accumulation in the edible parts. Transpiration is low in fruits and in the cabbage head (which is tightly packed in leaves). The material for their development is provided by the phloem sap which is produced in the leaves. It is thus to be expected that PFAAs will reach the fruits primarily via phloem sap. The efficiency of the leaf to fruit transfer of PFAAs can be assessed using the edible part/leaf transfer factor. All values were < 1 in all cases (SI Figure S5), with values close to 1 for PFBA and PFPeA for cabbage and tomato. Because these two compounds sorb very little and transpiration is low from the edible parts, values close to 1 can be expected. The edible part/leaf transfer factor was similar for most compounds in zucchini and cabbage. For tomato it decreased exponentially with increasing chain length and the difference between PFBA and PFUnA amounted to > 3 orders of magnitude. A possible explanation for the different transport in the phloem sap is differences in the phloem sap composition, e.g., different proteins.

Transpiration stream concentration factor. The transpiration stream concentration factor (TSCF) describes the translocation potential of compounds from roots to aerial plant parts. Normally the TSCF is the concentration of the chemical in the transpiration stream divided by concentration in soil pore water (Briggs et al. 1982; Trapp 2000). Because direct measurement of the concentration in the transpiration stream was not possible, we estimated the TSCF by dividing the mass of the PFAAs in

the aerial plant parts by the PFAA concentration in the nutrient solution multiplied with the volume of water transpired by the plant:

$$\mathrm{TSCF} = \frac{\text{Concentration in foliage (ng/g)} * \text{foliage weight (g)}}{\text{Concentration in nutrient solution (ng/mL)} * \text{water transpired (mL)}} \quad [4]$$

Estimating the TSCF in this manner requires the assumptions that the compounds are not degraded in the plant, no chemical elimination from the plant occurs (e.g., loss of the compounds from the leaves to the atmosphere or back to the roots), and that there is no other uptake pathway than through the roots (i.e., no atmospheric deposition). Due to the high persistence of the PFAAs and the low concentrations of the PFAAs in the above-ground parts of the control plants (no spiking with PFAAs of the nutrient solution), these assumptions are reasonable.

The TSCF values for cabbage, zucchini and tomato thus calculated ranged between 0.05 and 0.23, with much lower values for the C12-C14 PFCAs (Figure 5).

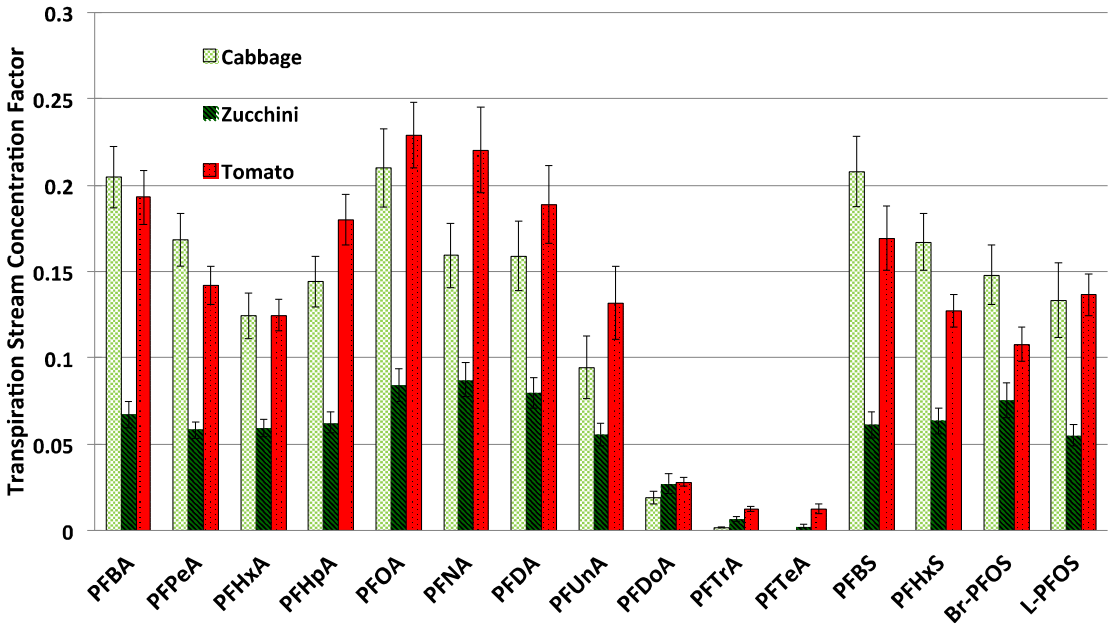

Figure 5: Transpiration stream concentration factors (TSCFs) for cabbage, zucchini and tomato. The mean RCFs from each spiking level were averaged. The error bars denote the standard error.

The TSCF values are relatively similar between compounds (generally a factor of <2) and between plant species (generally a factor of <2.5). There is, nevertheless, a consistent pattern in the TSCF values between the plants, with zucchini having significantly lower TSCF values than the other two tested species for all compounds except PFDoDA. Because the TSCF was always <1, the transfer from the nutrient solution to the vegetative parts of the plants was inhibited. The pattern over chain length is similar for all three plant species and shows a minimum for PFHxA and a maximum for PFOA. This is also comparable to the pattern for lettuce from our previous study, with the difference that markedly higher values were measured for PFBA and the long-chain PFCAs (C > 10) in lettuce.

There is no consistent trend with the carbon chain length of the compounds and there is no correlation between TSCF and measures of hydrophobicity such as log K_{OW} or log D_{OW} (SI Table S14). Briggs et al. reported that the TSCFs of non-ionized chemicals depend on their log K_{OW} values, and found the highest TSCF for compounds with a log K_{OW} of around 2 (Briggs et al. 1982). As we

discussed in our previous study (Felizeter et al. 2012), PFAAs do not fit this model, as the PFAAs with a log K_{OW} around 2 show the lowest TSCF values, with the exception of the long-chain PFCAs.

Recently, Krippner et al. (Krippner et al. 2014) reported a small influence of pH on the uptake of PFDA in maize roots, but found no influence of pH for C4-C9 PFCAs, PFBS, PFHxS, or PFOS. Due to very low pK_a values of PFAAs (Steinle-Darling et al. 2008b), the compounds are in their ionic form at environmentally relevant pH levels. All plants in this study were grown in nutrient solutions with the same pH, so that interspecies differences cannot be explained by the influence of the pH.

Differences between functional groups and isomers. To assess the influence of the PFAA functional group on PFAA uptake from soil and distribution in plants, PFBS was compared to PFPeA, PFHxS to PFHpA and L-PFOS to PFNA, because these are pairs of compounds with the same length of perfluorinated carbon chain. Furthermore, branched and linear PFOS were compared to provide insight into the uptake of different isomers.

All PFSAs had significantly higher RCFs than the PFCAs with the same number of fluorinated carbon atoms by a factor of about 2-3. This shows that the uptake or adsorption is not only governed by the length of the fluorinated C chain, but rather by a combination of chain length and functional group. Higgins et al. came to similar conclusions when investigating the sorption of PFAAs to soils (Higgins et al. 2006). They found that the sorption of sulfonates was stronger than the carboxylic analogues. The higher RCFs for PFSAs correspond to higher values of several measures of hydrophobicity. The modelled log K_{OW}, as well as the experimentally determined log k_0 and log $P^{0'}$ (see Table S14 in the SI) are generally higher for the PFSAs than for their carboxylic analogues. However, this does not apply for the log D_{OW} values of the compounds.

In contrast, there were few significant differences in the TSCF between the PFSAs and the corresponding PFCAs. This suggests that the functional group had a minor influence on the uptake of the chemical into the root vascular tissue. Consequently, the stronger contribution of the sulfonate functional group to the RCF noted above is likely due to its contribution to a higher sorption to root surface tissue.

The transfer from leaves to the edible part was significantly higher for PFPeA and PFHpA than for the corresponding PFSAs. No difference was found between PFNA and L-PFOS. A preference for the root uptake of the linear PFOS over the branched PFOS was also found for all species, but the difference was only significant for cabbage and tomato (Figure 1). This might be attributable to the smaller molecular volume of Br-PFOS resulting in lower root surface tissue/water sorption coefficients. However, no significant difference between branched and linear PFOS was found for the TSCF or the edible part/leaf concentration factor. Clearly, some elements of the root uptake of PFAAs and their distribution in plants are influenced by the PFAA's functional group as well as its chain length.

Implications for human exposure. PFAA concentrations in the edible part were relatively low. The European Food Safety Authority (EFSA) has defined tolerable daily intake values (TDIs) for PFOA and PFOS of 1500 ng/kg body weight and 150 ng/kg body weight, respectively (EFSA 2008). PFOA and PFOS concentrations in the edible parts of plants grown in the 1 µg/L exposure concentration did not exceed 0.5 ng/g and 0.2 ng/g fresh weight, respectively. Thus, to exceed the TDI for PFOA and PFOS, a person weighing 70 kg would need to eat around 210 kg and 50 kg, respectively, of these

contaminated crops daily, which is of course impossible. The concentrations of the short-chain PFCAs were higher than the PFOA concentrations by up to a factor of ~23 in cabbage and tomato and a factor of ~5 in zucchini. Although no TDIs for these compounds exist, their toxicity is reported to be lower than for PFOA (Renner 2006). Because the concentrations of the most abundant PFAAs in tap and surface water are usually in the lower nanogram per liter range (Eschauzier et al. 2012b), hydroponically grown crops generally should not be a danger to human health. However, should plants be exposed to much higher concentrations in soil or nutrient solution due to severe contamination, it is conceivable that TDI values could be exceeded and a risk for human health could occur.

All edible part/leaf transfer factors were below 1 in all cases (see above and Figure S5 in the SI), which indicates that leafy crops with open leaves, such as spinach or some lettuce varieties, accumulate higher amounts in the edible part than fruit bearing crops. Thus, leafy crops pose a higher risk for human exposure.

Mechanistic description. The results of the present study can be summarized in the following simple mechanistic description of plant uptake of PFAAs from soil or nutrient solution and their distribution in plant tissue. PFAAs are taken up by the roots via the transpiration stream. With the exception of long-chain PFCAs (C > 10), their concentration in the transpiration stream entering the stem is ~15% of the concentration in the nutrient solution. The majority of the PFAAs that enter the stem are carried with the transpiration stream to the leaves, where the transpiration of the water results in local accumulation of PFAAs. From the leaves, all PFAAs with the exception of long-chain PFCAs (C > 11) are transported via the phloem sap to the fruit and storage organs. Less PFAA accumulates in the fruit than in the leaves, which can in part be explained by the lower transpiration rate from fruits. There are species-specific differences in the effectiveness of this transfer: In cabbage and zucchini the effectiveness is similar both across chemicals and between species, whereas in tomato there is a pronounced decrease in transfer effectiveness with increasing chain length of the PFAAs. For the roots, there is a second accumulation mechanism in addition to the uptake with the transpiration stream, namely sorption to root surface tissue, which is especially important for the long-chain PFAAs. There was great consistency in this picture of PFAA uptake and distribution among the three plants studied here as well as the lettuce from the earlier study, the most notable difference being that lettuce also translocated long-chain PFCAs (C11-C14) to the above-ground plant parts.

This mechanistic description is plausible on the basis of the current understanding of contaminant behaviour in plants and PFAA properties. However, it is based on controlled laboratory experiments in hydroponic solutions. Its applicability to plants growing in soil under field conditions needs to be demonstrated. The soil-related studies found in the literature do not provide enough information to calculate pore-water concentrations. Consequently, we believe that further experimental work is required to further develop our understanding of soil to plant transfer of PFAAs.

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Supporting Information

Table S1: List of chemicals used, their purity and suppliers.

Chemical	Purity	Supplier
MPFAC-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
MPFAS-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
M5PFPeA (internal standard)		Wellington Laboratories, Ontario, Canada
M4PFHpA (internal standard)		Wellington Laboratories, Ontario, Canada
PFAC-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFAS/FOSA-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFBA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFPeA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHxA	≥97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHpA	99%	Sigma Aldrich, Zwijndrecht, Netherlands
PFOA	96%	Sigma Aldrich, Zwijndrecht, Netherlands
PFNA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFUnA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDoDA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTrDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTeDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFBS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFHxS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFOS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
Sodium carbonate	≥99%	Sigma Aldrich, Zwijndrecht, Netherlands
Sodium hydroxide	≥98,8%	J.T. Baker Chemicals, Deventer, Netherlands
Sodium hydrogencarbonate	≥99,5%	Merck, Darmstadt, Germany
Sodium sulfate	≥99%	Merck, Darmstadt, Germany
Tetrabutylammoniumhydrogensulfate (TBA)	≥99%	Merck, Darmstadt, Germany
Ammonium hydroxide		Sigma Aldrich, Zwijndrecht, Netherlands
Ammonium acetate	≥99,999%	Sigma Aldrich, Zwijndrecht, Netherlands
Methanol	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands
Water	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands
tert-Butyl methyl ether (MTBE)	HPLC-grade	Biosolve, Valkenswaard, Netherlands

Table S2: Chemical composition of the Hoaglands nutrient solution and the composition of the stock solutions used to achieve the final concentrations.

Component	Conc. Stock Solution g/L	mL Stock Solution per 1 L	final conc. in nutrient solution ppm	
KNO_3	202	2.5	N	210
$Ca(NO_3)_2$ x $4H_2O$	472	2.5	K	235
NH_4NO_3	32	2.5	Ca	200
$MgSO_4$ x $7H_2O$	493	1	Mg	48
KH_2PO_4 (pH to 6.0 with 3M KOH)	136	0.5	S	64
			P	31
Iron (Fe-EDTA sodium salt)	7.342	1	Fe	1,12
Minors:		1		
H_3BO_3	2.86		B	0.5
$MnCl_2$ x $4H_2O$	1.81		Mn	0.5
$ZnSO_4$ x $7H_2O$	0.22		Zn	0.05
$CuSO_4$	0.051		Cu	0.02
H_3MoO_4 x H_2O	0.09		Mo	0.01

Table S3: Dates of the seed sowing, the start and the end of the experiments, as well as dates when the nutrient solutions were exchanged.

	Date of sowing	Start of exposure	Dates of exchange of spiked nutrient solution	Harvest	End of experiment
Tomato	13.07.2010	27.07.2010	05.08.2010* 12.08.2010* 17.08.2010* 24.08.2010* 31.08.2010 09.09.2010 17.09.2010 28.09.2010 08.10.2010 20.10.2010 03.11.2010 22.11.2010	18.10.2010 to 12.11.2010	30.11.2010 to 17.12.2010
Cabbage	11.02.2011	25.02.2011	07.04.2011 13.04.2011* 20.04.2011 06.05.2011* 12.05.2011* 23.05.2011 08.06.2011*	07.06.2011 to 27.06.2011	07.06.2011 to 27.06.2011
Zucchini	22.03.2011	30.03.2011	27.04.2011 05.05.2011 13.05.2011 20.05.2011 27.05.2011	23.05.2011 to 07.06.2011	09.06.2011

* at this date not all the plants received new nutrient solution

Not all the plants grew equally fast or had ripe fruits at the same time. For tomato the fruits from the lowest branch were used for the paper. However, the experiment continued until all tomato plants had ripe fruits from all branches (low, medium and high). Cabbage plants were harvested when the cabbage heads started to crack open and no further growth could be expected. Zucchini fruits were harvested when they reached supermarket sizes. The experiment continued until at least 1 zucchini fruit of supermarket size was harvested from all plants.

Description of the instrumental method

The analytical methodology was according to the methods described by Eschauzier et al. (2010) [1]. The measurements were conducted in the scheduled MRM-mode (see Table S4). Briefly, instrumental settings included:

Ion Transfer Voltage:	-2000 V
Interface Temperature:	450°C
Curtain gas:	10 L min^{-1}
Collision gas:	6 L min^{-1}
Collision Energy:	-10 V for PFPeA to PFOA, -15 V for PFBA, -25 V for PFNA to PFTeDA and -70 V for the PFSAs

The concentrations of calibration standards ranged from 0.005 ng ml^{-1} (Calibration level 1) to 200 ng ml^{-1} (Calibration level 12). Peaks consisted of at least 24 scans and the smoothing width was 9 points.

For separation on the column a gradient elution with two mobile phases, A (40:60 methanol:water) and B (95:5 methanol:water; both with 2 mM ammonium acetate) was used. The system was equilibrated for 8 minutes with the initial mobile phase composition of 60 %A at a flow of 0.2 ml/min prior to sample injection. After injection the mobile phase composition changed linearly to 100% B at 10 minutes. This was held isocratic until 20 minutes. Afterwards the solvent composition was returned to initial condition within 2 minutes.

Table S4: List of the analytes, their abbreviations and molecular formula, the 13C-labelled internal standards used, and the mass transitions used in the MS/MS analysis of the analytes. Due to the lack of available mass-labelled standards for PFTrDA, PFTeDA and PFBS, these chemicals were corrected with the closest available standard, which could lead to under- or overestimated results due to different responses or extraction efficiencies.

Abbreviation	Compound	Transition 1	Transition 2	Quantification by internal Standard	Molecular Formula
PFBA	Perfluoro-n-butanoic acid	213 → 169	-	$^{13}C_4$ PFBA	$CF_3(CF_2)_2COOH$
PFPeA	Perfluoro-n-pentanoic acid	263 → 219	-	$^{13}C_5$ PFPeA	$CF_3(CF_2)_3COOH$
PFHxA	Perfluoro-n-hexanoic acid	313 → 269	313 → 119	$^{13}C_2$ PFHxA	$CF_3(CF_2)_4COOH$
PFHpA	Perfluoro-n-heptanoic acid	363 → 319	363 → 169	$^{13}C_4$ PFHpA	$CF_3(CF_2)_5COOH$
PFOA	Perfluoro-n-octanoic acid	413 → 369	413 → 169	$^{13}C_8$ PFOA	$CF_3(CF_2)_6COOH$
PFNA	Perfluoro-n-nonanoic acid	463 → 419	463 → 219	$^{13}C_9$ PFNA	$CF_3(CF_2)_7COOH$
PFDA	Perfluoro-n-decanoic acid	513 → 469	513 → 269	$^{13}C_6$ PFDA	$CF_3(CF_2)_8COOH$
PFUnA	Perfluoro-n-undecanoic acid	563 → 519	563 → 269	$^{13}C_7$ PFUnA	$CF_3(CF_2)_9COOH$
PFDoDA	Perfluoro-n-dodecanoic acid	613 → 569	613 → 319	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{10}COOH$
PFTrDA	Perfluoro-n-tridecanoic acid	663 → 619	663 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{11}COOH$
PFTeDA	Perfluoro-n-tetradecanoic acid	713 → 669	713 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{12}COOH$
PFBS	Perfluorobutane sulfonate	299 → 80	299 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_3SO_3$
PFHxS	Perlfuorohexane sulfonate	399 → 80	399 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_5SO_3$
PFOS	Perfluorooctane sulfonate	499 → 80	499 → 99	$^{13}C_8$ PFOS	$CF_3(CF_2)_7SO_3$
$^{13}C_4$ PFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	217 → 172	-		
$^{13}C_5$ PFPeA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	268 → 223	-		
$^{13}C_2$ PFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	315 → 270	315 → 119		
$^{13}C_4$ PFHpA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	367 → 323	367 → 169		
$^{13}C_8$ PFOA	Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	421 → 376	421 → 172		
$^{13}C_9$ PFNA	Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]nonanoic acid	472 → 427	472 → 223		
$^{13}C_6$ PFDA	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	519 → 474	519 → 219		
$^{13}C_7$ PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	570 → 525	570 → 270		
$^{13}C_2$ PFDoDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	615 → 570	615 → 369		
$^{18}O_2$ PFHxS	Perfluoro-1-hexane[$^{18}O_2$]sulfonate	403 → 84	403 → 103		
$^{13}C_8$ PFOS	Perfluoro-1-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanesulfonate	507 → 80	507 → 99		

Table S5: Concentrations of a repeated extraction of a cabbage leaf sample from the 500 ng L^{-1} nominal spiking concentration. All values in ng g^{-1} fresh weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	Br-PFOS	L-PFOS
Sample 1	3.93	2.55	2.94	4.54	6.16	5.90	3.52	1.16	0.082	0.021	0.017	10.2	7.99	1.32	3.41
Sample 2	4.36	2.52	3.58	4.31	5.50	5.42	3.11	1.06	0.091	0.024	0.020	10.8	6.89	1.17	3.18
Sample 3	4.53	2.43	3.59	4.15	5.79	4.81	3.16	0.93	0.089	0.025	0.015	10.9	7.16	1.09	2.94
Sample 4	4.37	2.35	3.28	4.31	5.58	5.76	3.71	1.07	0.091	0.019	0.015	10.7	6.99	1.19	3.00
Sample 5	4.51	2.41	2.98	4.89	6.13	4.59	2.82	0.99	0.076	0.020	0.017	10.6	6.89	1.09	3.16
Average	4.34	2.45	3.27	4.44	5.83	5.29	3.26	1.04	0.086	0.022	0.017	10.6	7.18	1.17	3.14
Standard deviation	0.22	0.07	0.28	0.26	0.27	0.52	0.32	0.08	0.006	0.002	0.002	0.23	0.41	0.08	0.16
% StDev	5%	3%	9%	6%	5%	10%	10%	7%	7%	10%	9%	2%	6%	7%	5%

Table S6: Recoveries (in %) of internal mass-labeled standards. They were determined by comparing the standard signal in the sample to the signal in matrix solutions which had been spiked with the same quantity of internal standard immediately prior to analysis. Mass labeled standards for PFPeA and PFHpA were not available at the time tomato roots and fruits were extracted. The bold entries are the mean recoveries (in %), while the non-bold entries are the respective standard deviations (in % of the mean).

	$^{13}C_4$ PFBA	$^{13}C_5$ PFPeA	$^{13}C_2$ PFHxA	$^{13}C_4$ PFHpA	$^{13}C_8$ PFOA	$^{13}C_9$ PFNA	$^{13}C_6$ PFDA	$^{13}C_7$ PFUnA	$^{13}C_2$ PFDoDA	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Cabbage											
Roots	**50**	**95**	**107**	**94**	**100**	**92**	**110**	**111**	**123**	**101**	**87**
	10	19	10	10	10	17	21	20	20	7	16
Stem	**43**	**88**	**93**	**92**	**94**	**108**	**127**	**126**	**133**	**112**	**103**
	11	19	13	11	14	17	19	17	18	8	16
Leaf	**34**	**106**	**96**	**103**	**103**	**107**	**99**	**93**	**90**	**70**	**76**
	4	19	15	16	13	17	17	16	17	11	11
Head	**134**	**101**	**96**	**90**	**104**	**97**	**102**	**98**	**106**	**96**	**89**
	23	14	10	10	15	10	17	30	19	10	12
Zucchini											
Roots	**43**	**92**	**136**	**102**	**102**	**86**	**101**	**85**	**140**	**93**	**101**
	17	20	14	19	18	13	29	22	22	14	21
Stem	**30**	**72**	**83**	**83**	**80**	**58**	**79**	**62**	**59**	**69**	**61**
	6	8	8	10	10	13	15	10	13	5	4
Twig	**32**	**73**	**81**	**90**	**75**	**61**	**74**	**56**	**62**	**79**	**67**
	8	12	14	12	12	14	11	7	10	4	6
Leaf	**41**	**84**	**93**	**100**	**96**	**67**	**99**	**72**	**67**	**78**	**75**
	5	13	9	13	14	15	24	9	15	8	12
Fruit	**31**	**64**	**78**	**85**	**79**	**65**	**78**	**71**	**73**	**60**	**54**
	8	11	16	16	17	15	19	15	19	19	17
Tomato											
Roots	**77**	n.a.	**105**	n.a.	**91**	**86**	**85**	**91**	**89**	**95**	**106**
	12		12		6	14	8	8	6	18	8
Fruit	**50**	n.a.	**102**	n.a.	**96**	**111**	**123**	**150**	**155**	**91**	**129**
	6		14		4	5	10	11	9	9	11
Stem	**46**	**74**	**79**	**72**	**69**	**64**	**86**	**64**	**73**	**68**	**62**
	5	10	7	13	14	13	18	16	11	11	17
Twig	**41**	**72**	**79**	**76**	**70**	**59**	**70**	**72**	**91**	**65**	**67**
	7	18	16	19	18	12	11	15	17	11	15
Leaf	**38**	**94**	**109**	**112**	**95**	**83**	**98**	**116**	**106**	**88**	**94**
	7	17	12	11	9	15	8	17	18	11	12

Table S7: Ionization enhancement and/or suppression for the internal standards added to purified extracts. Matrix effects are expressed as a percentage in relation to the signal area response of a solvent-based, matrix free, internal standard solution (100% = no matrix effect).

	$^{13}C_4$ PFBA	$^{13}C_5$ PFPeA	$^{13}C_2$ PFHxA	$^{13}C_4$ PFHpA	$^{13}C_8$ PFOA	$^{13}C_9$ PFNA	$^{13}C_6$ PFDA	$^{13}C_7$ PFUnA	$^{13}C_2$ PFDoDA	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Cabbage											
Root	49	83	102	104	111	93	88	76	70	112	91
Stem	49	87	101	102	111	97	86	78	74	106	88
Leaf	73	57	87	89	94	82	74	72	66	100	79
Head	19	56	83	94	97	90	77	61	56	105	80
Zucchini											
Root	19	33	60	77	78	61	42	49	39	96	60
Stem	107	84	97	99	104	101	90	102	107	111	91
Twig	103	94	102	102	108	102	96	107	98	109	101
Leaf	84	65	89	86	87	83	71	71	59	102	81
Fruit	106	92	93	93	102	95	87	94	91	105	93
Tomato											
Root	34		81		94	77	65	62	55	104	76
Stem	65	89	95	101	104	100	78	84	74	109	80
Twig	68	94	97	99	106	95	81	86	70	112	83
Leaf	84	72	87	85	89	68	56	47	42	100	63
Fruit	73		99		105	92	77	65	64	108	81

Table S8: Limits of Quantification (LoQ) in ng g^{-1} fresh weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	Br-PFOS	L-PFOS
Cabbage															
Root	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.002	0.002	0.005	0.018
Stem	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.002	0.002	0.005	0.018
Leaf	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.001	0.001	0.004	0.014
Head	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.001	0.001	0.004	0.014
Zucchini															
Root	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.001	0.001	0.004	0.014
Stem	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.001	0.001	0.004	0.014
Twig	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.001	0.001	0.004	0.013
Leaf	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.002	0.002	0.006	0.021
Fruit	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.001	0.001	0.002	0.009
Tomato															
Root	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.001	0.001	0.004	0.014
Stem	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.002	0.002	0.006	0.023
Twig	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.003	0.003	0.010	0.036
Leaf	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.003	0.003	0.010	0.036
Fruit	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.001	0.001	0.002	0.009

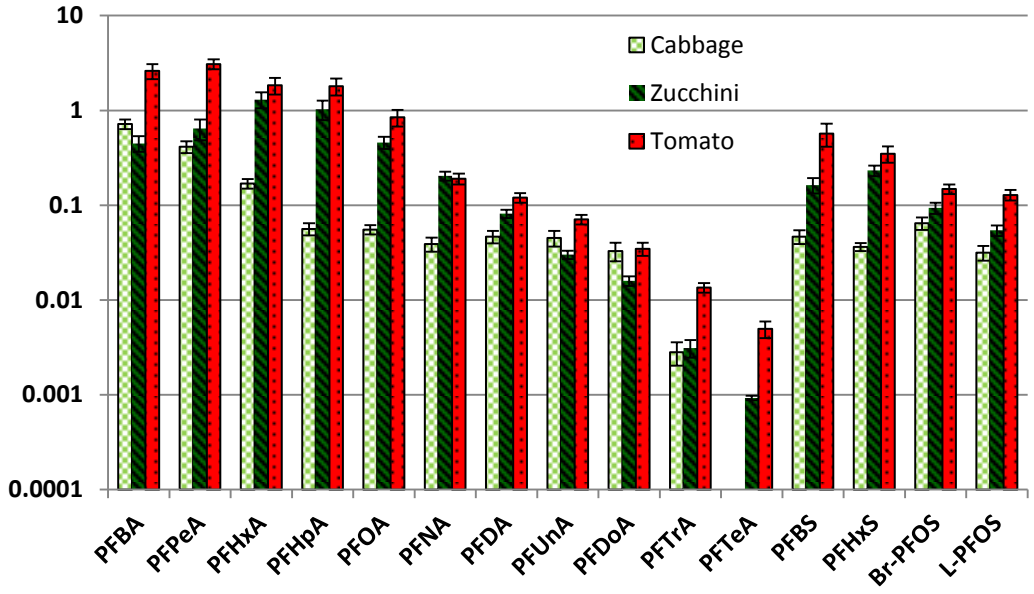

Figure S1: Stem/root concentration factor, calculated by dividing the PFAA concentration in the stem by the PFAA concentration in the root (both on a fresh weight basis). Logarithmic scale. The factor shown is the average of all plants with quantifiable concentrations (see tables S11-13). Error bars denote standard error.

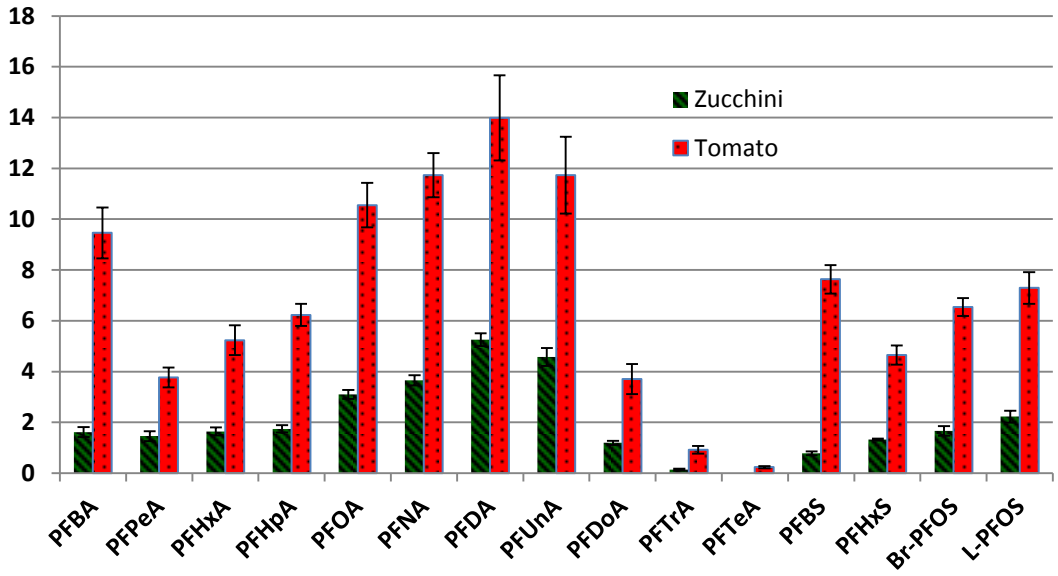

Figure S2: Twig concentration factor (TCF), calculated by dividing the fresh weight based PFAA concentration in the twig by the PFAA concentration in the nutrient solution. The factor shown is the average of all plants with quantifiable concentrations (see tables S11-13). Error bars denote standard error.

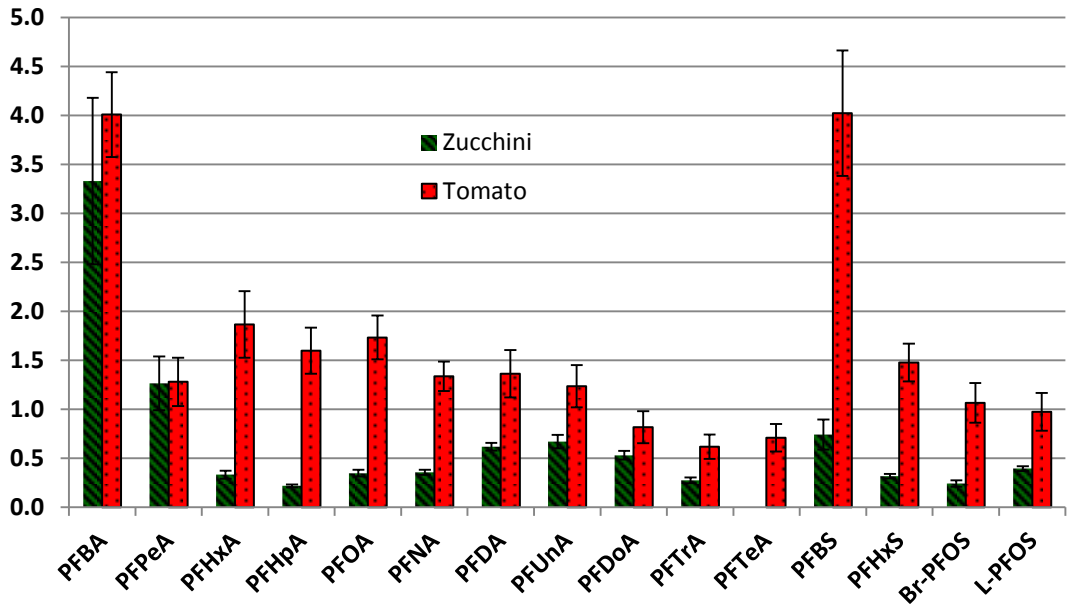

Figure S3: Twig/stem concentration factor, calculated by dividing the PFAA concentration in the twig by the PFAA concentration in the stem (both on a fresh weight basis). The factor shown is the average of all plants with quantifiable concentrations (see tables S11-13). Error bars denote standard error.

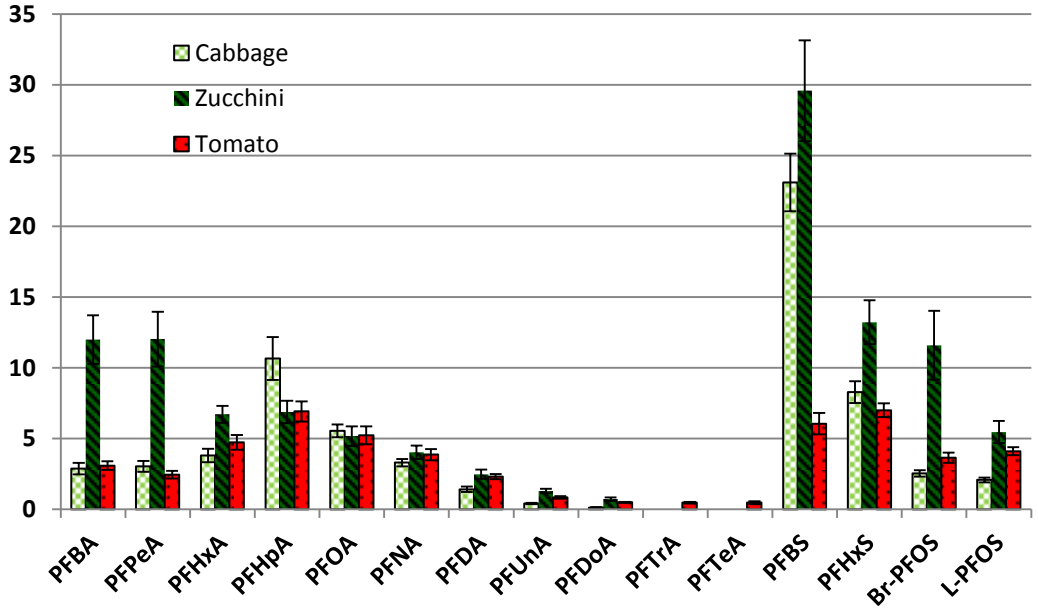

Figure S4: Leaf/twig concentration factor (for cabbage leaf/stem), calculated by dividing the PFAA concentration in the leaves by the PFAA concentration in the twig (stem) (all on a fresh weight basis). The factor shown is the average of all plants with quantifiable concentrations (see tables S11-13). Error bars denote standard error.

Table S9: Mass distribution of PFAAs in different tissues of cabbage plants, expressed as a percent of the total amount of PFAA found in the plant. Percent distributions were calculated for each plant. Values shown are the averages.

	Roots	Stem	Head	Leaf
PFBA	6%	4%	22%	67%
PFPeA	10%	3%	33%	53%
PFHxA	19%	3%	17%	62%
PFHpA	24%	1%	1%	74%
PFOA	39%	1%	1%	59%
PFNA	63%	1%	0%	35%
PFDA	79%	2%	0%	19%
PFUnA	91%	2%	0%	6%
PFDoDA	97%	2%	0%	2%
PFTrDA	99%	0%	0%	0%
PFTeDA	100%	0%	0%	0%
PFBS	20%	1%	1%	78%
PFHxS	38%	1%	1%	61%
Br-PFOS	64%	2%	0%	34%
L-PFOS	82%	1%	0%	16%

Table S10: Mass distribution of PFAAs in different tissues of zucchini plants, expressed as percent of the total amount of PFAA found in the plant. Percent distributions were calculated for each plant. Values shown are the averages.

	Roots	Stem	Twig	Leaf	Fruit
PFBA	7%	2%	8%	72%	11%
PFPeA	11%	4%	6%	57%	22%
PFHxA	17%	24%	6%	38%	15%
PFHpA	33%	24%	4%	32%	7%
PFOA	50%	19%	6%	24%	2%
PFNA	66%	13%	4%	16%	2%
PFDA	81%	7%	3%	8%	1%
PFUnA	93%	3%	2%	2%	0%
PFDoDA	98%	1%	1%	0%	0%
PFTrDA	100%	0%	0%	0%	0%
PFTeDA	100%	0%	0%	0%	0%
PFBS	32%	4%	3%	56%	5%
PFHxS	53%	11%	3%	32%	2%
Br-PFOS	77%	7%	2%	14%	1%
L-PFOS	85%	4%	2%	9%	0%

Table S11: PFAA concentrations (mean ± standard deviation) in samples from the cabbage experiment expressed in ng/L (water) and ng/g fresh weight (plant tissues). The nominal concentrations in the nutrient solution are given in the left hand column. The concentrations in the plant tissues were corrected for the concentrations in the control plants (data not shown).

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
Water								
10	10.4 ± 0.31	12.9 ± 0.57	16.0 ± 1.01	12.8 ± 0.67	10.2 ± 0.43	9.04 ± 0.30	7.15 ± 0.23	6.79 ± 0.24
100	143 ± 11.1	147 ± 10.7	180 ± 15.4	134 ± 9.56	110 ± 6.62	105 ± 5.85	83.8 ± 3.51	74.9 ± 2.99
500	726 ± 50.1	752 ± 47.9	930 ± 76.4	678 ± 41.4	551 ± 27.7	536 ± 25.9	440 ± 17.8	374 ± 14.0
1000	1313 ± 65.7	1375 ± 61.0	1671 ± 114	1253 ± 53.3	1038 ± 33.7	1015 ± 30.4	837 ± 18.2	715 ± 14.3
Root								
10	<0.026 ±	0.13 ± 0.058	0.28 ± 0.12	0.29 ± 0.10	0.82 ± 0.15	1.68 ± 0.34	2.40 ± 0.36	2.09 ± 0.44
100	0.68 ± 0.35	0.93 ± 0.46	1.91 ± 0.99	1.97 ± 1.02	4.03 ± 1.70	9.88 ± 2.51	17.1 ± 3.44	16.6 ± 2.97
500	3.99 ± 2.81	3.15 ± 0.20	5.23 ± 0.24	5.93 ± 0.37	10.9 ± 0.49	25.3 ± 0.79	52.0 ± 0.13	61.7 ± 16.0
1000	7.38 ± 3.07	7.57 ± 2.69	14.4 ± 5.65	15.0 ± 5.53	25.4 ± 10.4	49.6 ± 14.1	90.4 ± 18.2	124 ± 6.25
Stem								
10	<0.026 ±	0.037 ± 0.009	<0.026 ±	<0.026 ±	0.027 ± 0.001	<0.026 ±	<0.026 ±	<0.026 ±
100	0.37 ± 0.15	0.26 ± 0.14	0.32 ± 0.26	0.12 ± 0.092	0.21 ± 0.13	0.24 ± 0.15	0.36 ± 0.028	0.38 ± 0.17
500	1.40 ± 0.27	0.72 ± 0.20	0.55 ± 0.17	0.26 ± 0.13	0.89 ± 0.34	1.42 ± 0.59	2.82 ± 0.73	2.89 ± 1.22
1000	4.14 ± 1.68	2.58 ± 0.76	2.19 ± 1.16	0.76 ± 0.24	1.80 ± 0.45	2.96 ± 0.51	5.33 ± 1.13	8.11 ± 4.02
Leaf								
10	0.32 ± 0.11	0.18 ± 0.051	0.14 ± 0.033	0.11 ± 0.031	0.098 ± 0.036	0.073 ± 0.036	0.053 ± 0.016	<0.021 ±
100	1.00 ± 0.18	0.79 ± 0.13	0.89 ± 0.24	0.91 ± 0.29	0.96 ± 0.22	0.63 ± 0.16	0.43 ± 0.21	0.18 ± 0.016
500	4.39 ± 0.81	3.27 ± 1.16	3.78 ± 1.30	4.14 ± 0.62	4.85 ± 1.18	4.65 ± 1.24	3.46 ± 1.06	0.91 ± 0.11
1000	10.8 ± 2.84	8.23 ± 2.84	9.38 ± 3.16	9.81 ± 3.75	10.92 ± 3.40	9.65 ± 2.00	7.91 ± 3.14	2.49 ± 1.40
Head								
10	0.087 ± 0.010	0.12 ± 0.017	0.057 ± 0.007	<0.021 ±	<0.021 ±	<0.021 ±	<0.021 ±	<0.021 ±
100	0.63 ± 0.31	0.73 ± 0.20	0.32 ± 0.023	0.047 ± 0.003	0.042 ± 0.011	<0.021 ±	<0.021 ±	<0.021 ±
500	2.33 ± 0.60	2.37 ± 0.42	0.97 ± 0.34	0.14 ± 0.064	0.21 ± 0.035	0.077 ± 0.006	0.055 ± 0.005	0.024 ± 0.002
1000	7.21 ± 3.09	7.65 ± 3.42	3.04 ± 1.20	0.33 ± 0.10	0.34 ± 0.10	0.19 ± 0.051	0.15 ± 0.072	0.067 ± 0.031

Table S11: Continued

	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	Br-PFOS	L-PFOS
Water							
10	6.26 ± 0.26	4.57 ± 0.19	4.07 ± 0.18	19.9 ± 1.23	12.7 ± 0.70	1.90 ± 0.070	6.68 ± 0.23
100	65.8 ± 2.74	48.6 ± 1.84	41.0 ± 1.40	193 ± 13.7	124 ± 8.23	20.4 ± 1.04	72.8 ± 3.40
500	339 ± 13.9	242 ± 9.52	210 ± 8.24	967 ± 58.6	620 ± 34.4	106 ± 4.81	378 ± 16.3
1000	668 ± 20.5	481 ± 14.6	418 ± 13.0	1793 ± 75.6	1171 ± 44.4	206 ± 6.32	725 ± 18.2
Root							
10	1.26 ± 0.19	1.10 ± 0.39	0.92 ± 0.17	0.30 ± 0.16	0.59 ± 0.13	0.32 ± 0.065	1.56 ± 0.32
100	11.2 ± 2.36	8.96 ± 3.62	10.8 ± 3.56	3.84 ± 1.96	4.71 ± 2.03	2.35 ± 0.54	12.6 ± 1.91
500	34.8 ± 4.82	40.6 ± 5.20	35.4 ± 5.34	11.6 ± 1.56	13.1 ± 2.16	5.32 ± 0.61	38.4 ± 2.87
1000	52.2 ± 12.0	55.5 ± 13.8	49.8 ± 13.9	31.5 ± 10.3	31.2 ± 9.28	10.3 ± 3.85	79.3 ± 33.3
Stem							
10	<0.026 ±	<0.026 ±	<0.026 ±	<0.002 ±	<0.002 ±	<0.005 ±	<0.018 ±
100	0.21 ± 0.088	<0.026 ±	<0.026 ±	0.087 ± 0.042	0.15 ± 0.10	0.061 ± 0.024	0.15 ± 0.041
500	0.98 ± 0.68	0.14 ± 0.12	<0.026 ±	0.31 ± 0.063	0.58 ± 0.20	0.42 ± 0.11	1.32 ± 0.38
1000	2.26 ± 1.60	0.28 ± 0.12	<0.026 ±	0.90 ± 0.18	1.43 ± 0.65	0.71 ± 0.24	2.64 ± 0.84
Leaf							
10	<0.021 ±	<0.021 ±	<0.021 ±	0.21 ± 0.047	0.096 ± 0.032	0.011 ± 0.005	<0.014 ±
100	<0.021 ±	<0.021 ±	<0.021 ±	1.96 ± 0.67	1.03 ± 0.24	0.14 ± 0.041	0.24 ± 0.019
500	0.20 ± 0.17	<0.021 ±	<0.021 ±	10.1 ± 2.67	5.84 ± 1.31	0.99 ± 0.23	2.62 ± 0.74
1000	0.40 ± 0.27	<0.021 ±	<0.021 ±	21.4 ± 7.67	11.9 ± 3.90	1.72 ± 0.37	6.02 ± 2.18
Head							
10	<0.021 ±	<0.021 ±	<0.021 ±	<0.001 ±	<0.001 ±	<0.004 ±	<0.014 ±
100	<0.021 ±	<0.021 ±	<0.021 ±	0.078 ± 0.029	0.034 ± 0.007	<0.004 ±	<0.014 ±
500	<0.021 ±	<0.021 ±	<0.021 ±	0.30 ± 0.10	0.14 ± 0.049	0.015 ± 0.006	0.052 ± 0.011
1000	<0.021 ±	<0.021 ±	<0.021 ±	0.88 ± 0.28	0.27 ± 0.11	0.041 ± 0.005	0.10 ± 0.041

Table S12: PFAA concentrations (mean ± standard deviation) in samples from the zucchini experiment expressed in ng/L (water) and ng/g fresh weight (plant tissues). The nominal concentrations in the nutrient solution are given in the left hand column. The concentrations in the plant tissues were corrected for the concentrations in the control plants (data not shown).

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
Water								
10	16.1 ± 1.43	16.8 ± 1.30	19.6 ± 2.03	15.1 ± 1.08	12.2 ± 0.61	10.9 ± 0.39	7.99 ± 0.06	6.60 ± 0.03
100	174 ± 6.58	173 ± 5.37	209 ± 8.81	152 ± 4.18	124 ± 2.54	119 ± 2.23	93.0 ± 0.93	76.9 ± 0.40
500	857 ± 40.3	847 ± 31.5	1022 ± 54.4	743 ± 24.4	600 ± 13.9	603 ± 14.4	493 ± 7.63	371 ± 1.53
1000	1553 ± 45.0	1595 ± 43.5	1891 ± 58.7	1417 ± 36.2	1159 ± 23.6	1107 ± 20.1	935 ± 12.3	714 ± 1.75
Root								
10	<0.021 ±	0.10 ±	0.14 ±	0.16 ± 0.14	0.37 ± 0.16	0.89 ± 0.34	1.89 ± 0.77	2.28 ± 0.50
100	0.38 ± 0.22	0.64 ± 0.32	1.31 ± 0.24	1.69 ± 0.58	3.66 ± 1.24	6.74 ± 1.99	11.7 ± 3.26	18.2 ± 4.21
500	1.71 ± 0.35	4.58 ± 2.03	8.00 ± 4.19	8.09 ± 6.39	16.6 ± 12.3	36.9 ± 13.60	54.6 ± 8.94	112 ± 30.45
1000	2.58 ± 1.03	4.60 ± 2.38	8.41 ± 3.40	13.3 ± 1.95	28.9 ± 2.72	51.2 ± 13.18	86.6 ± 5.16	166 ± 35.46
Stem								
10	<0.021 ±	<0.021 ±	0.14 ± 0.04	0.11 ±	0.13 ± 0.05	0.18 ± 0.06	0.10 ± 0.022	0.05 ± 0.01
100	<0.021 ±	<0.021 ±	1.19 ± 0.25	1.16 ± 0.29	1.24 ± 0.27	1.25 ± 0.32	0.76 ± 0.15	0.49 ± 0.14
500	0.50 ± 0.37	1.33 ± 0.67	4.64 ± 1.13	6.22 ± 0.99	5.95 ± 1.23	6.04 ± 1.07	4.25 ± 1.03	2.63 ± 0.78
1000	1.45 ± 1.02	3.65 ± 1.68	9.59 ± 2.64	11.42 ± 1.25	11.90 ± 1.93	11.76 ± 2.99	9.45 ± 2.28	5.43 ± 2.03
Twig								
10	<0.019 ±	<0.019 ±	<0.019 ±	<0.019 ±	<0.019 ±	<0.019 ±	<0.019 ±	<0.019 ±
100	0.27 ± 0.10	0.19 ± 0.12	0.26 ± 0.07	0.19 ± 0.03	0.32 ± 0.04	0.38 ± 0.08	0.43 ± 0.11	0.28 ± 0.12
500	1.20 ± 0.49	1.17 ± 0.30	1.73 ± 0.45	1.52 ± 0.25	1.97 ± 0.18	2.41 ± 0.15	2.80 ± 0.16	2.04 ± 0.12
1000	3.24 ± 1.85	3.47 ± 1.84	3.95 ± 1.65	3.22 ± 0.42	3.69 ± 0.46	4.89 ± 0.77	5.36 ± 0.26	3.57 ± 0.89
Leaf								
10	0.54 ± 0.24	0.33 ± 0.27	0.18 ± 0.09	0.12 ± 0.12	0.19 ± 0.13	0.20 ± 0.10	0.18 ±	0.05 ±
100	2.62 ± 0.18	2.16 ± 0.25	1.86 ± 0.34	1.65 ± 0.37	2.06 ± 0.71	1.94 ± 0.66	1.44 ± 0.41	0.50 ± 0.21
500	13.9 ± 4.97	10.10 ± 2.25	11.2 ± 5.88	8.20 ± 3.97	10.6 ± 2.49	10.4 ± 2.27	6.74 ± 2.36	2.21 ± 0.84
1000	31.9 ± 6.29	22.11 ± 4.12	19.7 ± 4.06	16.0 ± 5.08	25.3 ± 11.12	22.0 ± 10.2	16.3 ± 7.03	4.18 ± 3.57
Fruit								
10	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±
100	0.12 ± 0.07	0.16 ± 0.03	0.15 ± 0.04	0.07 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	<0.013 ±	<0.013 ±
500	0.61 ± 0.29	0.76 ± 0.27	0.82 ± 0.23	0.38 ± 0.14	0.17 ± 0.04	0.21 ± 0.04	0.17 ± 0.10	0.066 ± 0.019
1000	1.24 ± 0.11	1.37 ± 0.01	1.61 ± 0.38	0.72 ± 0.26	0.29 ± 0.06	0.44 ± 0.19	0.27 ± 0.064	0.080 ± 0.004

Table S12: Continued

	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	Br-PFOS	L-PFOS
Water							
10	5.67 ± 0.02	5.09 ± 0.05	3.40 ± 0.05	22.1 ± 1.70	14.6 ± 1.03	2.15 ± 0.07	8.15 ± 0.27
100	63.6 ± 0.19	49.7 ± 0.17	32.4 ± 0.27	228 ± 7.13	143 ± 3.76	22.0 ± 0.30	82.6 ± 1.15
500	354 ± 3.21	261 ± 1.63	189 ± 0.58	1110 ± 41.3	694 ± 21.3	116 ± 2.23	415 ± 7.15
1000	698 ± 6.78	514 ± 3.47	407 ± 0.23	2139 ± 58.8	1354 ± 33.7	234 ± 4.67	805 ± 12.7
Root							
10	1.81 ± 0.77	0.89 ± 0.17	2.09 ± 0.48	0.21 ± 0.13	0.32 ± 0.07	0.28 ± 0.02	0.95 ± 0.20
100	16.4 ± 4.08	7.71 ± 5.27	24.2 ± 8.16	2.59 ± 1.18	4.00 ± 1.88	2.23 ± 0.63	8.20 ± 2.36
500	51.4 ± 13.3	36.9 ± 6.31	81.4 ± 44.4	13.91 ± 11.0	20.1 ± 13.4	8.97 ± 3.52	47.9 ± 12.2
1000	69.8 ± 11.7	58.6 ± 8.31	79.9 ± 51.3	19.04 ± 5.95	19.8 ± 9.41	9.70 ± 3.01	70.8 ± 7.50
Stem							
10	<0.021 ±	<0.021 ±	<0.021 ±	<0.001 ±	0.081 ± 0.007	0.007 ± 0.004	0.056 ± 0.029
100	0.17 ± 0.061	<0.021 ±	<0.021 ±	0.34 ± 0.16	0.69 ± 0.076	0.20 ± 0.066	0.44 ± 0.11
500	0.75 ± 0.11	0.071 ± 0.013	0.031 ±	1.38 ± 0.69	2.75 ± 0.71	0.73 ± 0.14	1.94 ± 0.39
1000	1.62 ± 0.61	0.21 ± 0.11	0.057 ±	2.69 ± 0.92	4.62 ± 0.18	1.15 ± 0.20	3.51 ± 0.94
Twig							
10	<0.019 ±	<0.019 ±	<0.019 ±	<0.001 ±	<0.001 ±	<0.004 ±	<0.014 ±
100	0.080 ± 0.025	<0.019 ±	<0.019 ±	0.15 ± 0.035	0.17 ± 0.010	0.030 ± 0.015	0.15 ± 0.039
500	0.43 ± 0.085	0.045 ± 0.03	<0.019 ±	0.93 ± 0.41	0.99 ± 0.10	0.25 ± 0.043	0.92 ± 0.081
1000	0.74 ± 0.21	0.056 ± 0.04	<0.019 ±	1.48 ± 0.62	1.83 ± 0.12	0.35 ± 0.12	1.33 ± 0.18
Leaf							
10	<0.030 ±	<0.030 ±	<0.030 ±	0.47 ± 0.02	0.24 ± 0.09	0.04 ±	0.10 ± 0.06
100	0.057 ± 0.012	<0.030 ±	<0.030 ±	4.81 ± 0.80	2.56 ± 0.65	0.46 ± 0.14	0.99 ± 0.36
500	0.20 ± 0.07	0.031 ±	<0.030 ±	22.8 ± 11.9	12.4 ± 7.24	2.09 ± 0.81	4.81 ± 2.71
1000	0.39 ± 0.33	0.081 ±	<0.030 ±	41.1 ± 1.60	25.9 ± 4.98	3.45 ± 1.50	11.1 ± 6.51
Fruit							
10	<0.013 ±	<0.013 ±	<0.013 ±	<0.001 ±	<0.001 ±	<0.002 ±	<0.009 ±
100	<0.013 ±	<0.013 ±	<0.013 ±	0.07 ± 0.04	<0.001 ±	0.007 ± 0.002	<0.009 ±
500	<0.013 ±	<0.013 ±	<0.013 ±	0.36 ± 0.11	0.19 ± 0.022	0.031 ± 0.007	0.07 ± 0.014
1000	<0.013 ±	<0.013 ±	<0.013 ±	0.74 ± 0.11	0.31 ± 0.093	0.049 ± 0.003	0.12 ± 0.023

Table S13: PFAA concentrations (mean ± standard deviation) in samples from the tomato experiment expressed in ng/L (water) and ng/g fresh weight (plant tissues). The nominal concentrations in the nutrient solution are given in the left hand column. The concentrations in the plant tissues were corrected for the concentrations in the control plants (data not shown).

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA
Water							
10	12.2 ± 0.59	13.4 ± 0.65	15.2 ± 1.00	12.0 ± 0.51	9.88 ± 0.25	9.30 ± 0.17	6.66 ± 0.09
100	137 ± 9.28	143 ± 7.91	165 ± 13.7	129 ± 6.43	106 ± 3.45	94.1 ± 1.61	79.8 ± 0.54
1000	1332 ± 29.0	1394 ± 24.2	1593 ± 44.6	1220 ± 16.7	1024 ± 8.99	986 ± 7.26	831 ± 2.98
10000	13518 ± 1218	14144 ± 1102	16445 ± 1811	11932 ± 676	10334 ± 455	9669 ± 304	8155 ± 114
Root							
10	<0.021 ±	<0.021 ±	0.061 ± 0.019	0.048 ± 0.026	0.066 ± 0.023	0.42 ± 0.15	0.98 ± 0.18
100	0.14 ± 0.042	0.18 ± 0.006	0.64 ± 0.034	0.50 ± 0.032	1.73 ± 0.85	5.69 ± 1.11	9.81 ± 2.92
1000	1.43 ± 0.98	1.93 ± 1.40	3.82 ± 3.60	3.67 ± 3.09	10.8 ± 6.46	49.0 ± 20.9	87.4 ± 23.1
10000	7.32 ± 4.31	14.2 ± 8.27	18.9 ± 14.6	26.8 ± 21.5	63.8 ± 36.5	309 ± 123	675 ± 69.9
Stem							
10	0.035 ±	<0.033 ±	0.042 ± 0.013	0.054 ± 0.017	0.092 ± 0.043	0.084 ± 0.014	0.11 ± 0.015
100	0.21 ± 0.18	0.31 ± 0.16	0.47 ± 0.20	0.75 ± 0.094	0.90 ± 0.18	0.79 ± 0.21	0.87 ± 0.37
1000	2.39 ± 1.01	1.90 ± 1.55	2.58 ± 2.27	4.77 ± 1.44	6.97 ± 1.67	9.25 ± 3.23	10.4 ± 3.22
10000	15.2 ± 6.58	20.2 ± 16.3	33.1 ± 21.2	51.0 ± 27.2	82.1 ± 37.6	111 ± 33.3	125 ± 11.3
Twig							
10	<0.053 ±	<0.053 ±	0.083 ± 0.026	0.058 ± 0.005	0.084 ±	0.11 ± 0.021	0.058 ± 0.004
100	0.93 ± 0.45	0.43 ± 0.28	0.72 ± 0.42	0.74 ± 0.32	0.93 ± 0.47	0.82 ± 0.14	0.77 ± 0.088
1000	13.4 ± 3.99	7.04 ± 2.46	10.9 ± 5.01	11.8 ± 6.20	11.5 ± 1.48	11.9 ± 2.84	11.7 ± 5.06
10000	99.1 ± 39.0	52.9 ± 16.4	72.0 ± 30.1	81.4 ± 23.6	101 ± 27.7	116 ± 34.7	144 ± 76.1
Leaf							
10	0.43 ± 0.15	0.10 ± 0.014	0.27 ± 0.18	0.53 ± 0.25	0.47 ± 0.21	0.23 ± 0.067	0.14 ± 0.026
100	2.18 ± 0.87	0.88 ± 0.45	2.50 ± 0.82	3.71 ± 0.84	3.55 ± 0.85	2.76 ± 0.72	1.61 ± 0.46
1000	42.6 ± 16.4	11.8 ± 3.37	49.2 ± 18.0	75.5 ± 21.2	85.8 ± 20.6	65.8 ± 15.2	39.3 ± 12.1
10000	332 ± 184	137 ± 74.8	233 ± 92.9	641 ± 261	575 ± 89.3	559 ± 161	372 ± 151
Fruit							
10	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±
100	0.58 ± 0.10	0.68 ± 0.071	0.43 ± 0.067	0.051 ± 0.010	0.029 ± 0.009	<0.013 ±	<0.013 ±
1000	5.56 ± 1.12	6.49 ± 1.18	3.40 ± 0.90	0.54 ± 0.10	0.28 ± 0.035	0.071 ± 0.011	0.025 ± 0.0004
10000	48.0 ± 39.1	65.7 ± 31.2	39.3 ± 27.2	10.3 ± 2.61	3.41 ± 0.74	0.97 ± 0.17	0.39 ± 0.066

Table S13: Continued

	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	Br-PFOS	L-PFOS
Water								
10	7.13 ± 0.037	5.98 ± 0.011	4.63 ± 0.009	4.02 ± 0.002	18.4 ± 0.99	11.7 ± 0.51	1.84 ± 0.024	6.38 ± 0.028
100	62.4 ± 0.57	59.5 ± 0.065	44.9 ± 0.074	38.2 ± 0.18	180 ± 8.59	113 ± 3.91	19.2 ± 0.33	67.8 ± 0.71
1000	674 ± 0.26	658 ± 2.20	495 ± 1.19	432 ± 0.88	1801 ± 29.2	1208 ± 18.1	213 ± 2.01	728 ± 4.39
10000	6569 ± 50.3	7175 ± 231	5283 ± 123	4556 ± 88.0	18799 ± 1487	12324 ± 871	2094 ± 88.8	7394 ± 240
Root								
10	1.26 ± 0.79	1.34 ± 0.56	0.69 ± 0.31	0.83 ± 0.44	<0.001 ±	0.15 ± 0.013	0.11 ± 0.029	0.63 ± 0.20
100	18.5 ± 8.38	13.0 ± 5.94	8.58 ± 2.28	8.48 ± 1.75	1.34 ± 0.88	2.66 ± 1.56	1.25 ± 0.23	6.99 ± 1.60
1000	150 ± 30.6	74.0 ± 10.6	60.0 ± 4.92	54.6 ± 7.79	9.21 ± 9.12	13.6 ± 8.52	6.45 ± 1.34	54.1 ± 12.7
10000	1752 ± 548	99.8 ± 40.1	102 ± 46.1	112 ± 48.0	78.7 ± 67.4	84.4 ± 48.7	114 ± 30.8	376 ± 65.7
Stem								
10	0.10 ± 0.044	0.046 ±	<0.033 ±	<0.033 ±	0.057 ± 0.055	0.046 ± 0.043	0.016 ± 0.014	0.068 ± 0.016
100	0.49 ± 0.13	0.23 ± 0.13	0.082 ± 0.059	<0.033 ±	0.56 ± 0.11	0.55 ± 0.18	0.26 ± 0.083	0.56 ± 0.44
1000	9.04 ± 2.86	4.00 ± 1.18	0.92 ± 0.30	0.18 ± 0.086	2.05 ± 1.41	5.50 ± 3.32	1.61 ± 0.45	4.69 ± 3.69
10000	104 ± 27.1	23.0 ± 4.54	5.39 ± 0.97	1.33 ± 0.45	36.7 ± 23.9	39.6 ± 20.9	10.6 ± 3.07	61.3 ± 43.9
Twig								
10	0.12 ± 0.038	<0.053 ±	<0.053 ±	<0.053 ±	0.17 ± 0.018	0.042 ± 0.025	0.013 ± 0.003	0.042 ±
100	0.38 ± 0.083	0.11 ± 0.027	0.082 ±	<0.053 ±	1.68 ± 0.41	0.70 ± 0.33	0.13 ± 0.024	0.38 ± 0.088
1000	7.83 ± 5.13	2.06 ± 1.21	0.34 ± 0.16	0.10 ± 0.035	18.3 ± 5.30	6.50 ± 1.21	1.30 ± 0.23	5.49 ± 1.42
10000	69.1 ± 36.6	20.2 ± 11.41	3.92 ± 2.93	0.89 ± 0.75	102 ± 62.6	45.0 ± 13.5	11.1 ± 3.27	69.6 ± 29.1
Leaf								
10	<0.053 ±	<0.053 ±	<0.053 ±	<0.053 ±	0.65 ± 0.37	0.27 ± 0.134	0.045 ± 0.005	0.090 ± 0.003
100	0.40 ± 0.20	0.15 ± 0.083	<0.053 ±	<0.053 ±	6.96 ± 1.84	3.37 ± 0.76	0.54 ± 0.16	1.43 ± 0.38
1000	11.3 ± 4.86	1.54 ± 0.69	0.25 ± 0.12	0.14 ± 0.072	126 ± 42.0	55.0 ± 10.8	6.39 ± 1.37	29.3 ± 6.97
10000	82.8 ± 25.3	10.1 ± 6.05	1.48 ± 0.91	0.60 ± 0.33	522 ± 192	322 ± 114	27.7 ± 9.69	303 ± 130
Fruit								
10	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	<0.001 ±	<0.001 ±	<0.002 ±	<0.009 ±
100	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	0.040 ± 0.011	<0.001 ±	<0.002 ±	<0.009 ±
1000	<0.013 ±	<0.013 ±	<0.013 ±	<0.013 ±	0.27 ± 0.11	0.056 ± 0.013	<0.002 ±	<0.009 ±
10000	0.037 ± 0.014	<0.013 ±	<0.013 ±	<0.013 ±	2.73 ± 1.71	0.80 ± 0.15	0.064 ± 0.039	0.22 ± 0.12

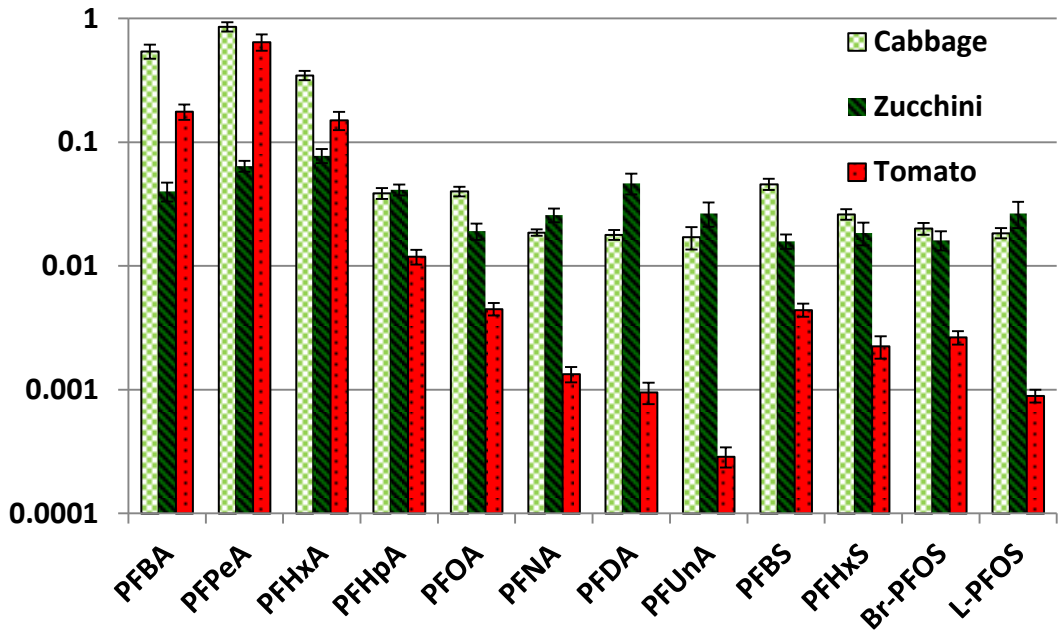

Figure S5: Edible part/leaf concentration factor, calculated by dividing the PFAA concentration in the edible part by the PFAA concentration in the leaves. Logarithmic scale. The factor shown is the average of all plants with quantifiable concentrations (see tables S11-13). Error bars denote standard error.

Table S14: Overview of various hydrophobicity parameters. Log k_0 by de Voogt et al. [2] , modelled log K_{OW} by Arp et al. [3], Wang et al. [4] and Kelly et al. [5], log $P^{0'}$ by Jing et al. [6] and log D_{OW} values modelled with ACD/PhysChem Suite, taken from www.chemspider.com.

	log k_0	*Calculated log K_{OW}*				*log $P^{0'}$*	*log D_{OW}*	
method	HPLC	COSMO-therm		Sparc		Voltammetry	pH 5.5	pH7.4
ref	de Voogt	Arp	Wang	Arp	Kelly	Jing	ACD/PhysChem Suite	
PFBA	1.83	-	2.82	-	-	-0.68	0.20	0.18
PFHxA	2.88	3.26	3.42	3.12	-	0.54	1.25	1.24
PFHpA	3.44	3.82	4.06	3.83	2.80	1.15	3.13	3.11
PFOA	4.22	4.30	4.67	4.59	3.60	1.76	4.02	4.00
PFNA	4.93	4.84	5.30	5.45	4.50	2.37	4.91	4.89
PFDA	5.73	5.30	6.50	6.38	5.40	2.98	5.80	5.78
PFUnA	6.22	5.76	7.15	7.40	6.40	3.59	-	-
PFDoDA	7.02	-	7.77	-	7.10	4.20	7.58	7.56
PFBS	2.32	-	3.90	-	-	-	0.18	0.18
PFHxS	3.63	-	5.17	-	-	-	1.75	1.75
PFOS	5.02	5.25	6.43	5.26	4.30	2.57	3.53	3.53

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Chapter 4

FATE OF A PERFLUOROALKYL ACID MIXTURE IN AN AGRICULTURAL SOIL STUDIED IN LYSIMETERS

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Abstract

Perfluoroalkyl acids (PFAAs) are environmental contaminants of concern in both food and drinking water. PFAA fate in agricultural soil is an important determinant of PFAA contamination of groundwater and crops. The fate of C4-C14 perfluorinated carboxylic acids (PFCAs) and two perfluorinated sulfonic acids (PFSAs) in an agricultural soil was studied in a field lysimeter experiment. Soil was spiked with PFAAs at four different levels and crops were planted. PFAA concentrations in soil were measured at the beginning and end of the growing season. Lysimeter drainage water was collected and analysed. The concentrations of all PFAAs decreased in the surface soil during the growing season, with the decrease being negatively correlated with the number of fluorinated carbons in the PFAA molecule. PFAA transfer to the drainage water was also negatively correlated with the number of fluorinated carbons. For the C11-C14 PFCAs most of the decrease in soil concentration was attributed to the formation of non-extractable residues. For the remaining PFAAs leaching was the dominant removal process. Leaching was concentration dependent, with more rapid removal from the soils spiked with higher PFAA levels. Model simulations based on measured K_d values under-predicted removal by leaching. This was attributed to mixture effects that reduced PFAA sorption to soil.

Keywords: PFAA, soil, leaching, sorption, mixture effect

Introduction

Perfluoroalkyl acids (PFAAs) are a group of highly persistent environmental contaminants (Moody et al. 2000; Prevedouros et al. 2006). Some PFAAs have been shown to have toxic effects (Lau et al. 2007; Domingo 2012; Saikat et al. 2013). As a result, the European Food Safety Authority has established tolerable daily intakes (TDIs) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) (Johansson et al. 2009) and is considering establishing them for other PFAAs. Human exposure to PFAAs occurs primarily via food and drinking water (Fromme et al. 2009; Klenow et al. 2013). PFAAs enter the agricultural food chain via root uptake from soil (Stahl et al. 2009). Agricultural soil can become contaminated with PFAAs via atmospheric deposition, the application of pesticides, or the addition of material for soil improvement such as sewage sludge or industrial waste (Scott et al. 2006; Wilhelm et al. 2008; Sepulvado et al. 2011; Gilljam et al. 2016). The fate of PFAAs in agricultural soil is therefore an important determinant of the PFAA contamination of groundwater and crops, and thereby of the potential for human exposure.

To understand the fate of PFAAs in soil, a variety of laboratory studies have been conducted. One focus has been on batch sorption experiments, which have been used to quantify the soil-water distribution coefficient K_d and to understand the soil and chemical properties that influence it (Higgins et al. 2006; You et al. 2010; Chen et al. 2012; Ferrey et al. 2012; Chen et al. 2013; Guelfo et al. 2013; Milinovic et al. 2015; Zhang et al. 2015; Chen et al. 2016). There has also been considerable research with soil column experiments to study the leaching and persistence of PFAAs under controlled conditions (Gellrich et al. 2012; Vierke et al. 2013; McKenzie et al. 2015; McKenzie et al. 2016). This work has shown PFAAs to be persistent in soil and provided insight into how leaching is influenced by the PFAA's structure (perfluoroalkyl chain length and functional group) and by soil properties (e.g.,, organic carbon content and pH). However, there have been comparatively few studies of PFAA behaviour under field conditions. A notable exception is a long term lysimeter experiment in which PFOA and PFOS were applied to the surface of 1.5 m deep soil columns in outdoor lysimeters and their concentrations in leachate were monitored. After 42 months only 3.1% of the PFOA and 0.013% of the PFOS had eluted (Stahl et al. 2013).

In this work the fate of PFAAs in an agricultural soil was studied in a field lysimeter experiment that provided a close approximation of environmental conditions. In parallel, K_d was measured in the laboratory in order to assess whether the leaching behaviour it predicts is consistent with that observed in the field experiment. A broad spectrum of PFAAs consisting of C4-C14 perfluorinated carboxylic acids (PFCAs) and two perfluorinated sulfonic acids (PFSAs) was included in the experiment in order to explore the impact of chemical structure on fate. The soil was spiked at four different levels, and the chemicals were uniformly mixed throughout the whole soil column, as this approximates agricultural situations where contaminants are mixed into soil via tilling. The lysimeters were planted with different crops and the chemical concentrations in soil, drainage water and plant parts were studied. Here we report on the results for the soil system (soil and drainage water).

Materials and Methods

Chemical reagents and lab materials

Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS) and perfluorooctane sulfonic acid (PFOS) were purchased. Each had a purity >95%. The suppliers and purities of these chemicals, their molecular formulas and the ^{13}C-labeled internal standards used for their quantification are listed in Tables S1 and S2 of the Supporting Information (SI) along with details about the other chemicals used.

Materials used for extraction and clean-up of the samples included Acrodisc LC13 GHP Pall 0.2 µm filters from Pall Corporation (Port Washington, NY, USA), 15 mL polypropylene (PP) tubes with screw caps from Sarstedt (Nümbrecht, Germany), centrifugation filter tubes (50 mL, 0.2 µm nylon filter) from Grace (Breda, Netherlands), and 2.0 mL PP vials from VWR International (Amsterdam, Netherlands).

Lysimeter experiment

The lysimeter experiment was conducted at the Fraunhofer Institute for Molecular Biology and Applied Ecology IME in Schmallenberg, Germany. A total of 20 lysimeters were employed. The soil of 16 of the lysimeters was spiked with equal concentrations of the 13 PFAAs studied to give four lysimeters at each of five different PFAA contamination levels: background concentrations (unspiked), 0.1 mg/kg, 1 mg/kg, 5 mg/kg, and 10 mg/kg of each PFAA. For comparison, PFOA and PFOS concentrations of ~1 mg/kg were measured in contaminated agricultural soil in Arnsberg, ~30 km from Schmallenberg (Wilhelm et al. 2008). Each lysimeter had a surface area of 1 m² and a total depth of 60 cm. Drainage water was collected in a stainless steel container. The lysimeters were outdoors and unprotected. Precipitation was measured at the site.

The lysimeters were each filled with 450 kg of sand (30-60 cm depth, pH 5-5.5, organic carbon content 0.3-0.5%, hereafter called the lower soil layer) and 450 kg of loamy sand (0-30 cm depth; 71% sand, 24% silt, 5% clay, pH 5.67, organic carbon content 0.93%, upper soil layer). This resembled a typical soil from northwestern Germany. The soil used for the upper layer is available as a reference soil (Refesol 01-A) from Fraunhofer IME (www.refesol.de/boden01a.shtml). The soil used for the lower layer is the soil that naturally occurs under the soil used for the upper layer.

The spiking of the soil was done stepwise. First a stock solution was prepared containing all PFAAs in methanol. Then 2 kg of soil were spiked with the stock solution and homogenized. Afterwards the 2 kg of spiked soil was mixed with 90 kg of soil in a cement mixer to achieve the desired concentration. This was repeated 5 times for each layer in each lysimeter. Samples were taken from each 90 kg batch, combined, and stored at –20 °C for later determination of the initial PFAA concentration in the soil of each lysimeter. The filled lysimeters were covered until planting, which occurred within one week.

The lysimeters were planted with onion (*allium cepa*), carrot (*daucus carota*), radish (*rapahnus sativus*), lettuce (*lactuca sativa*), pea (*pisum sativum*), or maize (*zea mays*). Each crop was planted in one lysimeter from each soil contamination level with the exception of onion, carrot and radish,

which were planted together in one lysimeter from each soil contamination level. On June 21, 2011, ca. 20 onion seeds, 20 carrot seeds, 20 radish seeds, 6 pea seeds, 20 lettuce seedlings or 9 maize seedlings (pre-grown in uncontaminated soil) were planted. The lysimeters were watered after planting, and kept humid by rain events and additional watering as needed (see Table S3 for water inputs to the lysimeters). When a significant quantity of drainage water had accumulated in the drainage container it was retrieved, weighed, and a sub-sample of approx. 10 mL was transferred to 15 ml centrifugation tubes and stored at 4 °C until analysis.

Radish, lettuce, pea and maize were harvested at maturity on Aug. 9, Sept. 1, Oct. 4 and Oct. 19, respectively, and soil samples were taken. This corresponds to lysimeter exposure periods of 49, 72, 105, and 120 days. For lettuce, pea and maize the soil samples were collected with a corer. The soil core was divided into two 30 cm lengths to provide average concentrations in the two soil types used. The soil was packed in freezer bags and stored at –20 °C until analysis. Onion and carrot did not germinate. For radish a sample of the top 1-2 cm of the soil was collected, as at the time of radish harvest it was still hoped that the onions and carrots seeded in the same lysimeters would germinate. PFAA concentrations were measured in the upper layer for lettuce, pea and maize, the surface layer for radish, and the lower layer for lettuce.

Determination of K_d

Soil-water distribution coefficients (K_d) were determined for each of the two soils according to OECD guideline 106 using 2 g of soil and 10 ml of water (OECD 2000). A mixture containing equal concentrations of the 13 PFAAs was tested. Seven different initial concentrations were used: 1, 5, 10, 50, 100, 500, and 1000 ng/mL. Each concentration level was measured in duplicate. PFAAs were analysed in both soil and water. An equilibration time of 3 days was used based on a preliminary experiment. Only results showing a PFAA mass balance between 70% and 140% were retained.

PFAA analysis

The soil was dried in an oven at 40 ˚C until no further weight loss was recorded. After homogenization, 1 g of soil was weighed in a 15 mL PP tube and spiked with internal standards. The soil was then extracted with 10 mL MeOH by vortex mixing for 1 minute and sonication for 10 minutes. Phase separation was achieved by centrifugation (10 min, 3000 RPM). The supernatant was transferred to a new 15 mL PP tube and concentrated in a Rapidvap (Labconco, Kansas City, US). The extraction was repeated twice with 5 mL MeOH. In a pre-experiment it was found that the third extraction contained only ~5% of the mass of PFAAs in the first extraction, so it was decided that three extractions were sufficient. The extracts were combined and concentrated in the Rapidvap to a final volume of 1 mL. They were diluted 1:1 with water prior to analysis to match the injection conditions of the HPLC.

For pore water analysis, 20 g of the soil was put in a 50 mL centrifugation filter tube with a 0.2 µm nylon filter. After 20 minutes of centrifugation at 2000 RPM, 0.5 mL of pore water was transferred to a vial. The internal standards and MeOH were added to achieve a final volume of 1 mL. Drainage water and water from the K_d determination was filtered and then treated like the pore water. The solutions were stored at 4 °C until instrumental analysis.

An HPLC system (LC-20AD XR pump, SIL-20A autosampler and SCL-10A VP system controller, Shimadzu, Kyoto, Japan) coupled with a tandem mass spectrometer (4000 QTrap, Applied Biosystems, Toronto, Canada) was used to analyze the samples for PFAAs. A pre-column (Pathfinder

300 PS-C18 column, ID 4.6 mm; length 50 mm; 3 µm particle diameter; Shimadzu, Duisburg, Germany) prior to the injection valve was used to remove potential background contamination.

Separation of the analytes was achieved using an ACE 3 C18-300 column (ID 2.1 mm; length 150 mm; 3 µm particle diameter; Advanced Chromatography Technologies, Aberdeen, Scotland) maintained at 30 °C with a mobile phase gradient consisting of two eluents A (40:60 MeOH:H2O, v:v) and B (95:5 MeOH:H2O; v:v), both containing 2 mM ammonium acetate. A volume of 20 µl was injected. The gradient used for separation and the mass transitions as well as other mass spectrometer settings can be found in the Supporting Information (Text S1). The mass spectrometer was equipped with an electrospray ionization interface operating in the negative ionization mode, and it was run in a scheduled MRM-mode.

Raw data were processed with the Analyst 1.5 software (Applied Biosystems).

Quality assurance of PFAA analysis

Each soil sample was extracted twice and each soil extract was injected in duplicate. The relative standard deviation of the concentrations derived from these four injections was <10% for all analytes in all samples.

Concentrations were quantified using a twelve-point calibration with fitted correlation lines that had R^2 values of >0.99 for all analytes.

Recoveries from the analytical procedure for soil were determined by comparison with a matrix free solution spiked with internal standard immediately prior to injection. Average recoveries of the internal standards in the samples were between 91% (PFPeA) and 112% (PFDoDA) (Table S4 in the Supporting Information).

Limits of quantification (LoQs) for soil (Table S5 in the Supporting Information) were calculated on the basis of the lowest validated calibration standard (signal to noise ratio ≥10). They were derived from the amount injected back calculated to an extract volume of 1 mL and divided by the average extracted sample quantities. Method blanks were prepared repeatedly with the same extraction procedure as the samples, but showed no quantifiable contamination. Solvent blanks were injected every ten injections to check for contamination of the LC system and for memory effects, but no contamination or memory effects were observed during the study. The LOQ for leachate was 0.5 µg/L for all substances. It was validated by replicate (five-fold) determination of fortified blank samples at the LOQ and at 10 x LOQ level.

Since PFOS is the only compound for which branched isomers were included in the standards used for the calibration curve, branched isomers could only be quantified for PFOS. All reported PFOS concentrations represent the sum of non-branched and branched isomers.

Model of PFAA fate in soil

The fate of PFAAs in soil was simulated using the one-dimensional model PELMO (Klein 1995). PELMO calculates the vertical transport of chemicals in the unsaturated soil system within and below the plant root zone. PELMO considers various environmentally relevant processes (run-off, erosion, plant uptake, sorption, leaching, degradation in soil and on plants, and volatilisation of pesticides). However, the model has been mainly used to estimate the leaching potential (described in more detail in e.g., (FOCUS 2000; FOCUS 2002; FOCUS 2014)), and this is the context in which it was

employed here. PELMO is presently officially used in European and national registration of pesticides (EFSA 2017).

To calculate the soil water regime, PELMO uses the field capacity approach (Carsel et al. 1984). For the simulations the soil was divided into different compartments (layers) of 5 cm each. All properties (e.g., soil density, soil moisture, temperature, but also the concentration of the chemical) are considered to be uniform within these compartments. Dependent on the soil depth, different processes determining the water content are considered. The model distinguishes between the surface layer, the segments in the root zone, and the compartments below the root zone. A time step of one day was considered for the simulations.

Since data on potential evapotranspiration were not available, it was estimated internally by the model using daily air temperature. Plants are characterised in the model by their maximum rooting depths and seasonal Kc-factors. The Kc-factors are used to calculate crop specific potential evapotranspiration. Plant growth is assumed to be linear. Actual evapotranspiration was calculated based on daily plant growth, the soil moisture at the current rooting depth, and the crop specific potential evapotranspiration.

Solute transport is calculated with the Convection-Dispersion-Equation (CDE). In the model non-linear sorption is implemented using a Freundlich isotherm. However, as the experimental data did not indicate any non-linear behaviour the Freundlich exponent was set to 1.0 for all PFAS. Sorption was described using the measured K_d values for the top and the bottom layer. Transformation was switched off.

Results and discussion

Quality assurance of the experiment

The initial concentrations in the soil were close to the nominal concentrations for all chemicals (98±12%, Figure 1, Table S6). This indicates that the soil contamination procedure was successful and that the soil analytical method was accurate. An exception was PFHxA, for which a deficit in initial concentrations was observed in the upper layer. This deficit increased with increasing contamination level, being negligible at the lowest spiking level and ~50% at the highest spiking level. We have no explanation for this observation.

At the end of the lettuce and peas experiments the concentrations in the uncontaminated soil were at least a factor of 10 less than the concentrations in the upper soil layer with the lowest spiking level with the exception of PFPeA (factor 5 less) and PFHxA (factor 8 less) in the pea experiment. In the maize experiment the smallest differences were a factor of 4-5 (for PFNA, PFDA, PFUnA, and PFOS), while in the radish experiment (where only the top 1-2 cm of soil was sampled) the differences were less than a factor of 4 for 10 of the analytes. This indicates that the influence of the surrounding environment on the PFAA concentrations in the contaminated soils was in all cases small or negligible. When quantifiable, PFAA concentrations from the non-spiked lysimeters were subtracted from the concentrations in the spiked lysimeters.

The different plant crops had an influence on the amount of evapotranspiration from the lysimeter. The amount of drainage water generated was markedly greater for lettuce (mean of 129 L) than for radish (99 L), pea (83 L) or maize (80 L). However, the crops did not play a significant role in the sequestration of PFAAs out of the soil. The quantity of PFAAs in the vegetation at harvest did not

exceed 1% of the amount added to the soil with the exception of PFBA, which had a maximum sequestration of 12% from the lowest spiking level with maize.

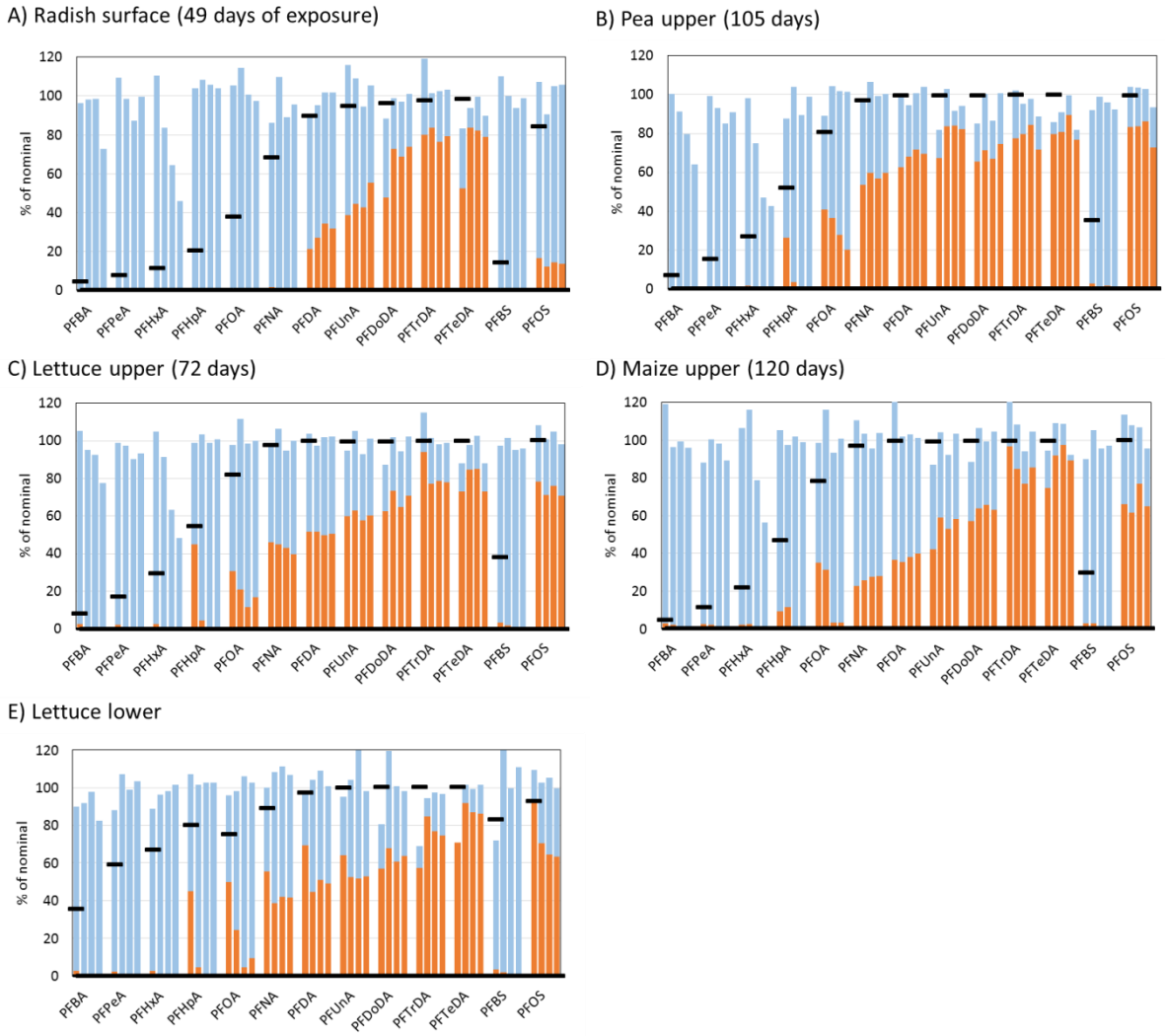

Figure 1: PFAA concentrations in the soil expressed as a percent of the nominal concentration at the start of the experiment. Blue bar = Measured initial concentration; Red bar = Measured final concentration (at harvest); Black symbol = Modeled final concentration. For each chemical the results are shown for spiking levels 1-4 (in order from left to right). For radish the final soil concentration refers to the surface layer (top 1-2 cm measured and top 2 cm modeled). The exposure period of the lysimeters is given in the panel headings.

Measured loss of PFAAs from soil

The concentrations of all of the chemicals decreased in soil during the 49-120 days from the start of the experiment to the end of the experiment (Figure 1, Table S7). There was a clear relationship between the final concentration in the soil as a fraction of the initial concentration and the number of perfluorinated carbon atoms in the molecule, with the median final fraction remaining in the upper soil layer increasing from <1% for PFBA to ~90% for PFTeDA (Figure S1).

The spiking level could potentially influence the fate of the PFAAs in soil. In this study the decrease in soil concentration was similar between the different spiking levels for most chemicals in the upper soil layer (Figure 1), suggesting that this was not the case. However, for those chemicals with high

removal rates from the upper soil layer (PFBA, PFPeA, PFHxA, PFHpA, PFBS), a dependency on the spiking levels was observed. The fraction of the nominal soil concentration remaining in the soil at the end of the experiment decreased as the spiking level increased, often by more than a factor of 10 between the lowest and the highest spiking level (Table S8). In other words, the removal of these chemicals became more efficient as the spiking level increased. A similar trend was observed in the lower soil layer, whereby it affected longer chained PFAAs (up to PFDA). Gellrich et al. (2012) observed interactions between PFAAs in soil. In laboratory soil column experiments they found that the addition of longer chained PFAAs increased the mobility of shorter chain PFAAs already in soil columns. Their explanation was that the longer chained PFAAs are able to displace the shorter chained PFAAs from their binding sites.

For the lysimeters growing lettuce, pea, and maize, the loss of the PFAAs from the soil was comparable. This indicates that the crop did not have a major influence on the PFAA fate in the soil. The lysimeters growing radish showed a greater loss of many chemicals, especially the C7-C11 PFCAs and PFOS. For radish, only the top 1-2 cm of the upper soil layer was sampled, whereas for the other crops the full 30 cm of each soil layer was sampled. Given that the length of the experiment was shorter for radish than for the other crops, this indicates that the loss of PFAAs was greater in the top 1-2 cm of the soil than in the top 30 cm. This can be explained by leaching removing a larger fraction of the PFAAs from the top 1-2 cm (see below).

PFAAs in drainage water

Three drainage samples were collected on July 29, August 11 and August 19. Not all of the results of the PFAA analysis of the drainage water could be used quantitatively. The extracts had to be diluted prior to analysis due to high concentrations of some analytes, which introduced uncertainty into the quantification. We therefore present only the results for Level 1, which had the lowest concentrations and thus were most proximate to the analyte:internal standard ratios in the calibration curve.

The drainage water data were used together with the concentrations in the soil at the beginning and the end of the experiment to assemble a mass balance of PFAAs in the lysimeter growing lettuce in soil with Level 1 contamination (Figure 2). The contribution of the residual in soil to the mass balance at the end of the experiment increased with PFAA chain length, in agreement with Figure 1. The drainage water made a major contribution to the mass balance, accounting for 30-40% of the original amount present in the lysimeter for PFBA, PFPeA, PFHxA, PFHpA and PFBS. For the remaining PFAAs the contribution of drainage water decreased with increasing chain length. There was also a strong correlation between the concentration in drainage water and the number of perfluorinated carbons in the PFAA molecule (nFC) for nFC > 5 (Figure S2), confirming that leaching of the PFAA was dependent on chain length. Finally, the mass balance did not close (Figure 2). Possible explanations include the fact that not all drainage water was analysed and the uncertainty in the drainage water analysis.

Evidence for chain length dependence of PFAA leaching has been reported previously. In soil plots to which sewage sludge contaminated with PFAAs had been applied, the ratio of the PFAA concentration at depth (60-120 cm) to that in surface soil decreased with increasing perfluoroalkyl chain length (Sepulvado et al. 2011). In a laboratory study in which PFAAs were applied to the top of 60 cm soil columns and then eluted with water for >100 weeks, PFAAs with nFC <6 eluted with or

shortly after the conservative tracer, while the elution of PFHpA, PFHxS, PFOA and PFOS took progressively longer with increasing nFC (Gellrich et al. 2012). Retardation factors for PFBA, PFPeA, PFHxA, PFOA and PFNA applied to the top of a column containing water saturated aquifer material increased with increasing nFC (Vierke et al. 2014). Similarly, retardation factors of C6-C11 PFCAs increased with increasing chain length in laboratory column studies (McKenzie et al. 2015). In a long term lysimeter experiment, the time trend for the concentrations of PFHxA, PFHpA, PFBS and PFHxS in drainage water indicated that these chemicals had largely eluted from the soil column after 27 months, while the concentrations of PFOA in drainage water peaked after 6 months, continuing at that level for 36 months, and the PFOS concentrations continued to increase over the 42 month monitoring period (Stahl et al. 2013). In that study, 3.1% of the PFOA applied to the soil eluted during the 42 months, while in our study only 20-30% was left determinable in the soil after < 4 months. This can be at least partly explained by the method of PFAA application (to the whole soil column in this study versus to the soil surface in Stahl et al., 2013).

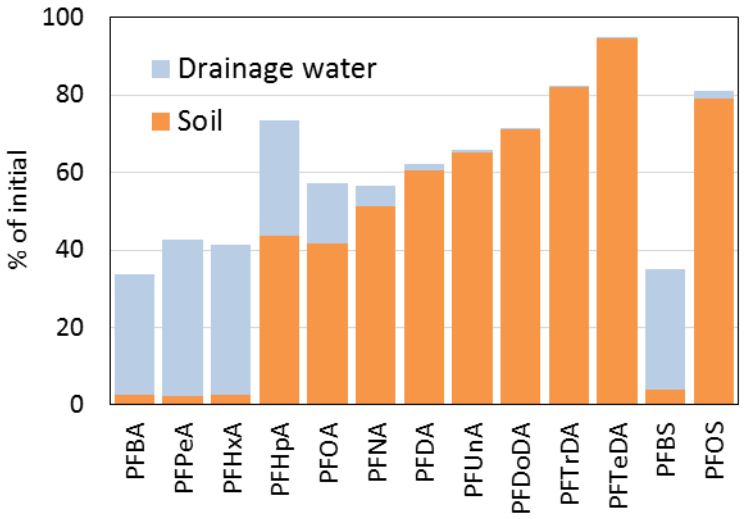

Figure 2: Quantity of PFAA in soil at harvest and in the accumulated leachate for the level 1 lysimeter planted with lettuce. The results are expressed as a percentage of the initial quantity of PFAA in the lysimeter.

Soil-water partition coefficients (K_d)

The measured K_d values ranged from 0.11 L kg^{-1} for PFBA to 330 L kg^{-1} for PFTeDA in the upper layer soil and 3700 L kg^{-1} in the lower layer soil (Table S9). K_{OC} values were calculated, and they lay within the range of K_{OC} values reported in the literature (see Table S10). There was a strong positive correlation between K_d and nFC. A linear regression of log K_d for the PFCAs against nFC gave correlation coefficients of 0.97 and 0.96 for the upper and lower layer soils, respectively (Figure 3). The slope of the regression line was 0.48 for the lower layer soil and 0.39 for the upper layer soil; at a first approximation the addition of two CF2 groups to the PFCA chain increased K_d by an order of magnitude. Higgins and Luthy observed this chain length dependence in batch sorption experiments of PFOA, PFNA, PFDA AND PFUnA to sediments. This relationship was confirmed for sorption of these chemicals to soil in later studies (Guelfo et al. 2013; McKenzie et al. 2015).

Both soils show a clearly weaker influence of nFC for shorter chain PFCAs (nFC < 7), with the addition of two CF2 groups increasing K_d by only half an order of magnitude. Higgins and co-workers have

reported K_d values for PFBA and PFPeA that were similar or higher than PFHxA, based on batch sorption experiments and soil column retention studies (Guelfo et al. 2013; McKenzie et al. 2015). On the other hand, K_d values derived from breakthrough times of PFAAs in sediment columns showed an increase from PFBA through PFPeA and PFHxA of more than an order of magnitude (Vierke et al. 2014). We observed a behaviour intermediate between these reports, with a K_d dependence on nFC that was positive but markedly weaker than reported by Vierke et al.

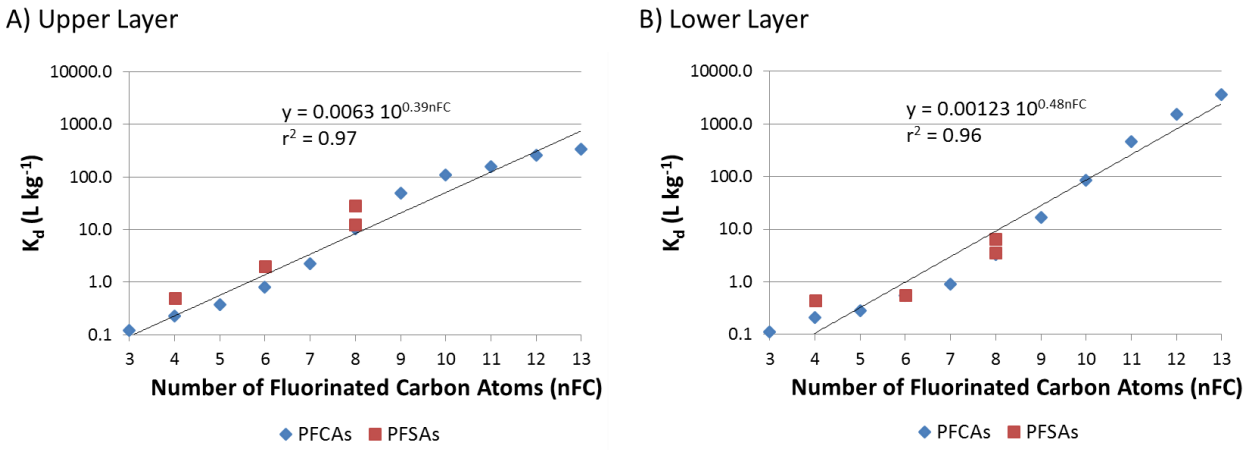

Figure 3: Soil water distribution coefficients K_d of the PFAAs versus the number of fluorinated carbon atoms in the PFAA molecule for the two soils studied. The line and equation show the linear regression of K_d against nFC for the PFCAs.

The PFSAs showed a similar dependence of K_d on nFC. From PFHxS to PFOS (i.e.,, nFC from 6 to 8), K_d increased by 1 order of magnitude in both soils, while the increase between PFBS and PFHxS was markedly smaller (Figure 3). K_d for the PFSAs was a factor of ~2 larger than K_d for the PFCA with the same nFC, with the exception of PFHxS in the lower layer soil (Figure 3, Table S9). Exchanging the carboxylic functional group for a sulfonate functional group thus had an influence on K_d. The literature provides contrasting reports on this subject. In their pioneering work, Higgins and Luthy (2006) concluded that K_d was on average a factor of 1.7 higher for PFSAs than for PFCAs with the same nFC, but a later reanalysis of their data indicated that there was no difference (Rayne et al. 2009a). Follow-up work by Higgins' group found that K_d values of PFSAs were 0.49 log units greater (McKenzie et al. 2015).

K_d was larger for the upper layer soil than for the lower layer soil for all PFSAs and the PFCAs with $6 \leq$ nFC ≤ 11. The difference was largest for PFNA, PFDA, and PFOS (a factor of 3-4). The upper layer soil was a loamy sand with an organic carbon content of 0.93% whereas the lower layer soil was sand with an organic carbon content of 0.3-0.5%. A range of studies have reported that these PFAAs sorb primarily to the organic matter in soil (Higgins et al. 2006; Chen et al. 2012; Chen et al. 2013; Milinovic et al. 2015), and it was recently shown that PFOA selectively binds to soil microbial protein (Masoom et al. 2015). Therefore the larger K_d values in the upper layer soil were expected.

In contrast, K_d was smaller for the upper layer soil than for the lower layer soil for the PFCAs with nFC ≥ 11. For PFTeDA the difference was more than an order of magnitude. Although organic carbon is believed to dominate sorption of PFAAs to soil, they have also been shown to sorb to soil minerals (Zhang et al. 2015). This sorption can be strong. A K_d value of 2.81 L kg^{-1} was measured for PFOS to organic carbon free Ottawa River sand (Johnson et al. 2007), which is similar to the K_d value

measured for PFOS to the lower layer soil in this study (3.15 L kg^{-1}). The much stronger sorption of the longer chain PFCAs to the lower layer soil suggests that sorption to soil minerals may be a comparatively more important process for long chain PFCAs. A recent review concluded that at least organic carbon content, pH, and clay content influence PFAS sorption to soil (Li et al. 2018b).

K_d was compared with soil / pore water distribution coefficients calculated from the PFAA concentrations measured in soil and soil pore water in the lettuce and maize lysimeters at the time of harvest. For the upper layer soil, the average soil / pore water distribution coefficient across all exposure levels agreed within a factor of 2.6 with the exception of PFPeA. For the lower layer soil good agreement was obtained for the shortest and longest chained compounds, while PFNA, PFDA, PFUnA and PFOS had average soil / pore water distribution coefficients that were about one order of magnitude greater than the K_d values. However, there was considerable variability between exposure levels, with the lower exposure levels tending to have higher soil / pore water distribution coefficients, particularly for the PFAAs with nFC ≥ 7 in the lower layer soil (Figure S3).

Modeled behaviour of PFAAs in the lysimeter

The model predicted the removal of the PFAAs from the lysimeter via the drainage water. It assumed that losses due to volatilization and transformation are negligible, assumptions which are consistent with current understanding of the environmental chemistry of PFAAs. It was also assumed that the formation of non-extractable residues was negligible. The modeled concentration in the upper soil layer at the end of the experiment increased with increasing chain length (Figure 1). Since removal via drainage water was the only modeled loss process, removal via drainage water decreased with increasing chain length. This is consistent with the measured concentration trend in the drainage water (Figure S2). However, the modeled and measured residual concentrations in soil differ greatly. Whereas the measurements indicated <5% of PFBA, PFPeA, PFHxA, PFHpA (except Level 1) and PFBS was left in the soil at the end of the experiment in all lysimeters, the model indicated the amount remaining was much higher (as high as 64% in the upper layer and 90% in the lower soil layer for PFHpA in lettuce (Figure 1)). Similarly, the PFAAs with nFC >7 were predicted by the model to be fully retained (>90%) in the soil, while the measurements indicated that just 80% of the PFTeDA and as little as 25% of PFNA were left in the soil at the end of the experiment (Figure 1). The model results changed little when the model was rerun using realistic worst case assumptions in the water mass balance that maximized leachate generation. Possible explanations for this inconsistency between theory and observations are discussed below.

Fate of longer chained PFAAs in soil

The loss of PFUnA, PFDoDA, PFTrDA and PFTeDA from the soil ranged from 14% to 40% (Figure 1). Given the high persistence and low volatility of PFAAs, the only mechanisms expected to have a major impact on their fate in soil are leaching and the formation of non-extractable residues. As noted above, a negligible fraction of the chemicals in the soil was sequestered into the crops.

The concentrations of PFUnA, PFDoDA, PFTrDA and PFTeDA in drainage water were <5% of the concentrations of the short chained PFCAs and PFBS (Figure S2). This indicates that only a small fraction of these chemicals was removed from the soil column by leaching. This is consistent with the high K_d values of these chemicals. Furthermore, for PFDoDA, PFTrDA and PFTeDA the loss from the surface soil (top 1-2 cm) in the radish lysimeters was not greater than the loss from the upper soil layer (30 cm) in the other lysimeters. This indicates that downward displacement of these chemicals

via leaching was negligible, and would suggest that the formation of non-extractable residues was a major loss process for the longer chained PFAAs. Since the initial concentrations in the soil were close to the nominal concentrations, the non-extractable residues would not have been primarily formed immediately after contamination of the soil. It is possible that they were formed during the experiment, perhaps as a result of natural weathering processes. We note that it may have been possible to extract more of these chemicals from the soil using a more aggressive extraction; non-extractable is relative to the extraction method employed.

Fate of shorter chained PFAAs in soil

The removal of the PFAAs with nFC <7 was much more rapid than predicted by the model, most particularly for the lower soil layer. This cannot be attributed primarily to the formation of non-extractable residues, as large fractions of these chemicals were found in the drainage water (Figure 2). Approximately equal concentrations of the PFAAs with nFC ≤ 6 were found in the leachate (Table S11), whereas the model predicted a pronounced dependency on chain length, with concentrations of PFHpA that were 3.2 times less than concentrations of PFBA in drainage water. This indicates that leaching of the chemical was apparently much more rapid than predicted by the model.

One possible explanation for the underestimation of leaching by the model is that the K_d values used were too high. We employed K_d values that had been measured with the same chemical mixture and the same soils as used in the study. However, the maximum PFAA concentrations in soil for the K_d measurements were at the lower end of the PFAA concentration range in the lysimeter experiment. Furthermore, the composition of the PFAA mixture sorbed to the soil was different as a result of the higher water:soil ratio in the K_d measurement. While the laboratory measurements showed no evidence that K_d decreases with increasing concentration (Table S9), the soil / pore water distribution coefficients measured at harvest tended to increase with increasing contamination level, and they were generally smaller than K_d for the shorter chained PFAAs (Figure S3). Hence the measured K_d may have overestimated soil / water distribution in the lysimeters.

Replacing the K_d values with the soil / pore water distribution coefficients yielded model predictions that agreed somewhat better with the observations, but the model continued to severely underpredict leaching (results not shown). As already noted, the loss of chemical from the soil increased with increasing level of soil contamination. This suggests that the process causing more rapid leaching of the chemicals is concentration dependent. Gellrich et al. showed that when PFBA was applied to a soil column that only about 80% could be eluted with water(Gellrich et al. 2012). However, when PFHxA and PFHxS were then added the remaining PFBA eluted immediately. They attributed this to the longer chained PFAAs out-competing PFBA for strong sorption sites in the soil. Such a mechanism could explain the concentration dependence observed in this study as well as the discrepancy between model predictions and observations. The longer chained PFAAs may have occupied sorption sites preferred by the PFAAs and reduced the sorption capacity of the soil for the shorter chained PFAAs. These PFAAs would have been more rapidly eluted from the lysimeters. For higher concentrations in the soil, the displacement would have been greater and would have affected PFAAs with longer chain lengths. As the experiment progressed and the concentrations of many of the PFAAs were depleted, the competition for sorption sites would be less intense and soil / pore water distribution coefficients would increase. This explanation is generally consistent with the observations. Interestingly, there was no time trend in the soil concentrations between the harvest dates for lettuce and maize (Figure S4), which could suggest that the accelerated leaching was largely

over by the time of the lettuce harvest. However, there was comparatively little leachate produced after the lettuce harvest, so little loss of chemical by leaching would be expected.

Implications of the findings

This study shows that the shorter chained PFCAs (PFBA, PFPeA, PFHxA) and PFBS are readily transported with water through soil. As a consequence, if these chemicals are introduced to agricultural soil with significant downward transport of water, they will reside in the surface soil for only a short period. The exposure of crops to the chemicals will be only transient. On the other hand, a large portion of these chemicals will be bioavailable for uptake by the roots. Furthermore, they will be rapidly transported to and with groundwater, where comparatively high concentrations will occur.

The longer chained PFCAs (PFDoDA, PFTrDA, PFTeDA) sorb strongly to soil and there is very limited transport with water. When introduced to agricultural soil, these chemicals will largely stay put. The exposure of crops to these chemicals will continue for many years, albeit with a low bioavailiability; they will be a long-term contamination problem. In addition, repeated inputs of these chemicals will result in their accumulation in soil; while the concentrations arising from one season's input may be of little concern, after several years or more of inputs they could become problematic. On the positive side, the transport of these PFAAs to and with groundwater will be limited, and the chemical concentrations in groundwater will be comparatively low. The slow rate of transport to groundwater will offer more time for remediation of surface soils before the groundwater becomes contaminated. In contrast to the shorter chain PFAAs such as PFBA and PFBS, there are water treatment technologies that efficiently remove longer chained PFCAs from water (Eschauzier et al. 2012a). However, groundwater contamination can be expected to persist for a much longer period of time.

This work suggests that non-extractable residues of PFAAs can form in soil under environmental conditions. In this study the PFAAs were applied to the soil using a solvent carrier. It is unknown whether non-extractable residues are also formed when the PFAAs enter the soil via other means more commonly encountered in the environment, such as atmospheric deposition or sewage sludge application. Sepulvado et al. (2011) conducted a mass balance of soil that had received PFAAs via sewage sludge over 3 years, but their study was not designed to identify losses of the PFAAs of the order of 20% via, e.g.,, formation of non-extractable residues.

Finally, this work highlights the necessity of measuring K_d values under conditions that closely approximate those in the environment of interest. Where the chemical contamination is a mixture, it can also be important that K_d is measured for the mixture, as mixture components can interact to influence sorption. For PFAAs, firefighting foams are mixtures that may warrant this treatment. Thereby it may not be sufficient to employ the mixture composition present in bulk soil in the K_d experiment; one should instead strive to have the same mixture composition in the sorbed phase in the K_d experiment and in the soil of interest.

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Supporting Information

Description of the instrumental method

The analytical methodology was according to the methods described by Eschauzier et al. (2010).[1] The measurements were conducted in the scheduled MRM-mode (see Table S2). Briefly, instrumental settings included:

Ion Transfer Voltage:	-2000 V
Interface Temperature:	450°C
Curtain gas:	10 L min^{-1}
Collision gas:	6 L min^{-1}
Collision Energy:	-10 V for PFPeA to PFOA, -15 V for PFBA, -25 V for PFNA to PFTeDA and -70 V for the PFSAs

The concentrations of calibration standards ranged from 0.005 ng ml^{-1} (Calibration level 1) to 200 ng ml^{-1} (Calibration level 12). Peaks consisted of at least 24 scans and the smoothing width was 9 points.

For separation on the column a gradient elution with two mobile phases, A (40:60 methanol:water) and B (95:5 methanol:water; both with 2 mM ammonium acetate) was used. The system was equilibrated for 8 minutes with the initial mobile phase composition of 60% A at a flow of 0.2 ml/min prior to sample injection. After injection the mobile phase composition changed linearly to 100% B at 10 minutes. This was held isocratic until 20 minutes. Afterwards the solvent composition was returned to initial condition within 2 minutes.

[1] Eschauzier, C.; Haftka, J.; Stuyfzand, P. J.; de Voogt, P., Perfluorinated Compounds in Infiltrated River Rhine Water and Infiltrated Rainwater in Coastal Dunes. *Environ. Sci. Technol.* **2010,** *44*, (19), 7450-7455.

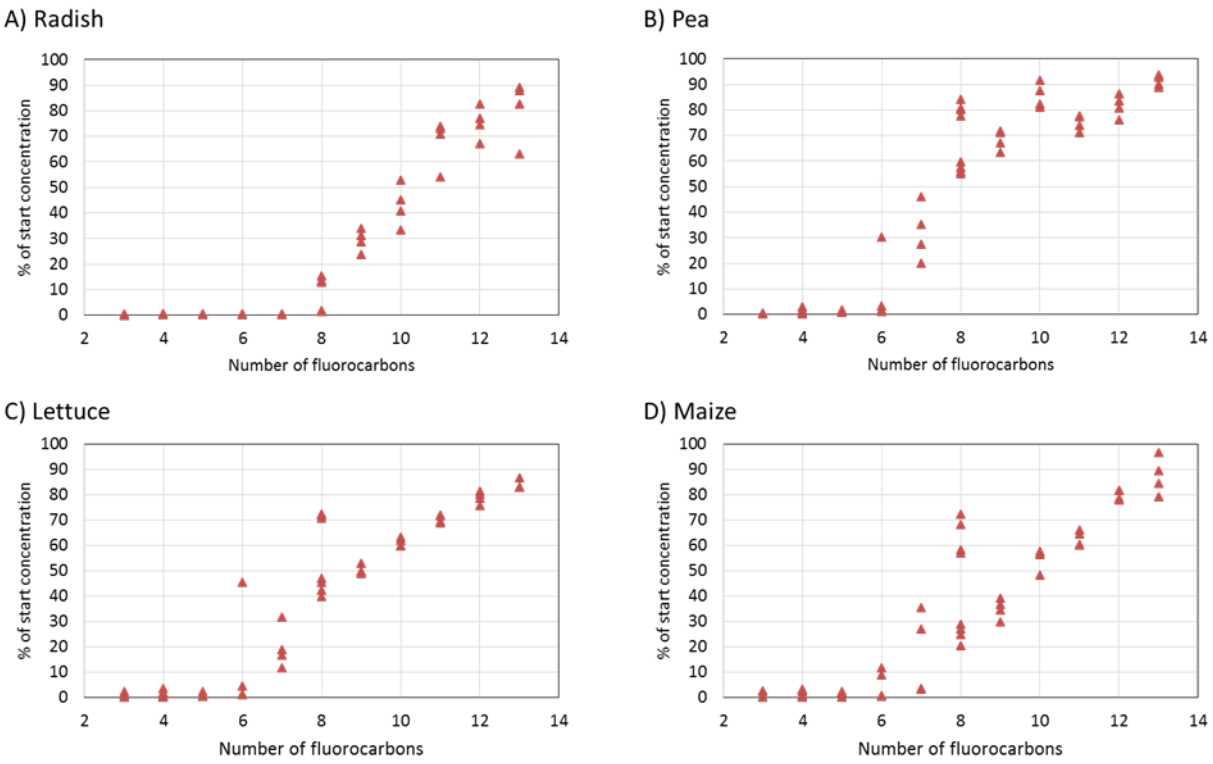

Figure S1: PFAA extracted from soil at harvest, expressed as a percentage of the amount extracted from the soil at the beginning of the experiment, plotted versus number of fluorocarbon atoms in the PFAA.

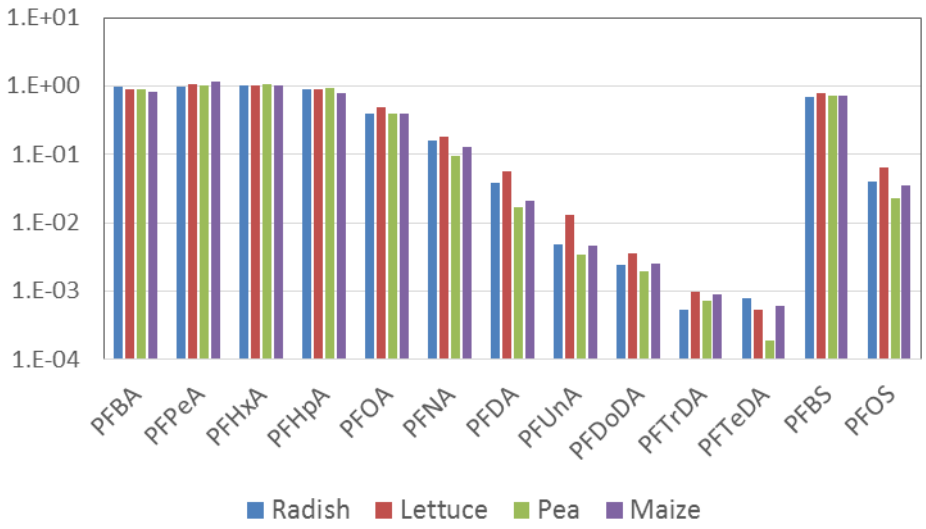

Figure S2: PFAA pattern in drainage water averaged according to the crop growing in the lysimeter. The results for the four different spiking levels are shown. The PFAA concentrations in the drainage water samples were first normalized to the initial soil concentration in the lysimeter, which facilitated comparison of the data across different spiking levels. Then the PFAA pattern in the each sample was extracted by normalizing the concentration ratio of each PFAA to the average of the ratios of the three most prominent PFCAs in the drainage water (PFBA, PFPeA, and PFHxA). This gave each sample equal weight in the final step, in which the patterns were averaged across the different drainage water samples collected for a given lysimeter.

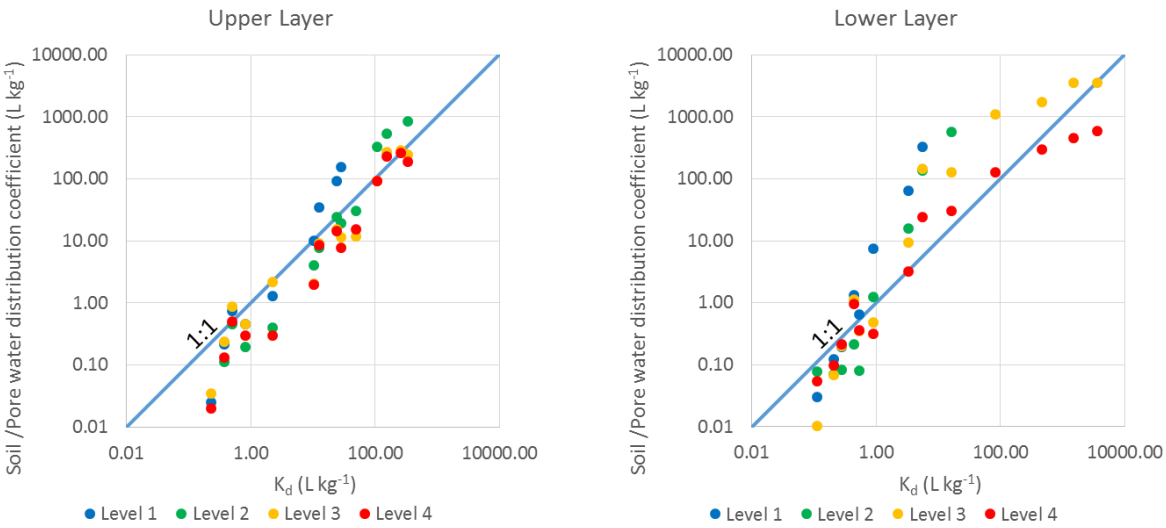

Figure S3: Soil / pore water distribution coefficient measured at harvest versus mean measured K_d. All K_d values are given in Table S9. The soil / pore water distribution coefficients are the means of the values measured in the lettuce and maize lysimeters.

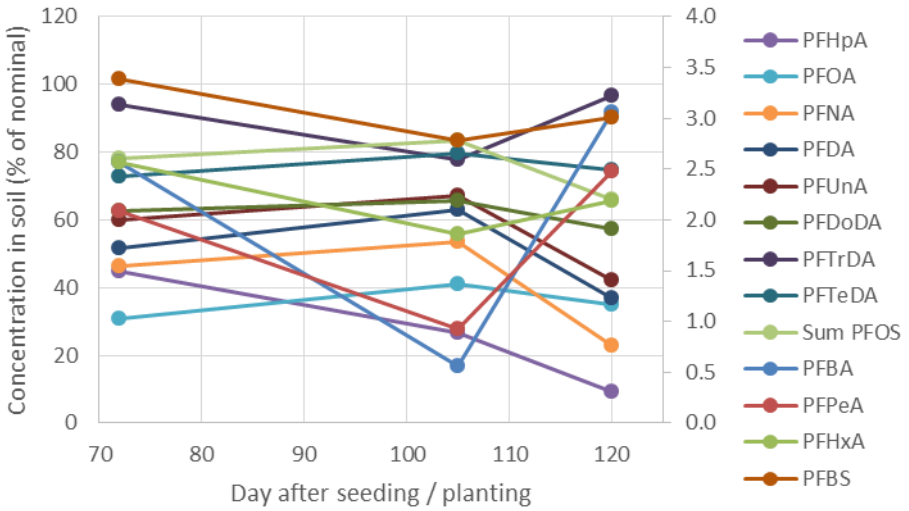

Figure S4: PFAA concentrations in upper layer soil at harvest for lettuce, pea and maize plotted versus the time of harvest. The data are for the level 1 lysimeters and are shown as percent of the nominal start concentration. PFBA, PFPeA, PFHxA and PFBS are plotted on the right hand y-axis.

Table S1: List of chemicals used, their purity and suppliers.

Chemical	Purity	Supplier
MPFAC-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
MPFAS-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
M5PFPeA (internal standard)		Wellington Laboratories, Ontario, Canada
M4PFHpA (internal standard)		Wellington Laboratories, Ontario, Canada
PFAC-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFAS/FOSA-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFBA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFPeA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHxA	≥97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHpA	99%	Sigma Aldrich, Zwijndrecht, Netherlands
PFOA	96%	Sigma Aldrich, Zwijndrecht, Netherlands
PFNA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFUnA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDoDA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTrDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTeDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFBS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFOS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
Ammonium acetate	≥99,999%	Sigma Aldrich, Zwijndrecht, Netherlands
Methanol	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands
Water	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands

Table S2: List of the analytes, their abbreviations and molecular formulas, the ^{13}C-labelled internal standards used for their quantification, and the mass transitions used in the MS/MS analysis of the analytes.

Abbreviation	Compound	Transition 1	Transition 2	Quantification by internal Standard	Molecular Formula
PFBA	Perfluoro-n-butanoic acid	213 → 169	-	$^{13}C_4$ PFBA	$CF_3(CF_2)_2COOH$
PFPeA	Perfluoro-n-pentanoic acid	263 → 219	-	$^{13}C_5$ PFPeA	$CF_3(CF_2)_3COOH$
PFHxA	Perfluoro-n-hexanoic acid	313 → 269	313 → 119	$^{13}C_2$ PFHxA	$CF_3(CF_2)_4COOH$
PFHpA	Perfluoro-n-heptanoic acid	363 → 319	363 → 169	$^{13}C_4$ PFHpA	$CF_3(CF_2)_5COOH$
PFOA	Perfluoro-n-octanoic acid	413 → 369	413 → 169	$^{13}C_8$ PFOA	$CF_3(CF_2)_6COOH$
PFNA	Perfluoro-n-nonanoic acid	463 → 419	463 → 219	$^{13}C_9$ PFNA	$CF_3(CF_2)_7COOH$
PFDA	Perfluoro-n-decanoic acid	513 → 469	513 → 269	$^{13}C_6$ PFDA	$CF_3(CF_2)_8COOH$
PFUnA	Perfluoro-n-undecanoic acid	563 → 519	563 → 269	$^{13}C_7$ PFUnA	$CF_3(CF_2)_9COOH$
PFDoDA	Perfluoro-n-dodecanoic acid	613 → 569	613 → 319	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{10}COOH$
PFTrDA	Perfluoro-n-tridecanoic acid	663 → 619	663 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{11}COOH$
PFTeDA	Perfluoro-n-tetradecanoic acid	713 → 669	713 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{12}COOH$
PFBS	Perfluorobutane sulfonate	299 → 80	299 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_3SO_3$
PFOS	Perfluorooctane sulfonate	499 → 80	499 → 99	$^{13}C_8$ PFOS	$CF_3(CF_2)_7SO_3$
$^{13}C_4$ PFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	217 → 172	-		
$^{13}C_5$ PFPeA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	268 → 223	-		
$^{13}C_2$ PFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	315 → 270	315 → 119		
$^{13}C_4$ PFHpA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	367 → 323	367 → 169		
$^{13}C_8$ PFOA	Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	421 → 376	421 → 172		
$^{13}C_9$ PFNA	Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]nonanoic acid	472 → 427	472 → 223		
$^{13}C_6$ PFDA	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	519 → 474	519 → 219		
$^{13}C_7$ PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	570 → 525	570 → 270		
$^{13}C_2$ PFDoDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	615 → 570	615 → 369		
$^{18}O_2$ PFHxS	Perfluoro-1-hexane[$^{18}O_2$]sulfonate	403 → 84	403 → 103		
$^{13}C_8$ PFOS	Perfluoro-1-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanesulfonate	507 → 80	507 → 99		

Table S3: Water inputs to the lysimeters (L per lysimeter)

Date	Precipitation	Watering radish	Watering lettuce	Watering pea	Watering maize
2011-06-21	2.8	5	5	5	5
2011-06-22	18.4				
2011-06-23	1.3				
2011-06-24	2.7				
2011-06-25	2.4				
2011-06-26	1.3				
2011-06-27	0				
2011-06-28	0				
2011-06-29	6.7				
2011-06-30	0.3				
2011-07-01	0.4				
2011-07-02	0				
2011-07-03	0				
2011-07-04	0				
2011-07-05	0		3		6
2011-07-06	0				3
2011-07-07	0	3	3	3	6
2011-07-08	4.8				
2011-07-09	0				
2011-07-10	0.1	3	3	3	6
2011-07-11	0				
2011-07-12	0	3	3	3	6
2011-07-13	1.9				
2011-07-14	0.7				
2011-07-15	2.8				
2011-07-16	0.8				
2011-07-17	13				
2011-07-18	2.9				
2011-07-19	0				
2011-07-20	2.6				
2011-07-21	18.9				
2011-07-22	0.1				
2011-07-23	0.5				
2011-07-24	11.5				
2011-07-25	3.1				
2011-07-26	12.5				
2011-07-27	13.7				
2011-07-28	21.6				
2011-07-29	0.1				
2011-07-30	0.5				
2011-07-31	0				
2011-08-01	0				
2011-08-02	0				

2011-08-03	15.3				
2011-08-04	4.4				
2011-08-05	0.8				
2011-08-06	7.4				
2011-08-07	0.6				
2011-08-08	15.1				
2011-08-09	8.2				
2011-08-10	0.2				
2011-08-11	0				
2011-08-12	11.5				
2011-08-13	6				
2011-08-14	12				
2011-08-15	0.1				
2011-08-16	0.3				
2011-08-17	0.1				
2011-08-18	28.9				
2011-08-19	2.4				
2011-08-20	0				
2011-08-21	2.1				
2011-08-22	1.3				
2011-08-23	0.5				
2011-08-24	0.6				
2011-08-25	0.1				
2011-08-26	11.8				
2011-08-27	9.6				
2011-08-28	1.6				
2011-08-29	0				
2011-08-30	0.1				
2011-08-31	0				
2011-09-01	0				
2011-09-02	0				
2011-09-03	0				
2011-09-04	16.7				
2011-09-05	0.5				
2011-09-06	1.2				
2011-09-07	6.6				
2011-09-08	17.4				
2011-09-09	0.3				
2011-09-10	0				
2011-09-11	10.4				
2011-09-12	0.1				
2011-09-13	0				
2011-09-14	0				
2011-09-15	0				
2011-09-16	0				

2011-09-17	0.2				
2011-09-18	1.6				
2011-09-19	0.2				
2011-09-20	0				
2011-09-21	0				
2011-09-22	0.1				
2011-09-23	0				
2011-09-24	0				
2011-09-25	0				
2011-09-26	0				
2011-09-27	0				
2011-09-28	0				
2011-09-29	0				
2011-09-30	0				
2011-10-01	0				
2011-10-02	0				
2011-10-03	0				
2011-10-04	0				
2011-10-05	0				
2011-10-06	5.2				
2011-10-07	8.5				
2011-10-08	2.5				
2011-10-09	2.3				
2011-10-10	3				
2011-10-11	12.3				
2011-10-12	19.2				
2011-10-13	0.1				
2011-10-14	0				
2011-10-15	0				
2011-10-16	0				
2011-10-17	0				
2011-10-18	2.5				
2011-10-19	4				

Table S4: Recoveries (in %) of the mass-labeled internal standards. The recoveries were determined by comparing the standard signal in the sample to the signal in matrix-free solutions which had been spiked with the same quantity of internal standard immediately prior to analysis. The bold (upper) entries are the mean recoveries, while the non-bold (lower) entries are the respective coefficient of variation (in %).

	$^{13}C_4$ PFBA	$^{13}C_5$ PFPeA	$^{13}C_2$ PFHxA	$^{13}C_4$ PFHpA	$^{13}C_8$ PFOA	$^{13}C_9$ PFNA	$^{13}C_6$ PFDA	$^{13}C_7$ PFUnA	$^{13}C_2$ PFDoDA	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Soil	**103**	**91**	**92**	**94**	**92**	**93**	**106**	**109**	**112**	**105**	**101**
	13	12	10	9	6	11	8	12	13	4	9

Table S5: Limits of Quantification (LoQ) in ng g^{-1} dry weight for soil and ng ml^{-1} for water.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Soil	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.089	0.092
Pore water	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.089	0.092

Table S6: Concentrations in soil at the start of the experiment in ng g^{-1} dry weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS	Water content (mL g^{-1} dw)
Radish S[#]														
Level 1	96.4	109.4	110.3	104.0	105.5	86.4	89.1	115.8	88.4	119.3	83.2	110.2	107.4	0.014
Level 2	981.3	984.4	837.1	1083	1146	1098	952.8	1089	988.6	1015	938.1	999.34	906.5	0.034
Level 3	4929	4358	3218	5286	5044	4455	5089	4722	4857	5123	4977	4693	5257	0.027
Level 4	7287	9961	4590	10400	9735	9572	10198	10530	10122	10339	8969	9877	10581	0.032
Lettuce U														
Level 1	105.3	99.0	105.0	98.9	97.8	98.2	103.8	94.9	87.3	115.2	87.9	97.4	108.3	0.022
Level 2	952.7	972.5	916.2	1033	1116	1066	973.3	1054	1019	1017	978.9	1015	1008	0.004
Level 3	4626	4511	3170	4950	4937	4737	5096	4640	4717	4905	5131	4757	5244	0.041
Level 4	7769	9321	4832	10063	9990	9999	10239	10102	10212	9888	8790	9607	9834	0.028
Pea U														
Level 1	100.3	99.3	98.3	87.6	89.2	97.6	99.0	81.9	85.0	102.1	85.9	92.0	103.9	0.006
Level 2	914.5	929.5	748.4	1040	1042	1066	946.7	1030	1003	953.7	909.5	990.3	1036	0.026
Level 3	3984	4252	2349	4473	5095	4971	5042	4591	4331	4885	4980	4793	5138	0.008
Level 4	6406	9087	4269	9897	10143	10030	10396	9417	10072	8876	8183	9250	9354	0.027
Maize U														
Level 1	119.2	88.2	106.4	105.2	98.8	110.7	123.2	87.1	88.6	124.2	94.6	90.1	113.7	0.005
Level 2	962.1	1004	1163	976.3	1161	1035	1020	1043	1065	1083	1089	1055	1080	0.014
Level 3	4966	4921	3942	5091	4671	4786	5156	4608	4963	4709	5437	4785	5335	0.017
Level 4	9613	8916	5638	9892	10092	10397	10125	10359	10441	10447	9217	9695	9566	0.020
Lettuce L														
Level 1	90	88.2	89	107.1	95.8	100	96.3	95.2	80.6	69	63.8	71.9	109.4	0.027
Level 2	918.6	1070.1	964.3	1015	980.5	1082	1043	1043	1195	944	1014	1199	1026	0.044
Level 3	4896	4953	4904	5136	5294	5574	5444	6033	5045	4869	4965	4981	5270	0.048
Level 4	8263	10331	10137	10253	10277	10679	10080	9833	9819	9671	10156	11080	9960	0.054

[#] S = surface layer; U = upper layer; L = lower layer

Table S7: Concentrations in soil at the time of the harvest in ng g^{-1} dry weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS	Water content (mL g^{-1} dw)
Radish S[#]														
Level 1	<LoQ	0.27	0.42	0.62	0.34	1.63	21.3	38.7	47.9	80.0	52.6	0.51	16.6	0.163
Level 2	5.23	6.11	4.07	6.08	4.93	16.2	273.0	444.6	729.9	838.6	837.1	5.56	123.7	0.169
Level 3	6.70	7.33	6.78	10.6	24.8	71.0	1727	2129	3439	3819	4108	17.1	716.0	0.168
Level 4	18.4	9.89	5.31	9.60	28.6	158.1	3177	5559	7384	7951	7885	14.1	1373	0.155
Lettuce U														
Level 1	2.57	2.09	2.57	44.9	30.9	46.2	51.7	60.0	62.7	93.9	72.9	3.39	78.2	0.146
Level 2	10.1	8.40	7.76	46.2	210.8	450.0	516.1	629.9	735.0	770.5	848.5	17.1	712.3	0.136
Level 3	10.4	11.4	20.1	60.8	583.9	2150	2494	2883	3245	3928	4259	38.1	3801	0.132
Level 4	10.8	14.2	31.8	132.9	1683	3973	5072	6037	7098	7779	7299	62.6	7075	0.140
Pea U														
Level 1	0.56	0.93	1.85	26.5	41.0	53.7	62.8	67.3	65.7	77.6	79.8	2.79	83.3	0.054
Level 2	1.95	3.51	6.51	35.1	365.8	597.9	679.5	835.8	713.6	795.5	808.6	9.33	836.2	0.071
Level 3	15.8	20.9	33.6	76.6	1394	2852	3591	4206	3357	4218	4478	82.8	4317	0.042
Level 4	11.3	16.1	32.5	121.0	2035	5980	6962	8237	7454	7180	7674	67.2	7271	0.038
Maize U														
Level 1	3.06	2.48	2.19	9.42	35.0	22.7	36.7	42.1	57.2	96.7	74.9	3.01	66.2	0.135
Level 2	21.4	23.2	26.6	114.8	312.7	258.2	353.4	590.5	639.5	849.6	918.5	31.2	616.4	0.127
Level 3	12.5	10.8	11.9	22.0	159.2	1386	1897	2651	3279	3847	4868	20.0	3850	0.135
Level 4	16.6	18.5	27.6	79.1	347.5	2813	3978	5826	6324	8547	8919	65.7	6513	0.134
Lettuce L														
Level 1	2.57	2.26	2.75	45.1	49.7	55.4	69.5	64.2	56.8	57.3	71	3.48	94.2	0.081
Level 2	10.2	9.05	7.59	46.2	244	385.6	447.4	526.2	679.3	846.8	919.9	18.4	703	0.090
Level 3	12.2	11.8	20.5	54.6	233.2	2096	2541	2580	3028	3844	4352	43.5	3214	0.066
Level 4	12.4	15.1	32.4	130.2	926.3	4156	4918	5297	6384	7454	8619	67.2	6349	0.074

[#] S = surface layer; U = upper layer; L = lower layer

Table S8: Concentrations in soil at the time of the harvest as percentage of initial nominal concentration.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish S[#]													
Level 1		0.27	0.42	0.62	0.34	1.63	21.29	38.66	47.91	80.02	52.58	0.51	16.57
Level 2	0.52	0.61	0.41	0.61	0.49	1.62	27.30	44.46	72.99	83.86	83.71	0.56	12.37
Level 3	0.13	0.15	0.14	0.21	0.50	1.42	34.55	42.58	68.78	76.38	82.16	0.34	14.32
Level 4	0.18	0.10	0.05	0.10	0.29	1.58	31.77	55.59	73.84	79.51	78.85	0.14	13.73
Lettuce U													
Level 1	2.57	2.09	2.57	44.9	30.9	46.2	51.7	60	62.7	93.9	72.9	3.39	78.2
Level 2	1.01	0.84	0.776	4.62	21.08	45	51.61	62.99	73.5	77.05	84.85	1.71	71.23
Level 3	0.208	0.228	0.402	1.216	11.678	43	49.88	57.66	64.9	78.56	85.18	0.762	76.02
Level 4	0.108	0.142	0.318	1.329	16.83	39.73	50.72	60.37	70.98	77.79	72.99	0.626	70.75
Pea U													
Level 1	0.56	0.93	1.85	26.50	41.01	53.68	62.85	67.33	65.68	77.62	79.79	2.79	83.35
Level 2	0.19	0.35	0.65	3.51	36.58	59.79	67.95	83.58	71.36	79.55	80.86	0.93	83.62
Level 3	0.32	0.42	0.67	1.53	27.88	57.04	71.81	84.11	67.15	84.36	89.56	1.66	86.34
Level 4	0.11	0.16	0.32	1.21	20.35	59.80	69.62	82.37	74.54	71.80	76.74	0.67	72.71
Maize U													
Level 1	3.06	2.48	2.19	9.42	34.96	22.72	36.74	42.13	57.22	96.73	74.86	3.01	66.17
Level 2	2.14	2.32	2.66	11.48	31.27	25.82	35.34	59.05	63.95	84.96	91.85	3.12	61.64
Level 3	0.25	0.22	0.24	0.44	3.18	27.72	37.94	53.01	65.58	76.94	97.36	0.40	77.00
Level 4	0.17	0.18	0.28	0.79	3.48	28.13	39.78	58.26	63.24	85.47	89.19	0.66	65.13
Lettuce L													
Level 1	2.57	2.26	2.75	45.10	49.70	55.40	69.50	64.20	56.80	57.30	71.00	3.48	94.20
Level 2	1.02	0.91	0.76	4.62	24.40	38.56	44.74	52.62	67.93	84.68	91.99	1.84	70.30
Level 3	0.24	0.24	0.41	1.09	4.66	41.92	50.82	51.60	60.56	76.88	87.04	0.87	64.28
Level 4	0.12	0.15	0.32	1.30	9.26	41.56	49.18	52.97	63.84	74.54	86.19	0.67	63.49

[#] S = surface layer; U = upper layer; L = lower layer

Table S9: Soil-water distribution coefficients (K_d, L kg^{-1}) measured at different solution concentrations.

Upper layer soil

C_{init} ng/mL	Rep	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	L-PFOS	Br-PFOS
1	A							41	57	159	127			2.1	36	22
1	B						10.4	36	74	149	177	333	0.29	1.94	32	19.0
5	A	0.14	0.24	0.36	0.71	2.5	12.5		77		349	325		2.1	34	23
5	B	0.07	0.20	0.32	0.66	1.96	8.5			103	227	393		1.96	20	15.0
10	A	0.14	0.21	0.34	0.84	2.6	12.4	49	90	212	375			2.5	33	22.6
10	B	0.14	0.26	0.32	0.69	1.92	9.9		84	198	306			2.0	25	17.4
50	A		0.25	0.38	0.94	2.5	11.6	58	147	138	269	319		2.1	38	10.6
50	B			0.37	0.91	2.4	12.3	56	129	136	268	299		2.2	33	11.4
100	A	0.06	0.23	0.46	0.88	2.1	9.4	61	164				0.55	1.83	30	8.3
100	B		0.19	0.37	0.79	1.89	8.7	51	125				0.52	1.66	29	7.5
500	A	0.19	0.19	0.61	0.86	2.5	10.4	54	65				0.49	1.66	27	5.4
500	B		0.18	0.25	0.74	2.1	9.4	60					0.62	1.88	24	5.3
1000	A	0.12	0.31		0.79	2.2	7.7	43	135				0.57		19.0	4.3
1000	B	0.10	0.22		0.96	1.83	10.7	34	159				0.49		21	4.5
Mean		0.12	0.23	0.38	0.81	2.2	10.3	50	109	156	262	334	0.50	2.0	29	12.6
Standard Deviation		0.041	0.042	0.097	0.101	0.29	1.59	9.7	36	37	84	36	0.105	0.23	5.9	7.1
RSD		34%	19%	26%	12%	13%	15%	20%	33%	24%	32%	11%	21%	12%	21%	56%
5th Percentile		0.04	0.14	0.18	0.61	1.64	7.1	30	36	81	95	263	0.29	1.54	16.6	-1.63
95th Percentile		0.20	0.31	0.57	1.02	2.8	13.5	69	182	231	429	405	0.72	2.46	40	27

Table S9 continued

Lower layer soil

C_{init} ng/mL	Rep	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	L-PFOS	Br-PFOS
1	A						1.81	10.6	46	695				0.61	6.7	4.8
1	B						2.9	13.4	50	381				0.60	7.4	5.3
5	A	0.08	0.18	0.19	0.29	0.53	2.8	14.0	66	395	1086			0.47	6.2	5.0
5	B	0.13	0.21	0.23	0.30	0.55	2.5	14.6	76	402	1659			0.55	8.2	5.7
10	A	0.18	0.22	0.28	0.29	0.50	2.5	13.0	64	427	1201			0.53	6.5	4.0
10	B	0.14	0.21	0.24	0.25	0.54	3.1	13.3	60	466	1642			0.56	7.2	4.7
50	A		0.21	0.27	0.62	1.28	4.5	22	107		1094	2122		0.54	7.2	3.1
50	B		0.21	0.22	0.53	1.17	4.3	24	93		1905	4480	0.31	0.51	7.3	3.2
100	A		0.21	0.33	0.64	1.43	4.1	20	87	494	2160	4406	0.35	0.55	7.0	2.0
100	B		0.22	0.32	0.64	1.35	4.1	19.2	102				0.30	0.51	6.1	2.1
500	A	0.10	0.21	0.44	0.81				127				0.49	0.57	4.6	1.88
500	B		0.27	0.32	0.65			18.0	95				0.55	0.67		
1000	A	0.06	0.23		0.61				101				0.58		3.8	1.56
1000	B	0.10	0.11		0.92				105				0.52			
Mean		0.11	0.21	0.28	0.55	0.92	3.3	16.6	84	466	1535	3670	0.44	0.56	6.5	3.6
Standard Deviation		0.040	0.038	0.072	0.22	0.42	0.93	4.4	24	109	421	1341	0.119	0.052	1.24	1.48
RSD		35%	18%	26%	40%	46%	28%	26%	29%	23%	27%	37%	27%	9%	19%	41%
5th Percentile		0.03	0.13	0.14	0.11	0.08	1.42	7.9	36	248	693	988	0.20	0.45	4.0	0.64
95th Percentile		0.19	0.28	0.43	0.99	1.76	5.1	25	132	683	2377	6351	0.68	0.66	9.0	6.6

Table S10: log K_{OC} values (L kg^{-1}) from this study and the literature.

Source	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFHxS	PFOS
(1)					2.06	2.39	2.76	3.30						2.57
(2)														3.10
(3)					2.50									3.70
(4)			2.10	2.10		2.90	3.80	4.70	5.60				2.20	3.70
(5)			1.91	2.19	2.31	2.33	3.17						2.70	3.34
(6)	1.88	1.37	1.31	1.63	1.89	2.36	2.96	3.56				1.79	2.05	2.80
(7) 40 cm depth	0.80	1.80	3.00		4.00	3.80							1.20	
(7) 80 cm depth	2.70		3.60		3.90	3.80						2.70	2.80	
(8)					1.98							1.23		2.85
(9) at 2.5 µg L^{-1}					2.05	2.81	3.58						1.82	3.30
This study, upper layer soil	1.11	1.39	1.61	1.94	2.38	3.04	3.73	4.07	4.23	4.45	4.56	1.73	2.33	3.49
This study, lower layer soil	1.45	1.72	1.85	2.14	2.36	2.91	3.62	4.32	5.07	5.58	5.96	2.04	2.14	3.21

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Table S11: PFAA concentrations in leachate from exposure level 1 lysimeters in µg L^{-1} (average from the 3 collected samples). Level 1 results are shown because these were least influenced by mixture effects.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish	369	414	428	345	158	49.0	12.1	1.80	0.70	0.40	0.40	286	15.3
Lettuce	236	293	291	237	116	41.8	12.5	3.13	0.87	0.50	0.20	203	16.3
Pea	323	365	389	302	129	32.7	5.87	1.60	0.90	0.40	<LoQ	241	8.3
Maize	340	378	380	268	111	34.0	4.80	1.30	0.80	0.30	0.20	229	8.3

Chapter 5

INFLUENCE OF SOIL ON THE UPTAKE OF PERFLUOROALKYL ACIDS BY LETTUCE: A COMPARISON BETWEEN A HYDROPONIC STUDY AND A FIELD STUDY

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Abstract

This study explores whether mechanistic understanding of plant uptake of perfluoroalkyl acids (PFAAs) derived from hydroponic experiments can be applied to soil systems. Lettuces (*Lactuca sativa*) were grown in outdoor lysimeters in soil spiked with 4 different concentrations of 13 PFAAs. PFAA concentrations were measured in soil, soil pore water, lettuce roots, and foliage. The PFAA uptake by the lettuce was compared with uptake measured in a hydroponic study. The foliage:pore water concentration ratios in the lysimeter were similar to the foliage:water concentration ratios from the hydroponic experiment. In contrast, the root:pore water concentration ratios in the lysimeter were 1-2 orders of magnitude lower than in the hydroponic study for PFAAs with 6 or more perfluorinated carbons. Hence, hydroponic studies can be expected to provide a good quantitative measure of PFAA transfer from soil to foliage if one accounts for soil:pore water partitioning and differences in transpiration rate. However, hydroponic studies will be of little value for estimating PFAA transfer from soil to roots because sorption to the root surface is greatly enhanced under hydroponic conditions.

Keywords: PFAA, root uptake, translocation, PFOA, PFOS

Introduction

Perfluoroalkyl acids (PFAAs) have been detected ubiquitously in water (Ahrens 2011; Eschauzier et al. 2012b; Xiao 2017), biota (Giesy et al. 2001; Langberg et al. 2019) and the atmosphere (Dreyer et al. 2009; Rauert et al. 2018) as well as in human blood serum and breast milk (Volkel et al. 2008; Winkens 2017; Jin et al. 2020). They have known and suspected toxic effects (Lau et al. 2007; Anderko et al. 2020), and human exposure occurs via food (Fromme et al. 2009; D'Hollander et al. 2010a; Klenow et al. 2013). In response to concerns about these chemicals, the European Food Safety Authority established tolerable daily intakes (TDIs) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), and they have recently presented a proposal to add perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) while reducing the TDI for the sum of all four (Johansson et al. 2009; Knutsen 2018). To ensure that the TDIs are not exceeded, we must understand the sources of PFAAs in food. Crops are one possible vector for PFAAs into the food supply. Crops have been shown to take up PFAAs from soil (Stahl et al. 2009; Zhao et al. 2013) and soils can be contaminated with PFAAs (Wilhelm et al. 2008; Rankin et al. 2016). This work aims to further our understanding of how PFAAs are transferred from soils into crops.

Plant uptake of PFAAs via the roots has been studied using several experimental designs. The first studies published were soil-based experiments. Stahl et al. (2009) and Lechner et al. (2011) showed that the concentration of PFOA and PFOS in several crops was linearly proportional to the concentration in the soil in which they were grown. Since then there have been several reports of uptake of a broad spectrum of PFAAs in vegetation growing in biosolids-amended soils. They show that the length of the perfluoroalkyl chain is the dominant variable influencing PFAA uptake in foliage. Foliage concentration factors (FCFs) are negatively correlated with chain length (Navarro et al. 2011; Yoo et al. 2011; Blaine et al. 2013; Wen et al. 2014). For grasses, an average decrease in FCF of 0.24 log units per CF_2 group was observed (Yoo et al. 2011), while for lettuce and tomato plants the average decrease was 0.3 log units per CF_2 group (Blaine et al. 2013). Regarding PFAA accumulation in root tissue, a much weaker influence of chain length has been observed. For instance, the variation in root concentration factors (RCFs) for C5-C10 perfluoroalkyl carboxylic acids (PFCAs) was just 0.5 log units for radish, celery, tomato and pea (Blaine et al. 2014a). A similarly small variation was found between PFHxA, PFOA, PFBS, PFHxS and PFOS in wheat (see Table S1 and S2 for a list of the abbreviations of the different PFAAs and their full chemical names) (Lan et al. 2018). In contrast, root concentration factors in chicory showed a pronounced dependence on the chain length, suggesting that root accumulation is influenced by species and soil type (Gredelj et al. 2020a).

Hydroponic experiments provide an opportunity to obtain a more systematic understanding of contaminant accumulation in plants. For instance, a hydroponic experiment was used to assess the influence of different metabolic inhibitors on the uptake of PFOA and PFOS in maize shoots (Wen et al. 2013). The influence of pH on PFAA uptake into maize roots was also elucidated in a hydroponic experiment, showing no effect in a pH range of 5-7 for nine of the ten PFAAs studied (Krippner et al. 2014). A hydroponic study was used to explore the effect of temperature and salinity on PFAA uptake in wheat, identifying a positive effect for both, which was attributed to increased evapotranspiration (Zhao et al. 2016).

Hydroponic experiments have also been used to study how perfluoroalkyl chain length influences uptake in plants. PFAAs with perfluoroalkyl chain lengths ranging from 3 to 13 were all transferred

via the roots to the plant foliage in lettuce, tomato, cabbage and zucchini (Felizeter et al. 2012; Felizeter et al. 2014). Transpiration stream concentration factors (TSCFs, the quotient of the concentration in the xylem flow and that in the nutrient solution) for C4-C10 PFAAs ranged over just a factor of two for three of the four species. Relatively high TSCFs of 0.05-0.8 showed that the PFAAs were clearly able to cross the Casparian strip and plasma membranes that prevent the passive entry of many polar molecules into the vascular tissue of the root (Felizeter et al. 2012). A weak influence of chain length on TSCF was also observed in grass (García-Valcárcel et al. 2014).

Hydroponic studies have also been used to study PFAA uptake into roots. In lettuce, the root-nutrient solution concentration factor decreased with chain length for C4-C6 PFCAs before increasing by almost 3 orders of magnitude from PFHxA to PFUnA. While the accumulation of the shorter chained compounds was explained by uptake with the transpiration stream, the uptake of the longer chained compounds was attributed to sorption to the surface tissue of the roots (Felizeter et al. 2012). Hydroponic experiments with tomato, cabbage and zucchini showed a strong positive relationship between root-hydroponic solution concentration factor and chain length for C4-C11 PFAAs, indicating that root-surface sorption was the dominant uptake mechanism for all of the PFAAs in these species (Felizeter et al. 2014). In detailed experiments with a hydroponic model plant system (*Arabidopsis thaliana*), Müller et al. (2016) also concluded that the root uptake of all but the shortest PFAAs was governed by sorption and observed that the dead root-hydroponic solution concentration factor increased by almost 3 orders of magnitude from PFBA to PFOS.

Comparing the results from hydroponic and soil experiments, there are clear differences in the chain length dependence of PFAA uptake. In foliage, the hydroponic studies show a weak dependence of uptake on chain length, while soil studies show a very strong dependence. The opposite is the case in roots; the hydroponic studies show a strong positive chain length dependence that is attributed to sorption to root surfaces, while the soil studies show a weak dependence.

It is unclear what the reasons for these differences are, and how and to what extent findings from hydroponic studies can be transferred to natural soil systems. Sorption of PFAAs to soil solids is certainly an important factor, as this reduces the fraction of chemical available for uptake by the roots. To be able to sorb to the root surface or be taken up with the transpiration stream, the compounds first need to be present in pore water. Long chain compounds sorb strongly to the soil; hence, for a long chain PFAA much higher concentrations in soil are required to generate a given concentration in pore water than for short chain PFAAs (Yoo et al. 2011; Zhao et al. 2013; Blaine et al. 2014a; Wen et al. 2014). However, there may be other factors that affect the comparability of hydroponic and soil systems. For instance, some contaminants appear to be taken up through the action of root exudates (Campanella et al. 2000), which would be highly diluted or not present under hydroponic conditions. Another possibility is that differences in the nature of root tissue when grown under hydroponic conditions influence PFAA uptake and translocation. The uptake of the PFAAs could also be influenced by other solutes present in the soil.

To explore these questions, we conducted a lysimeter experiment in which lettuce was grown in soil containing PFAAs, and compared this with our previous hydroponic experiment conducted with the same plant species, chemicals, sample preparation and analysis. The lysimeter soil was spiked with 11 PFCAs and 2 perfluoroalkane sulfonates (PFSAs). Four lysimeters were used, each with a different spiking level. At maturity the lettuce was harvested and the roots and leaves were analyzed

separately. Additionally, the PFAA concentrations in soil and pore water were determined. The measurement of concentrations in pore water facilitated comparison of this experiment with our earlier hydroponic greenhouse study, and thereby identification of differences in the uptake into roots and leaves between soil and hydroponic growth environments.

Materials and methods

Chemical reagents and lab materials

Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutane sulfonic acid (PFBS) and perfluorooctane sulfonic acid (PFOS) were studied. All standards had a purity >95%. The suppliers and purities of the chemicals, their molecular formulas and the ^{13}C-labeled internal standards used for their quantification can be found in Tables S1 and S2 of the Supporting Information (SI).

Materials used for extraction and clean-up of the samples included Florisil SPE cartridges (1000 mg, 6 mL) from Applied Separations (Allentown, PA, USA); Acrodisc LC13 GHP Pall 0.2 µm filters from Pall Corporation (Port Washington, NY, USA); 50 and 15 mL polypropylene (PP) tubes with screw caps from Sarstedt (Nümbrecht, Germany); and Supelclean ENVI-Carb 120/140 from Supelco (Bellefonte, PA, USA). Tetrabutylammonium hydrogensulfate and sodium hydrogencarbonate were purchased from Merck (Darmstadt, Germany). Sodium carbonate and ammonium hydroxide a.c.s. reagent were from Sigma Aldrich; 2.0 and 0.3 mL PP vials were purchased from VWR International (Amsterdam, Netherlands). Centrifugation filter tubes (50 mL, 0.2 µm nylon filter) were obtained from Grace (Breda, Netherlands).

Field experiment

The field experiment was conducted at the Fraunhofer Institute for Molecular Biology and Applied Ecology IME in Schmallenberg, Germany. Lettuce plants were grown in 5 lysimeters, one containing soil with background concentrations of PFAAs (unspiked), and 4 with intended concentrations of individual PFAAs in soil of 0.1 mg/kg, 1 mg/kg, 5 mg/kg and 10 mg/kg (all soil concentrations on a dry weight basis). This compares with PFOA and PFOS concentrations of ~1 mg/kg measured in contaminated agricultural soil in Arnsberg, ~30 km from Schmallenberg (Vestergren et al., 2012). The results from the highest spiking level were not used because the lettuce plants were significantly smaller at the time of harvest than those growing in the lower exposure levels, indicating that PFAAs had phytotoxic effects (see Table S3). Phytotoxic effects of PFAAs have been reported elsewhere (Stahl et al. 2009; Qu et al. 2010; Zhao 2011; Qian et al. 2019; Zhang 2019; Chen et al. 2020; Lin et al. 2020).

Each lysimeter had a surface area of 1 m² and a total depth of 60 cm. The lysimeters were each filled with ~450 kg sand (30-60 cm depth) and ~450 kg of loamy sand (0-30 cm depth; 71% sand, 24% silt, 5% clay, pH 5.67, organic carbon content 0.93%). This resembled a typical soil from northwestern Germany. The soil used for the upper layer is available as a reference soil (Refesol 01-A) from Fraunhofer IME (www.refesol.de/boden01a.shtml).

The spiking of the soil was done stepwise. First a stock solution was prepared containing all PFAAs in methanol. With this stock solution 2 kg of soil were spiked. Afterwards the 2 kg spiked soil was mixed with approximately 90 kg of soil in a concrete mixer to achieve the desired concentration. This was repeated 5 times for each layer in each lysimeter. Samples were taken from each batch and combined to determine the initial PFAA concentration in the soil of each lysimeter.

The lettuce plants were pre-grown in a greenhouse for 2 weeks in non-spiked soil before they were transferred to the lysimeters. Within one week of preparing the spiked soil, 20 lettuce seedlings were put in each lysimeter (on June 21, 2011). The seedlings were watered after planting, and kept humid by rain events until harvest with supplementary watering when needed (a total of 17 L of tap water per lysimeter distributed over 5 occasions). After 72 days the lettuce plants were harvested (on September 1, 2011). The plants were divided into roots and foliage, packed in freezer bags and stored at –20 °C until analysis. Soil samples were taken with a soil corer when the plants were harvested. The soil core, which was taken from the top to the bottom of the lysimeter, was divided between the upper and lower soil layers, and the soil was packed in freezer bags and stored at –20 °C for later separation of pore water and analysis.

Extraction and clean-up

Before homogenization with a household blender (Braun Multiquick MX 2050) the roots were rinsed with demineralized water to wash off residual soil and then carefully dried superficially with paper towels. As no residual soil was visibly apparent on the leaf samples, no cleaning was performed.

The extraction method used is based on the modification Vestergren et al. (2012) proposed for the method published by Hansen et al. (2001). Briefly, 10 g of the homogenate were weighed into a 50 mL PP tube and spiked with mass-labeled surrogate standards. After adding 5 mL of 0.4M NaOH solution and vortex-mixing, the samples were left in the refrigerator (4 °C) over night to allow the internal standards to distribute in the slurry. Next, 4 mL of 0.5M tetrabutylammonium hydrogensulfate solution and 5 mL of a carbonate buffer (0.25M Na_2CO_3/$NaHCO_3$) were added to the samples and thoroughly mixed. After adding 10 mL MTBE and vortex-mixing for 1 minute the samples were sonicated for 10 minutes. Phase separation was achieved by centrifuging for 10 minutes at 3000 rpm. The MTBE phase was transferred to a new 50 mL PP tube and the extraction repeated two times. The extracts were combined and concentrated to approximately 2 mL using a Rapidvap (Labconco Corp., Kansas City, MO, USA). After adding 1 g of sodium sulfate to Florisil SPE-cartridges to remove any remaining water in the extracts, the cartridges were conditioned with 10 mL MeOH and 10 mL MTBE before they were loaded with the extract. The elution of the non-polar matrix was done with 10 mL MTBE before the target compounds were washed off the cartridge with 10 mL MeOH/MTBE (30:70, v:v). This extract was again evaporated to 1 mL final volume. An additional clean-up step following the Powley method with ENVI-Carb (Powley et al. 2005) was added when the final extract was still strongly colored.

For the analysis of PFAAs in soil, the soil was dried in an oven at 40 ˚C until no further weight loss was recorded. After homogenization, 1 g of soil was weighed in a 15 mL PP tube and spiked with internal standards. The soil was then extracted with 10 mL MeOH by vortex mixing for 1 minute and sonication for 10 minutes. Phase separation was achieved by centrifugation (10 min, 3000 RPM). The supernatant was transferred to a new 15 mL PP tube and concentrated in the Rapidvap. The

extraction was repeated twice with 5 mL MeOH. The extracts were combined and concentrated in the Rapidvap to a final volume of 1 mL.

For pore water analysis 20 g of the soil was put in a 50 mL centrifugation filter tube with a 0.2 µm nylon filter. After 20 minutes of centrifugation at 2000 RPM, 0.5 mL of pore water was transferred to a vial. The internal standards and MeOH were added to achieve a final volume of 1 mL.
All final extracts were passed through an Acrodisc LC 13 GHP Pall nylon filter into 2 mL PP vials and stored at 4°C until analysis.

Analysis
An HPLC system (LC-20AD XR pump, SIL-20A autosampler and SCL-10A VP system controller, Shimadzu, Kyoto, Japan) coupled with a tandem mass spectrometer (4000 QTrap, Applied Biosystems, Toronto, Canada) was used to analyze the samples for PFAAs. A pre-column (Pathfinder 300 PS-C_{18} column, ID 4.6 mm; length 50 mm; 3 µm particle diameter; Shimadzu, Duisburg, Germany) prior to the injection valve was used to remove potential background contamination from the LC system.

Separation of the analytes was achieved using an ACE 3 C18-300 column (ID 2.1 mm; length 150 mm; 3 µm particle diameter; Advanced Chromatography Technologies, Aberdeen, Scotland) maintained at 30 °C with a mobile phase gradient consisting of two eluents A (40:60 MeOH:H2O, v:v) and B (95:5 MeOH:H2O; v:v), both containing 2 mM ammonium acetate. The gradient used for separation and the mass transitions as well as other mass spectrometer settings can be found in the Supporting Information. The mass spectrometer was equipped with an electrospray ionization interface operating in the negative ionization mode, and it was run in a scheduled MRM-mode.

The purified extracts were diluted 1:1 with water prior to analysis to match the injection conditions of the HPLC. A volume of 20 µl was injected.

Raw data were processed with the Analyst 1.5 software (Applied Biosystems).

Quality assurance and control
Each sample was extracted three times and each extract was injected in duplicate. The relative standard deviation of the concentrations derived from these six injections was <10% for all analytes in all samples.

Concentrations were quantified using a twelve-point calibration with fitted correlation lines that had r^2 values of >0.99 for all analytes; no weighting was applied. Further information on quality assurance and quality control is provided in our previous studies (Felizeter et al. 2012; Felizeter et al. 2014).

Recoveries were determined by comparison with a matrix free solution spiked with internal standard immediately prior to injection. Average recoveries of the internal standards in the samples were between 22% (PFBA) and 112% (PFDoDA). Since mass labeled internal standards were used for quantifying the analytes, no correction for recovery was necessary. See Table S4 in the Supporting Information for detailed information on recoveries.

Limits of quantification (LoQs) (Table S5 in the Supporting Information) were calculated on the basis of the lowest validated calibration standard (signal to noise ratio ≥10). They were derived from the

amount injected back calculated to an extract volume of 1 mL and divided by the average extracted sample quantities. Method blanks were prepared repeatedly with the same extraction procedure as the samples, but showed no quantifiable contamination. Solvent blanks were injected every ten injections to check for contamination of the LC system and for memory effects, but no contamination or memory effects were observed during the study.

All PFAA concentrations from the non-spiked lysimeters (in plant parts as well as in soil or pore water) were subtracted from the concentrations in the spiked lysimeters. Any resulting concentrations below the LoQ were neglected.

Since PFOS is the only compound for which branched isomers were included in the standards used for the calibration curve, branched isomers could only be quantified for PFOS. All reported PFOS concentrations are sum concentrations of non-branched and branched isomers.

Results and discussion

PFAA fate in soil

The soil concentrations at the time of planting were generally within the intended concentration range (Figure S1). The soil concentrations at the harvest date show that the shortest chain PFAAs, the C4-C6 PFCAs and PFBS, were depleted. Less than 3% of the initial mass was left in the soil (Figure S1). Depletion occurred in both the upper and lower soil layers (see Tables S6 and S7). In contrast, some 80-90% of the longer chain PFCAs dosed were still present in the soil at the harvest date.

We analyzed the behaviour of the PFAAs in the lysimeter soil in another paper in which we include data from 12 other lysimeters prepared in the same manner but planted with different crops (McLachlan et al. 2019). That work showed that the depletion of the shorter chained PFAAs was due to leaching, and that the leaching was greater than anticipated due to interactions between the PFAAs. This accelerated leaching increased with the initial PFAA contamination level of the soil. Lower precipitation towards the end of the growth period contributed to reduced leaching and more stable conditions; two weeks before harvest the lysimeters had already received 91% of the water input for the whole growth period (Figure S2). Hence, although the lettuce was exposed to changing PFAA concentrations in soil, the evidence indicates that the concentrations were more stable towards the end of the growth period when the plants were largest and transpiring (and thus taking up PFAAs) most.

Uptake factors

To evaluate the plant uptake of the PFAAs, the PFAA concentrations in the plant tissues were compared with the PFAA concentrations in the sampled exposure media, soil and pore water, using uptake factors. Concentrations in soil were only available for the start of the experiment and at the time of harvest, and concentrations in pore water were only available at harvest. We chose to use the concentrations in exposure media measured at harvest because a much larger portion of the plant growth and transpiration occurred during the latter part of the growth period and because soil concentrations were judged to be more stable (see above). It is nevertheless possible that the uptake factors for the shortest chain PFAAs are somewhat overestimated due to the depletion of these chemicals in the soil over the course of the experiment.

Root uptake

Root uptake was assessed using RCFs, calculated as the ratio between the PFAA concentration in the roots (on a fresh weight basis) at the time of harvest and the concentration in the corresponding exposure medium (i.e.,, soil). Two RCFs were calculated, one using the PFAA concentration in the upper layer of the soil (on a dry weight basis) at the time of harvest (RCF_{soil}) to represent the exposure medium, and the other using the concentration in the pore-water in the upper soil layer at the time of harvest ($RCF_{porewater}$). For a given chemical, there was some variability between the RCFs from the different contamination levels, with relative standard deviations averaging 0.64 for $RCF_{porewater}$ and 0.35 for RCF_{soil} (Table 1). The higher variability for $RCF_{porewater}$ could be due to a larger uncertainty in the determination of the PFAA concentration in pore water, which could have arisen from the separation procedure, small sample quantity and lower concentrations.

Table 1: Root concentration factors (RCFs) for PFAAs in lettuce roots from the three lowest exposure levels, calculated with respect to the concentration in soil pore water ($RCF_{porewater}$) and dry soil (RCF_{soil}).

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
$RCF_{porewater}$ (L/kg root fresh weight)[§]													
Level 1	16.2	11.2	0.43	0.02	0.1	5.0						0.11	
Level 2	9.4	3.3	0.37	0.07	0.1	2.0	23				28	0.16	8.8
Level 3	5	1.14	0.14	0.09	0.24	1.57	8.1	61	61	27	9.5	0.28	3.6
Average	**10.2**	**5.2**	**0.31**	**0.06**	**0.14**	**2.9**	**15.5**	**61**	**61**	**27**	**18.8**	**0.18**	**6.2**
RSD[‡]	0.56	1.02	0.49	0.60	0.58	0.65	0.68				0.70	0.49	0.60
RCF_{soil} (kg dry soil/kg root fresh weight)[†]													
Level 1	152	68	1.51	0.04	0.08	0.36	0.55	0.51	0.41	0.09	0.07	0.15	0.21
Level 2	92	27	1.41	0.17	0.15	0.39	0.71	0.77	0.36	0.14	0.08	0.21	0.43
Level 3	40	8	0.6	0.17	0.14	0.64	0.84	0.89	0.36	0.15	0.08	0.24	0.38
Average	**95**	**34**	**1.17**	**0.13**	**0.12**	**0.46**	**0.70**	**0.72**	**0.38**	**0.13**	**0.08**	**0.20**	**0.34**
RSD[‡]	0.59	0.89	0.43	0.58	0.32	0.33	0.21	0.27	0.08	0.25	0.07	0.23	0.34

[§] Concentrations in roots and pore water are given in Table S10 and Table S8, respectively.
[‡] Relative standard deviation
[†] Concentrations in roots and soil are given in Table S10 and Table S7, respectively.

The variability in RCF between chemicals exceeded three orders of magnitude for both RCF_{soil} and $RCF_{porewater}$, and was thus much greater than the variability due to the different contamination levels. The lowest and highest values of RCF_{soil} were measured for PFTeDA (0.08) and PFBA (95), respectively, while the lowest and highest values of $RCF_{porewater}$ were measured for PFHpA (0.06) and PFUnA/PFDoDA (61) (Table 1). RCF_{soil} was particularly elevated for PFBA and PFPeA, which is consistent with other reports for wheat, radish, celery, tomato and pea (Blaine et al. 2014a; Lan et al. 2018). However, some of the elevation could be due to the uncertainty in the concentrations of these chemicals in soil (see above). No chain length dependence of RCF_{soil} was observed in another study of wheat (Wen et al. 2014).

Mechanistic insight into the influence of soil on root uptake was obtained by comparing $RCF_{porewater}$ with the RCF values from our previous experiment in which lettuce was grown in a hydroponic solution ($RCF_{hydroponic}$) (Wen et al. 2014). Both $RCF_{porewater}$ and $RCF_{hydroponic}$ are referenced to water, which facilitates comparison. The maximum concentrations in the nutrient solution in the hydroponic experiment (~1 µg L^{-1}) were somewhat below the concentration range observed in pore water in the field experiment (2-900 µg L^{-1}). $RCF_{hydroponic}$ and $RCF_{porewater}$ show a similar pattern with PFAA carbon chain length, characterized by minimum values for PFHxA and PFHpA, with increasing values towards shorter and longer chain lengths (Figure 1).

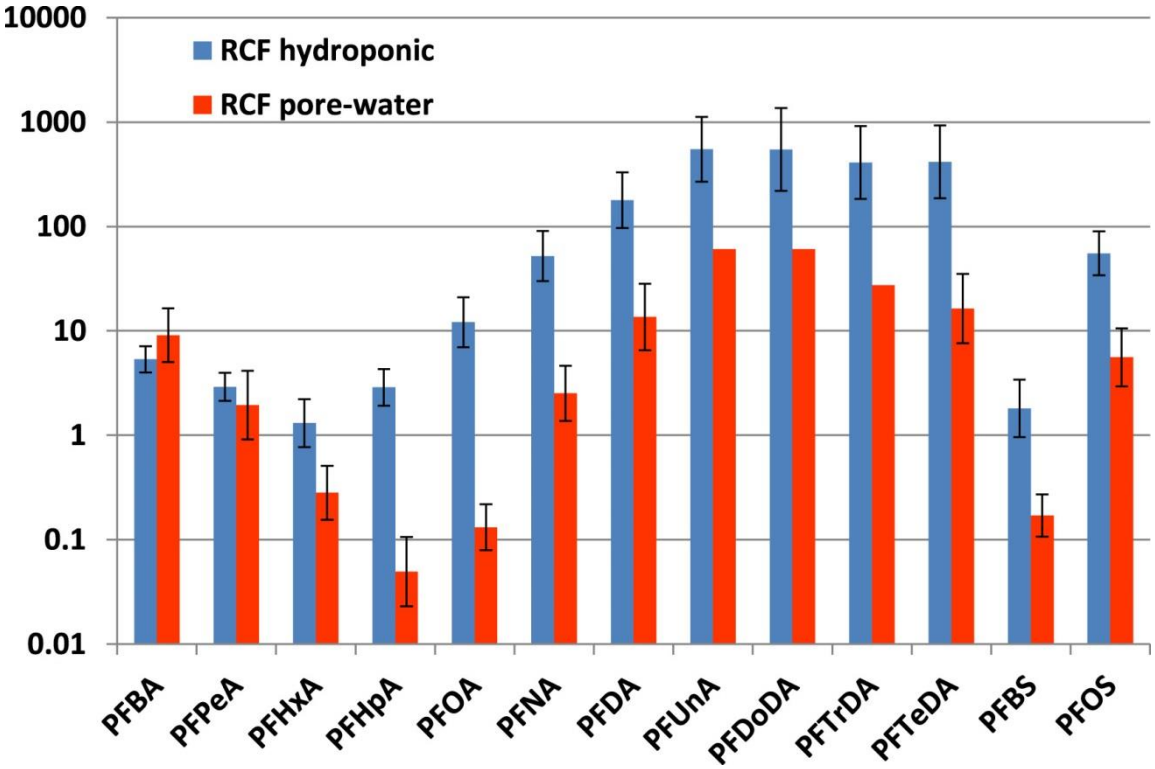

Figure 1: Comparison of PFAA root concentration factors: RCF_{soil}, based on concentrations in soil from this experiment; $RCF_{porewater}$, based on concentrations in pore water from this experiment; $RCF_{hydroponic}$, from a hydroponic experiment (Felizeter et al., 2012). Average values from experiments conducted at different exposure levels are shown. Error bars denote the standard error.

For PFBA and PFPeA there was good agreement between $RCF_{hydroponic}$ and $RCF_{porewater}$ (5.6/10.2 and 3.0/5.2, respectively). For the longer chain PFCAs and the PFSAs, $RCF_{porewater}$ was 1-2 orders of magnitude less than $RCF_{hydroponic}$. For these chemicals the hydroponic study greatly overestimated the root uptake under field conditions.

The similar chain length pattern for $RCF_{hydroponic}$ and $RCF_{porewater}$ suggests that similar processes govern the root uptake of PFAAs from these two media. In the hydroponic study it was concluded that PFBA and PFPeA readily crossed the Casparian strip and accumulated in the vascular tissue of the roots (Felizeter et al. 2012). The similar values of $RCF_{hydroponic}$ and $RCF_{porewater}$ for these two chemicals suggest that their transport from solution in pore water across the Casparian strip is similar under hydroponic and soil conditions.

In the hydroponic study it was further concluded that the uptake of the longer chain PFAAs was dominated by sorption to the root surface (Felizeter et al. 2012). In the soil experiment the RCF of PFAAs with perfluoroalkyl chain lengths of six and longer is 1-2 orders of magnitude lower than in the hydroponic experiment. It follows that there are marked differences between hydroponic and soil conditions with respect to sorption to the root surface. This cannot be attributed to differences in the preparation of the root samples, as the same procedure was used in both studies (rinsing with demineralized water, drying on paper towel, homogenization, extraction). One possible explanation is that a significant fraction of the chemical in the pore water was not freely dissolved but rather sorbed to colloidal matter. Since presumably only the freely dissolved PFAAs are available for

sorption to the root surface, this would have reduced the root uptake. However, although this hypothesis is plausible for PFDoDA, PFTrDA and PFTeDA for which the concentrations in soil are more than two orders of magnitude greater that the concentrations in pore water (Tables S7 and S8), it cannot explain the differences between $RCF_{hydroponic}$ and $RCF_{porewater}$ for medium chain length PFAAs like PFHpA and PFOA for which the concentration in pore water is similar to or even higher than the concentration in soil. Wen et al. (2013) reported that uptake of PFOA and PFOS into maize roots is modulated by metabolic inhibitors, aquaporin inhibitors and anion channel blockers (Wen et al. 2013), but it is not apparent how an absorption model could explain the observed difference in RCF between soil and hydroponic exposure. Zhao et al. (2013) stated in their work that water chemistry variables such as pH and salinity can have an effect on the RCF. The pH dependence of root uptake was also investigated by Krippner et al. (2014). However, the effects that they reported (maximum a factor of 1.7 between pH=5 and pH=7) are much smaller than the discrepancy between $RCF_{hydroponic}$ and $RCF_{porewater}$ observed in the present study. This suggests that other soil pore water properties play a more important role for the sorption of PFAAs to roots.

The one plausible explanation that we have for the discrepancy between $RCF_{hydroponic}$ and $RCF_{porewater}$ is competitive sorption. The pore water contains a multitude of other solutes besides the PFAAs. If they successfully compete for sorption sites on the root surfaces, this would result in less sorption of the PFAAs. To explain interspecies differences in RCF of PFOS and PFOA of a factor 3.5 and 6, respectively, Wen et al have proposed that lipids in plant root tissue compete with PFAAs for sorption sites in root proteins (Wen et al. 2016). Our results suggest that competition from other solutes in the soil may have an even stronger effect.

Foliage accumulation

Foliage uptake was assessed using FCFs that were calculated analogously to the RCFs using the PFAA concentrations in the foliage (on a fresh weight basis) at the time of harvest. For a given chemical, there was some variability between the FCFs from the different contamination levels, with relative standard deviations averaging 0.67 for $FCF_{porewater}$ and 0.45 for FCF_{soil} (Table 2). As for RCF, the higher variability for $FCF_{porewater}$ could be due to a larger uncertainty in the determination of the PFAA concentration in pore water.

The variability in $FCF_{porewater}$ between chemicals was much less than for RCF, amounting to a factor of 26. The variability in FCF_{soil}, on the other hand, was a factor 8800 and exceeded the variability in RCF_{soil}. It showed a strong inverse correlation with chain length, with the lowest and highest values measured for PFTeDA and PFBA, respectively (Table 2). This pronounced inverse correlation for FCF_{soil} with chain length is consistent with other reports in the literature (Yoo et al. 2011; Blaine et al. 2014a; Krippner et al. 2015; Navarro et al. 2017; Lan et al. 2018). As with the roots, the properties of the PFAAs clearly have a strong influence on their transfer from the soil environment to lettuce foliage.

In analogy to the RCF, we first compared $FCF_{porewater}$ with $FCF_{hydroponic}$ to obtain mechanistic insight into the influence of soil on the uptake of PFAAs in foliage (Figure 2). $FCF_{porewater}$ and $FCF_{hydroponic}$ show a similar pattern with chain length; minimum FCFs were obtained for PFHpA and PFOA, with increasing values towards shorter and longer chain lengths. The magnitudes of $FCF_{porewater}$ and $FCF_{hydroponic}$ were also similar; the standard deviations overlapped for all substances except for PFOS

(no measure of uncertainty was available for $FCF_{porewater}$ for PFUnA, PFDoDA and PFTrDA), and the median difference was a factor 1.6 (Figure 2).

Table 2: Foliage concentration factors (FCFs) for PFAAs in lettuce from the three lowest exposure levels, calculated with respect to the concentration in soil pore water ($FCF_{porewater}$) and dry soil (FCF_{soil}).

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
FCFporewater (L/kg foliage fresh weight) §													
Level 1	31	37	4.8	0.13	0.21	0.5						0.91	
Level 2	16.3	12.3	3.7	0.65	0.34	2.2	4.3				6.1	1.75	5.2
Level 3	9.6	4.9	0.93	1.38	1.59	2.1	3.1	7.5	9.4	5.5	2.4	4.9	8.2
Average	18.8	18.1	3.1	0.72	0.71	1.58	3.7	7.5	9.4	5.5	4.3	2.5	6.7
RSD‡	0.57	0.94	0.63	0.87	1.07	0.59	0.22				0.61	0.83	0.32
FCFsoil (kg dry soil/kg foliage fresh weight) †													
Level 1	290	230	16.8									1.22	
Level 2	161	99	14.1	1.58	0.51	0.41	0.13	0.09	0.05	0.04	0.02	2.4	0.19
Level 3	78	34	3.9	2.7	0.95	0.85	0.32	0.11	0.06	0.03	0.02	4.2	0.88
Average	175	120	11.6	2.1	0.73	0.63	0.23	0.1	0.05	0.03	0.02	2.6	0.53
RSD‡	0.6	0.82	0.59	0.36	0.43	0.49	0.58	0.14	0.14	0.24	0	0.57	0.92

§ Concentrations in foliage and pore water are given in Table S10 and Table S8, respectively.
‡ Relative standard deviation
† Concentrations in foliage and soil are given in Table S10 and Table S7, respectively.

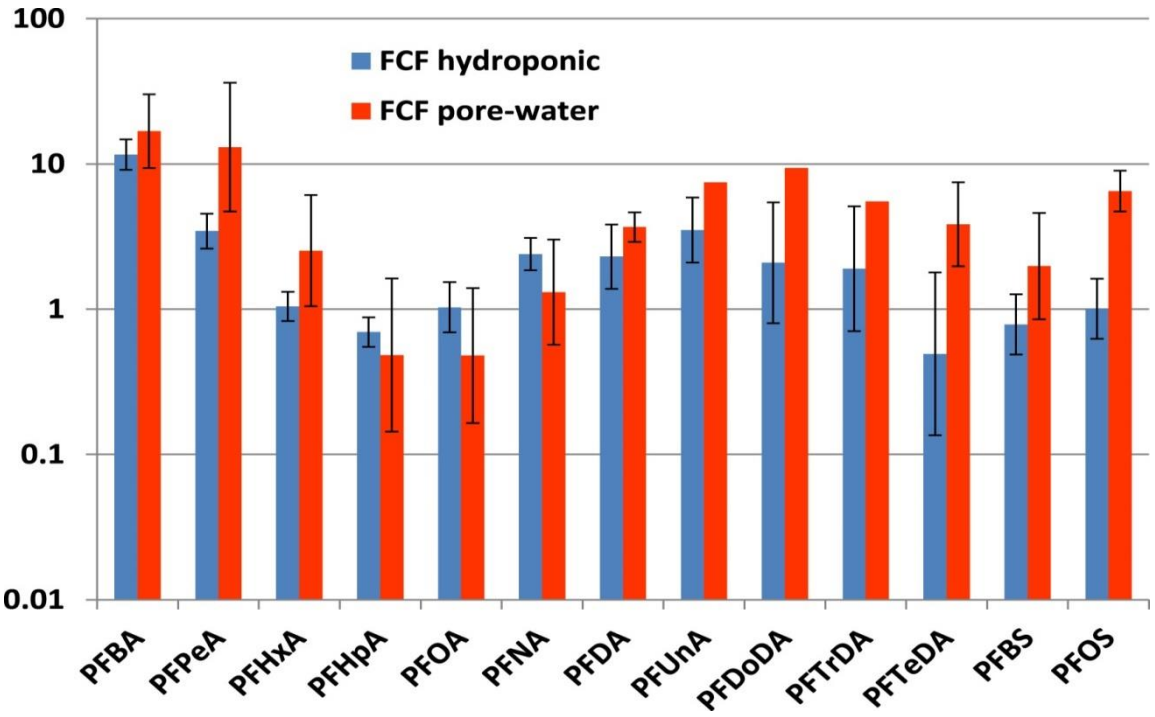

Figure 2: Comparison of PFAA foliage concentration factors: FCF_{soil}, based on concentrations in soil from this experiment; $FCF_{porewater}$, based on concentrations in pore water from this experiment; $FCF_{hydroponic}$, from a hydroponic experiment (Felizeter et al., 2012). Average values from experiments conducted at different exposure levels are shown. Error bars denote the standard error.

Transport from the soil solution to the foliage requires that a chemical first crosses the Casparian strip in the root endodermis and then is translocated with the xylem flow through the roots to the foliage. Underway the chemical can be sequestered into the root tissue. In the hydroponic study the overall effectiveness of this transport was evaluated using TSCF, the quotient of the concentration in the xylem flow and that in the nutrient solution. This is equivalent to the fraction of the chemical originally in the water taken up by the roots that arrives in the foliage. It showed a maximum (0.8) for PFBA, decreasing values with increasing perfluoroalkyl chain length to a minimum (0.05) for PFHpA, followed by increasing values again to PFDoDA (0.3) and thereafter decreasing values to PFTeDA (0.06). It was concluded that the TSCF minimum for PFHpA was the result of lower efficiency of the transport across the Casparian strip (Felizeter et al. 2012). A similar U-shaped dependency of TSCF on perfluoroalkyl chain length was observed in chicory, and retardation factors for root uptake of different PFAAs were determined (Gredelj et al. 2020b).

To calculate the TSCF for the lysimeter study, the amount of water transpired (Q_W, L) must be known in order to convert the concentration in the foliage (C_F, mol kg^{-1}) into the concentration in xylem flow (C_X, mol L^{-1}).

$$C_X = C_F \frac{Q_F}{Q_W} \quad (1)$$

where Q_F is the mass of the foliage (kg). However, Q_F is not known, so the TSCF cannot be calculated. Nevertheless, the TSCF is related to $FCF_{porewater}$ by a constant (Q_F / Q_W), and therefore the PFAA chain length pattern for $FCF_{porewater}$ corresponds to the PFAA chain length pattern for TSCF. The similarity in the chain length patterns for $FCF_{porewater}$ and $FCF_{hydroponic}$ (Figure 2) indicates that the relative efficiency of transport across the Casparian strip and translocation through the roots was similar for lettuce grown in the soil and hydroponic environments. Consequently, and in contrast to the results for root uptake, the hydroponic experiment yielded information on PFAA uptake in foliage that could be transferred to field conditions.

With this finding, measurements of foliage accumulation from hydroponic experiments can be integrated in a simple and sensible structure to quantify foliage uptake in the field. In order to quantitatively transfer $FCF_{hydroponic}$ for a given PFAA to the field, two pieces of information are required. One is the ratio of the specific cumulative transpiration in the field to that in the hydroponic study, where the specific cumulative transpiration is defined as the total amount of water transpired during the period of exposure to the PFAAs per gram of foliage biomass. This ratio will vary with the duration of the exposure and the climate, as well as with the conditions in the laboratory experiment (in this study the ratio was ~1.6). The second important piece of information is the soil/pore water distribution coefficient. Since we generally only have information on contaminant levels in soil, we need to understand the soil/pore water distribution in order to employ FCF from hydroponic experiments to estimate levels in foliage in the environment.

Transferring FCF results from hydroponic experiments to other plant species is subject to larger uncertainty than transferring for the same species. Considerable interspecies variation in FCF_{soil} has been observed (Blaine et al. 2014a; Gobelius et al. 2017). Some of this can be due to differences in the specific cumulative transpiration of different species. However, marked differences in relative FCF_{soil} for different PFAAs have also been reported. One explanation for this is species specific

differences in the TSCF. For instance, the TSCF for lettuce varies widely as a function of chain length, with a very pronounced minimum for PFHpA (a factor of 5 and 4 lower than for PFPeA and PFDA, respectively) (Felizeter et al. 2012), while the TSCF for tomato, cabbage, and zucchini varied by less than a factor of 2 among the C3-C10 PFAAs (Felizeter et al. 2014). This suggests that there are chain length specific differences in the permeability of the Casparian strip barrier for lettuce. It is also conceivable that differences in sorption coefficients to viable root tissue could contribute to species differences in the relative FCFs of PFAAs, as this would create different chromatography-like retention effects during xylem (i.e.,, the mobile phase) transport through viable root tissue (i.e.,, the stationary phase) to the foliage. Despite these limitations, hydroponic experiments remain a useful tool for studying the accumulation of PFAAs in plant foliage.

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Supporting Information

Description of the instrumental method

The analytical methodology was according to the methods described by Eschauzier et al. (2010)[1]. The measurements were conducted in the scheduled MRM-mode (see Table S2). Briefly, instrumental settings included:

Ion Transfer Voltage:	-2000 V
Interface Temperature:	450°C
Curtain gas:	10 L min^{-1}
Collision gas:	6 L min^{-1}
Collision Energy:	-10 V for PFPeA to PFOA, -15 V for PFBA, -25 V for PFNA to PFTeDA and -70 V for the PFSAs

The concentrations of calibration standards ranged from 0.005 ng ml^{-1} (Calibration level 1) to 200 ng ml^{-1} (Calibration level 12). Peaks consisted of at least 24 scans and the smoothing width was 9 points.

For separation on the column a gradient elution with two mobile phases, A (40:60 methanol:water) and B (95:5 methanol:water; both with 2 mM ammonium acetate) was used. The system was equilibrated for 8 minutes with the initial mobile phase composition of 60% A at a flow of 0.2 ml/min prior to sample injection. After injection the mobile phase composition changed linearly to 100% B at 10 minutes. This was held isocratic until 20 minutes. Afterwards the solvent composition was returned to initial condition within 2 minutes.

[1] Eschauzier, C.; Haftka, J.; Stuyfzand, P. J.; de Voogt, P., Perfluorinated Compounds in Infiltrated River Rhine Water and Infiltrated Rainwater in Coastal Dunes. *Environ. Sci. Technol.* **2010,** *44*, (19), 7450-7455.

Table S12: List of chemicals used, their purity and suppliers.

Chemical	Purity	Supplier
MPFAC-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
MPFAS-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
M5PFPeA (internal standard)		Wellington Laboratories, Ontario, Canada
M4PFHpA (internal standard)		Wellington Laboratories, Ontario, Canada
PFAC-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFAS/FOSA-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFBA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFPeA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHxA	≥97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHpA	99%	Sigma Aldrich, Zwijndrecht, Netherlands
PFOA	96%	Sigma Aldrich, Zwijndrecht, Netherlands
PFNA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFUnA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDoDA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTrDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTeDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFBS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFOS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
Sodium carbonate	≥99%	Sigma Aldrich, Zwijndrecht, Netherlands
Sodium hydroxide	≥98,8%	J.T. Baker Chemicals, deventer, Netherlands
Sodium hydrogencarbonate	≥99,5%	Merck, Darmstadt, Germany
Sodium sulfate	≥99%	Merck, Darmstadt, Germany
Tetrabutylammoniumhydrogensulfate (TBA)	≥99%	Merck, Darmstadt, Germany
Ammonium hydroxide		Sigma Aldrich, Zwijndrecht, Netherlands
Ammonium acetate	≥99,999%	Sigma Aldrich, Zwijndrecht, Netherlands
Methanol	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands
Water	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands
tert-Butyl methyl ether (MTBE)	HPLC-grade	Biosolve, Valkenswaard, Netherlands

Table S2: List of the analytes, their abbreviations and molecular formulas, the 13C-labelled internal standards used for their quantification, and the mass transitions used in the MS/MS analysis of the analytes.

Abbreviation	Compound	Transition 1	Transition 2	Quantification by internal Standard	Molecular Formula
PFBA	Perfluoro-n-butanoic acid	213 → 169	-	$^{13}C_4$ PFBA	$CF_3(CF_2)_2COOH$
PFPeA	Perfluoro-n-pentanoic acid	263 → 219	-	$^{13}C_5$ PFPeA	$CF_3(CF_2)_3COOH$
PFHxA	Perfluoro-n-hexanoic acid	313 → 269	313 → 119	$^{13}C_2$ PFHxA	$CF_3(CF_2)_4COOH$
PFHpA	Perfluoro-n-heptanoic acid	363 → 319	363 → 169	$^{13}C_4$ PFHpA	$CF_3(CF_2)_5COOH$
PFOA	Perfluoro-n-octanoic acid	413 → 369	413 → 169	$^{13}C_8$ PFOA	$CF_3(CF_2)_6COOH$
PFNA	Perfluoro-n-nonanoic acid	463 → 419	463 → 219	$^{13}C_9$ PFNA	$CF_3(CF_2)_7COOH$
PFDA	Perfluoro-n-decanoic acid	513 → 469	513 → 269	$^{13}C_6$ PFDA	$CF_3(CF_2)_8COOH$
PFUnA	Perfluoro-n-undecanoic acid	563 → 519	563 → 269	$^{13}C_7$ PFUnA	$CF_3(CF_2)_9COOH$
PFDoDA	Perfluoro-n-dodecanoic acid	613 → 569	613 → 319	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{10}COOH$
PFTrDA	Perfluoro-n-tridecanoic acid	663 → 619	663 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{11}COOH$
PFTeDA	Perfluoro-n-tetradecanoic acid	713 → 669	713 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{12}COOH$
PFBS	Perfluorobutane sulfonate	299 → 80	299 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_3SO_3$
PFOS	Perfluorooctane sulfonate	499 → 80	499 → 99	$^{13}C_8$ PFOS	$CF_3(CF_2)_7SO_3$
$^{13}C_4$ PFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	217 → 172	-		
$^{13}C_5$ PFPeA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	268 → 223	-		
$^{13}C_2$ PFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	315 → 270	315 → 119		
$^{13}C_4$ PFHpA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	367 → 323	367 → 169		
$^{13}C_8$ PFOA	Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	421 → 376	421 → 172		
$^{13}C_9$ PFNA	Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]nonanoic acid	472 → 427	472 → 223		
$^{13}C_6$ PFDA	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	519 → 474	519 → 219		
$^{13}C_7$ PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	570 → 525	570 → 270		
$^{13}C_2$ PFDoDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	615 → 570	615 → 369		
$^{18}O_2$ PFHxS	Perfluoro-1-hexane[$^{18}O_2$]sulfonate	403 → 84	403 → 103		
$^{13}C_8$ PFOS	Perfluoro-1-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanesulfonate	507 → 80	507 → 99		

Table S3: Lettuce biomass at harvest (g fresh weight/plant, average for the plants analyzed from each level).

	Foliage	Roots
Level 1	250	50
Level 2	250	50
Level 3	250	50
Level 4	150	30

Table S4: Recoveries (in %) of the mass-labeled internal standards. The recoveries were determined by comparing the standard signal in the sample to the signal in matrix-free solutions which had been spiked with the same quantity of internal standard immediately prior to analysis. The bold (upper) entries are the mean recoveries, while the non-bold (lower) entries are the respective coefficient of variation (in %).

		$^{13}C_4$ PFBA	$^{13}C_5$ PFPeA	$^{13}C_2$ PFHxA	$^{13}C_4$ PFHpA	$^{13}C_8$ PFOA	$^{13}C_9$ PFNA	$^{13}C_6$ PFDA	$^{13}C_7$ PFUnA	$^{13}C_2$ PFDoDA	$^{18}O_2$ PFHxS	$^{13}C_8$ PFOS
Soil		**103**	**91**	**92**	**94**	**92**	**93**	**106**	**109**	**112**	**105**	**101**
		13	12	10	9	6	11	8	12	13	4	9
Lettuce	**Root**	**23**	**75**	**98**	**81**	**67**	**86**	**58**	**50**	**85**	**102**	**43**
		8	10	9	10	6	10	10	9	6	14	7
	Foliage	**22**	**95**	**71**	**71**	**63**	**50**	**58**	**53**	**91**	**94**	**52**
		9	13	12	14	14	13	13	14	14	10	15

Table S5: Limits of Quantification (LoQ) in ng g^{-1} fresh weight for roots and foliage, ng g^{-1} dry weight for soil, and ng ml^{-1} for pore water.

		PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Lettuce	Roots	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.022	0.023
	Foliage	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.017	0.017
Soil		0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.089	0.092
Pore-water		0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.089	0.092

Table S6: Concentrations[#] in soil at the start of the experiment in ng g^{-1} dry weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Upper layer													
Level 1	105.3	99.0	105.0	98.9	97.8	98.2	103.8	94.9	87.3	115.2	87.9	97.4	108.3
Level 2	952.7	972.5	916.2	1033	1116	1066	973.3	1054	1019	1017	978.9	1015	1008
Level 3	4626	4511	3170	4950	4937	4737	5096	4640	4717	4905	5131	4757	5244
Level 4	7769	9321	4832	10063	9990	9999	10239	10102	10212	9888	8790	9607	9834
Lower layer													
Level 1	90.0	88.2	89.0	107.1	95.8	100.0	96.3	95.2	80.6	69.0	63.8	71.9	109.4
Level 2	918.6	1070.1	964.3	1015	980.5	1082	1043	1043	1195	944.0	1014	1199	1026
Level 3	4896	4953	4904	5136	5294	5574	5444	6033	5045	4869	4965	4981	5270
Level 4	8263	10331	10137	10253	10277	10679	10080	9833	9819	9671	10156	11080	9960

Table S7: Concentrations[#] in soil at the time of the harvest of the lettuce plants in ng g^{-1} dry weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Upper layer													
Unspiked	<LoQ	0.18	0.18	0.21	0.31	0.35	0.41	0.52	0.69	0.52	0.64	0.09	0.48
Level 1	2.57	2.09	2.57	44.9	30.9	46.2	51.7	60.0	62.7	93.9	72.9	3.39	78.2
Level 2	10.1	8.40	7.76	46.2	210.8	450.0	516.1	629.9	735.0	770.5	848.5	17.1	712.3
Level 3	10.4	11.4	20.1	60.8	583.9	2150	2494	2883	3245	3928	4259	38.1	3801
Level 4	10.8	14.2	31.8	132.9	1683	3973	5072	6037	7098	7779	7299	62.6	7075
Lower layer													
Level 1	2.57	2.26	2.75	45.1	49.7	55.4	69.5	64.2	56.8	57.3	71.0	3.48	94.2
Level 2	10.2	9.05	7.59	46.2	244.0	385.6	447.4	526.2	679.3	846.8	919.9	18.4	703
Level 3	12.2	11.8	20.5	54.6	233.2	2096	2541	2580	3028	3844	4352	43.5	3214
Level 4	12.4	15.1	32.4	130.2	926.3	4156	4918	5297	6384	7454	8619	67.2	6349

Table S8: Concentrations[#] in pore water at the time of harvest in ng ml^{-1}.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Upper layer													
Unspiked	0.46	0.81	0.58	0.28	0.26	1.36	3.31	4.45	4.24	3.19	2.76	0.15	1.38
Level 1	24.0	12.7	9.09	93.9	24.8	3.28	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	4.53	<LoQ
Level 2	99.2	67.9	29.6	113.2	322.7	86.4	16.0	<LoQ	<LoQ	<LoQ	2.39	23.3	35.2
Level 3	84.8	80.0	84.5	118.0	346.7	875.6	258.7	42.0	19.2	22.0	34.5	32.7	402.7
Level 4	96.2	101.8	127.4	368.6	3994	1777	293.1	81.5	47.8	50.7	74.8	175.5	547.9
Lower layer													
Level 1	23.2	10.1	15.6	63.8	5.00	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	2.52	<LoQ
Level 2	57.9	56.1	76.5	667.0	119.1	61.5	10.7	<LoQ	<LoQ	<LoQ	<LoQ	49.6	33.1
Level 3	206.8	71.7	57.3	104.6	415.8	151.6	11.0	1.34	<LoQ	<LoQ	<LoQ	25.9	11.4
Level 4	95.7	77.8	96.0	253.0	3030	880.5	100.5	43.0	20.4	14.3	11.6	49.3	179.8

Table S9: Fraction of chemical in the bulk soil that was present in the pore water at the time of harvest in %.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Upper layer													
Level 1	119	78	45	27	10	1						17	
Level 2	118	96	46	29	18	2	0				0	16	1
Level 3	95	82	49	23	7	5	1	0	0	0	0	10	1
Level 4	109	88	49	34	29	5	1	0	0	0	0	34	1
Lower layer													
Level 1	67	33	42	11	1							5	
Level 2	47	51	83	119	4	1	0					22	0
Level 3	106	38	17	12	11	0	0	0				4	0
Level 4	53	35	20	13	22	1	0	0	0	0	0	5	0

Table S10: Concentrations[#] in the roots and the foliage of the lettuce plants at the end of the experiment in ng g^{-1} fresh weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Roots													
Unspiked	0.70	0.36	0.28	0.32	0.52	1.31	1.39	1.66	1.30	1.12	0.57	0.24	1.85
Level 1	389.2	141.9	3.88	1.94	2.50	16.4	28.4	30.5	25.8	8.83	5.08	0.51	16.2
Level 2	929.8	225.4	10.9	7.68	31.0	176.3	366.7	486.0	267.6	107.0	67.1	3.67	308.7
Level 3	420.0	91.3	12.0	10.1	81.7	1376	2096	2552	1160	601.4	327.5	9.24	1433
Level 4	158.1	28.3	5.95	7.77	61.8	1173	2569	4140	2096	1194	612	4.01	2410
Foliage													
Unspiked	0.50	0.12	0.09	0.08	0.32	0.80	0.67	0.63	0.67	0.54	0.43	0.05	1.04
Level 1	732.4	474.0	43.2	12.0	5.11	1.64	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	4.14	<LoQ
Level 2	1621	833.0	109.1	73.1	108.4	185.8	69.4	58.2	35.6	27.1	14.7	40.8	134.2
Level 3	810.3	388.1	78.3	162.3	552.5	1837	806.2	313.9	179.8	121.1	82.8	159.2	3337
Level 4	292.1	97.9	35.3	83.5	819.8	4184	2156	619.2	355.6	251.7	150.5	16.5	6571

[#] The concentrations in the sample from the unspiked lysimeter have been subtracted from the concentrations in Levels 1-4.

<LoQ arises when the correction above gives a number <LOQ.

This applies to Tables S6, S7, S8 and S10.

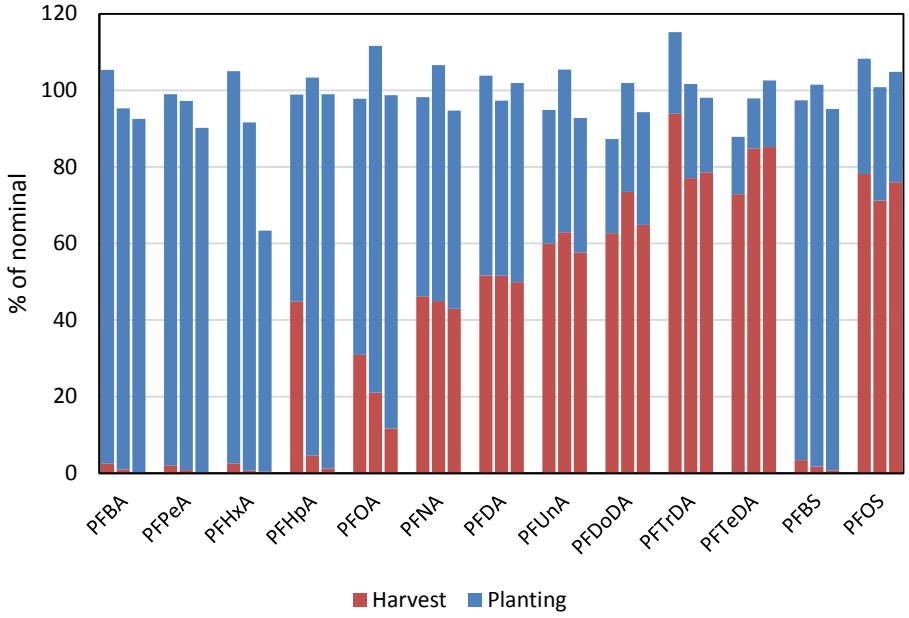

Figure S1: Concentrations in the surface soil layer at planting and at harvest, expressed as the percentage of the nominal concentration. Three bars are shown for each chemical representing the 3 exposure levels (nominal concentrations of 0.1 mg/kg, 1 mg/kg and 5 mg/kg, left to right). The soil concentrations are provided in Tables S6 and S7.

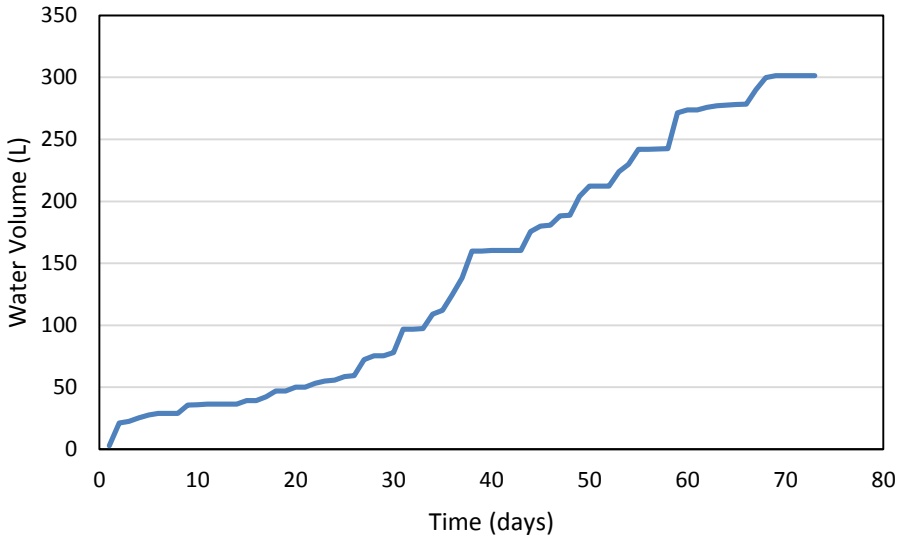

Figure S2: Cumulative volume of water (L) added to the lysimeter as a function of time.



Chapter 6

UPTAKE OF PERFLUORINATED ALKYL ACIDS BY CROPS:

RESULTS FROM A FIELD STUDY

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Abstract

Four crops with different edible plant parts (radish, lettuce, pea and maize) were grown in outdoor lysimeters on soil spiked with 13 perfluorinated alkyl acids (PFAAs) at 4 different levels. PFAA concentrations were measured in soil, soil pore water, and different plant parts at harvest. Edible part/soil concentration factors ranged over seven order of magnitude and decreased strongly with increasing PFAA chain length, by a factor of 10 for each additional fluorinated carbon (n_{CF}) for pea. Three processes were responsible for most of the variability. The first was sorption to soil; calculating whole plant concentration factors on the basis of concentration in pore water instead of soil reduced the variability from five orders of magnitude to two. Second, the journey of the PFAAs with the transpiration stream to the leaves was hindered by retention in the roots driven by sorption; root retention factors increased by a factor 1.7 for each n_{CF}. Third, transfer of PFAAs from the leaves to the fruit via the phloem flow was also hindered – presumably by sorption; fruit/leaf concentration factors decreased by a factor 2.5 for each n_{CF}. A simple mathematical model based on the above principles described the measured concentrations in roots, leaves, fruits and radish bulbs within a factor 4 in most cases. This indicates that the great diversity in PFAA transfer from soil to crops can be largely described with simple concepts for four markedly different species.

Keywords: PFAA, root uptake, translocation, crops, plants, PFOA, PFOS

Introduction

In addition to having been detected ubiquitously in several environmental compartments including water (Ahrens 2011; Eschauzier et al. 2012b), biota (Giesy et al. 2001) and the atmosphere (Dreyer et al. 2009), perfluorinated alkyl acids (PFAAs) have also been found in human blood serum and breast milk (Volkel et al. 2008; Karrman et al. 2010; Antignac et al. 2013; Barbarossa et al. 2013). Because of their known and suspected toxic effects (Lau et al. 2007; Domingo 2012; Saikat et al. 2013), it is important to understand the pathways of human exposure to minimize the risk for exposure and possible adverse health effects. The European Food Safety Authority therefore established tolerable daily intakes (TDIs) for perfluorooctanoic acid (PFOA), and perfluoroctane sulfonic acid (PFOS) in response to concerns about these chemicals (Johansson et al. 2009). They recently revised these TDIs and established a new and much lower tolerable weekly intake rate of 4.4 ng per kg bw per week for the extended group of PFOA, PFNA, PFHxS and PFOS (EFSA 2020). Food has been identified as the main source of human exposure (Ericson et al. 2008; Fromme et al. 2009; D'Hollander et al. 2010a; Dellatte et al. 2013; Herzke et al. 2013; Klenow et al. 2013), and crops are one possible vector for PFAAs into the food supply. PFAAs are taken up by crops when grown in contaminated soil that has been contaminated, for instance via water reuse irrigation or biosolids application (Stahl et al. 2009; Felizeter et al. 2012; Zhao et al. 2013), and there are two known cases where agricultural sites have been widely contaminated with PFAAs in Germany (Wilhelm et al. 2008; Regierungspräsidium-Karlsruhe 2018). The aim of the presented work is to further our understanding of how PFAAs are transferred from soils to crops.

Current knowledge of plant uptake of PFAAs has been summarized in several recent reviews (Ghisi et al. 2019; Jiao et al. 2020). In early research on this subject, Stahl et al. (Stahl et al. 2009) and Lechner et al. (Lechner et al. 2011) showed that the concentration of PFOA and PFOS in the vegetative parts of several crops was linearly proportional to the concentration in the soil they were grown in. Yoo et al. found that the foliage/soil concentration factors of C6-C14 perfluoroalkyl carboxylic acids (PFCAs) in grass decreased logarithmically with increasing chain length, while the foliage/soil pore water concentration factors increased with chain length (Yoo et al. 2011). This evidence for a pronounced influence of soil-pore water partitioning on uptake was supported by Blaine et al., who found that uptake by crops varied between soils (Blaine et al. 2013). In another field experiment Blaine and coworkers studied the distribution of C4-C10 PFAAs in four crops. They found that root/soil concentration factors (RCFS) varied little or not at all with chain length, but in agreement with Yoo et al. they found that both the shoot/soil concentration factors and the fruit/soil concentration factors decreased with increasing chain length, the first by 0.11-0.36 log units per CF2 group, the second by 0.54-0.58 log units. They incorporated these relationships into a simple conceptual model of PFAA uptake, attributing the lower accumulation of longer chained PFAAs in fruit compared to roots and shoot to an increased number of biological barriers that must be crossed (e.g., the cambium during loading into the phloem for transport to the fruit, and the Casparian strip separating root epidermis and cortex from root vascular tissue) (Blaine et al. 2014a). Wen et al., on the other hand, postulated that transport within the plant was mediated by the sorption of PFAAs to plant tissue and explored correlations between shoot/soil and root/soil concentration factors and the lipid and protein content of the respective tissue (Wen et al. 2016).

Further mechanistic insight into plant uptake of PFAAs can be obtained by studying hydroponic systems without the influence of soil. Transport of organic chemicals from the root zone to foliage is governed by the transpiration stream concentration factor (TSCF, the quotient of the concentration in the xylem flow and that in the nutrient solution) and the quantity of water transpired (Briggs et al. 1982; Dodgen et al. 2015; Zhang et al. 2020). In hydroponic experiments the TSCFs for C4-C11 PFCAs were similar in tomato, cabbage and zucchini and only weakly influenced by chain length, varying by less than a factor of 2.5 within a given species (Felizeter et al. 2014). In a hydroponic study with grass, the foliage-solution accumulation factors also varied by less than a factor 2.5 for C4-C10 PFAAs (Valcárcel et al. 2014). This suggests that the combined effects of biological barriers hindering PFAA uptake into the root and sorption to root vascular tissue retarding transport to foliage are similar across chain lengths and between species. Lettuce, on the other hand, showed a decidedly different, V-shaped, chain length dependency, with TSCF values for perfluorododecanoic acid (PFDoDA) that were 6 times greater than for perfluoroheptanoic acid (PFHpA) suggesting the presence of a chain length specific barrier to root uptake (Felizeter et al. 2012). Salinity and temperature were shown to be positively correlated with PFAA uptake rate, whereby this was attributed to the influence of these variables on transpiration rate (Zhao et al. 2016). Concerning accumulation in roots, root uptake factors from soil calculated on the basis of concentrations in pore water were up to two orders of magnitude lower than root uptake factors for the same crop (lettuce) in a hydroponic exposure. This was attributed to lower sorption to the roots surface as a result of competition from other molecules in the pore water and indicates that hydroponic experiments are of limited value for studying accumulation in roots (Felizeter et al. 2020). It has also been suggested that differences in root development between hydroponic and soil growing conditions lead to differences in PFAA accumulation (Gredelj et al. 2020a).

Despite this wealth of research, there is still limited understanding of the dominant factors controlling uptake of PFAAs from soil into edible plant parts under real-world exposure conditions. To contribute to progressing knowledge in the area, we grew four crops in outdoor lysimeters containing soil spiked with PFAAs. Radish, lettuce, pea and maize were chosen because their edible parts come from different parts of the plant (roots vs. leaves vs. pulses vs. cereal grain). A broad range of PFAAs was studied: 11 PFCAs (C4-C14) and 2 perfluoroalkane sulfonates (C4 and C8). Five lysimeters were used for each crop, four of which were spiked at different PFAA levels plus one non-spiked control. The different plant parts (roots, stems, leaves, etc.) were analyzed separately. Uptake factors based on PFAA concentrations in soil and pore water were calculated and compared across chain length and species.

Materials and methods

Chemical reagents and lab materials

The names, abbreviations and molecular formulas of the test chemicals, their suppliers and purities, and the ^{13}C-labeled internal standards used for their quantification can be found in Tables S1 and S2 of the Supporting Information (SI). All standards had a purity >95%.

Materials used for extraction and clean-up of the samples included Florisil SPE cartridges (1000 mg, 6 mL) from Applied Separations (Allentown, PA, USA); Acrodisc LC13 GHP Pall 0.2 µm filters from Pall Corporation (Port Washington, NY, USA); 50 and 15 mL polypropylene (PP) tubes with screw caps from Sarstedt (Nümbrecht, Germany); and Supelclean ENVI-Carb 120/140 from Supelco (Bellefonte,

PA, USA). Tetrabutylammoniumhydrogensulfate and sodium hydrogencarbonate were purchased from Merck (Darmstadt, Germany). Sodium carbonate and ammonium hydroxide a.c.s. reagent were from Sigma Aldrich; 2.0 and 0.3 mL PP vials were purchased from VWR International (Amsterdam, Netherlands). Centrifugation filter tubes (50 mL, 0.2 µm nylon filter) were obtained from Grace (Breda, Netherlands). For solvents see Table S1.

Field experiment

The field experiment was conducted at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Schmallenberg, Germany (51.15N, 8.29E) between June and October 2011. Each plant species was grown in 5 lysimeters, one containing soil with background concentrations of PFAAs (non-spiked), and 4 spiked with a mixture of PFAAs in which each PFAA had a nominal concentration in soil of 0.1 mg/kg dw, 1 mg/kg dw, 5 mg/kg dw and 10 mg/kg dw, respectively. For comparison, the PFOA and PFOS concentrations measured in contaminated agricultural soil in Arnsberg (51.41N, 8.05E), ~30 km from Schmallenberg, were ~1 mg/kg dw (Wilhelm et al. 2008). The lysimeters had a surface area of 1 m² and a total depth of 60 cm and were each filled with ~450 kg sand (30-60 cm depth) and ~450 kg of loamy sand (0-30 cm depth; 71% sand, 24% silt, 5% clay, pH 5.67, organic carbon content 0.93%). This resembled a typical soil in northwestern Germany. The soil used for the upper layer is available as a reference soil (Refesol 01-A) from Fraunhofer IME (www.refesol.de/boden01a.shtml). The lysimeters were outdoors and unprotected (see Figure S1). Precipitation was measured with a rain gauge located close to the lysimeters (Table S3).

The spiking of the soil was described in a previous paper (McLachlan et al. 2019). Briefly, a stock solution was prepared containing equal concentrations of all PFAAs. This stock solution was spiked into 2 kg of soil which was homogenized and then mixed with approx. 90 kg of soil in a concrete mixer to achieve the desired concentration. This was repeated 5 times for each layer in each lysimeter. Samples were taken from each batch and combined to determine the final PFAA concentration in the soil of each lysimeter.

The lysimeters were planted with onion, carrot, radish, lettuce, pea, bean or maize (Table 1). Each crop was planted in one lysimeter of each spiked soil level. Onion, carrot and radish were planted together as were pea and bean, while lettuce and maize were the only crops in their respective lysimeters. Within one week of preparing the spiked soil, 6 bean seeds, 20 radish seeds, 20 onion seeds, 20 carrot seeds and 6 pea seeds plus 9 maize seedlings and 20 lettuce seedlings (pre-grown in uncontaminated soil) were planted in the respective lysimeters (on June 21, 2011). The lysimeters were watered after planting, and kept humid by natural precipitation and additional watering if needed (see Table S3 for water inputs).

Onion, carrot and bean did not germinate in either the exposed or the control lysimeters. The other plants were harvested at maturity (see schedule in Table S4). At harvest soil samples were also taken. The soil samples were collected with a corer for lettuce, pea and maize. The soil core was separated into the upper (30 cm loamy sand) and lower (30 cm sand) layers, and the soil was packed in freezer bags and stored at –20 °C until analysis. It was not possible to sample the lower soil layer in some of the lysimeters. Consequently, only the results for the upper soil layer are presented here. For radish only the top 1-2 cm of the soil were sampled, because at the time of radish harvest it was still hoped that the onions and carrots seeded in the same lysimeters would germinate. The whole plant was

harvested and divided into plant parts as detailed in Table 1. All plant parts were packed in polyethylene freezer bags and stored at –20 °C until analysis.

Table 1: Summary of the field experiment

Crop	Soil concentration (nominal, mg/kg)	Plant compartments sampled
onion (*allium cepa*)	B[§], 0.1, 1, 5, 10	Did not germinate
carrot (*daucus carota*)	B[§], 0.1, 1, 5, 10	Did not germinate
radish (*rapahnus sativus*)	B[§], 0.1, 1, 5, 10	Roots, bulb, foliage
Lettuce[#] (*lactuca sativa*)	B[§], 0.1, 1, 5, 10	Roots, foliage
pea (*pisum sativum*)	B[§], 0.1, 1, 5, 10	Roots, stem, twigs, leaves, pods, peas
bean (*phaseolus vulgaris*)	B[§], 0.1, 1, 5, 10	Did not germinate
maize (*zea mays*)	B[§], 0.1, 1, 5, 10	Roots, stem, leaves, hull leaves, cobs, kernels

§ B = the background contamination present in the test soil

The results for lettuce have already been published in connection with a comparison of hydroponic and soil uptake.[39]

Extraction

Root and radish bulb samples were gently washed (no brushes were used) under running demineralized water to remove adherent soil and afterwards dried superficially by patting with kitchen towels. No cleaning of the other plant samples was necessary. The material was homogenized with a household blender (Braun Multiquick MX 2050). For the extraction of the PFAAs from the samples, the method by Hansen et al. (Hansen et al. 2001) was used with modifications proposed by Vestergren et al. (Vestergren et al. 2012) as described in our previous work (Felizeter et al. 2012; Felizeter et al. 2014). Briefly, 10 g of the homogenate were weighed in a 50 mL PP tube, spiked with isotope-labeled surrogate standards and mixed with 5 mL of 0.4M NaOH solution. The samples were then left in the fridge over night to allow the internal standards to distribute in the slurry. Next, the samples were mixed with 4 mL of 0.5M tetrabutylammonium hydrogen sulfate solution and 5 mL of a carbonate buffer (0.25M Na_2CO_3/$NaHCO_3$) and extracted with 10 mL Methyl tert-butyl ether (MTBE). After centrifugation for 10 minutes at 3000 rpm and room temperature the MTBE phase was transferred to a new 50 mL PP tube and the extraction with MTBE was repeated two times. The extracts were combined and concentrated to approximately 2 mL using a Rapidvap (Labconco Corp., Kansas City, MO, USA). After a clean-up step using Florisil SPE-cartridges to remove non-polar matrix, the final extract was evaporated to 1 mL using a Rapidvap. If the extract was still strongly colored then an additional clean-up step following the Powley method with ENVI-Carb was added (Powley et al. 2005).

For the analysis of PFAAs in soil, the soil was dried in an oven at 40 ˚C until no further weight loss was recorded. After homogenization, 1 g of soil was weighed in a 15 mL PP tube and spiked with internal standards. The soil was then extracted with 10 mL MeOH by vortex mixing for 1 minute and

sonication for 10 minutes. The supernatant after centrifugation (10 min, 3000 RPM) was transferred to a new 15 mL PP tube and concentrated in the Rapidvap. The extraction was repeated twice with 5 mL MeOH. The extracts were combined and concentrated in the Rapidvap to a final volume of 1 mL. For pore water analysis, 20 g of the soil was put in a 50 mL centrifugation filter tube with a 0.2 µm nylon filter. After 20 minutes of centrifugation at 2000 RPM, 0.5 mL of pore water was transferred to a vial. The internal standards and MeOH were added to achieve a final volume of 1 mL.

All final extracts were passed through an Acrodisc LC 13 GHP Pall nylon filter into 2 mL PP vials and stored at 4°C until analysis.

Analysis

For PFAA analysis an HPLC system (LC-20AD XR pump, SIL-20A autosampler and SCL-10A VP system controller, Shimadzu, Kyoto, Japan) coupled with a tandem mass spectrometer (4000 QTrap, Applied Biosystems, Toronto, Canada) was used. A pre-column (Pathfinder 300 PS-C18 column, ID 4.6 mm; length 50 mm; 3 µm particle diameter; Shimadzu, Duisburg, Germany) prior to the injection valve was used to remove potential background contamination.

The analytes were separated on an ACE 3 C18-300 column (ID 2.1 mm; length 150 mm; 3 µm particle diameter; Advanced Chromatography Technologies, Aberdeen, Scotland) maintained at 30 °C with a mobile phase gradient consisting of two eluents A (40:60 MeOH:H2O, v:v) and B (95:5 MeOH:H2O; v:v), both with 2 mM ammonium acetate. The gradient used for separation and the mass transitions as well as other mass spectrometer settings can be found in our previous papers (Felizeter et al. 2012; Felizeter et al. 2014; Felizeter et al. 2020). The mass spectrometer was equipped with an electrospray ionization interface operating in the negative ionization mode, and it was run in the scheduled MRM-mode.

The purified extracts were diluted 1:1 with UPLC grade water prior to analysis to match the injection conditions of the HPLC. As pore water samples already had a water:methanol ratio of 1:1, no further dilution was performed for these samples. A volume of 20 µl was injected.

Raw data were processed with the Analyst 1.5 software package (Applied Biosystems).

Quality assurance and control

Each sample was extracted in duplicate and each extract was injected in duplicate. The relative standard deviation of the concentrations derived from these four injections was <10% for all analytes in all samples.

Concentrations were quantified using a twelve-point calibration, with R^2 >0.99 for all analytes; no weighting was applied. Further information on quality assurance and quality control is provided in our previous studies (Felizeter et al. 2012; Felizeter et al. 2014).

Recoveries were determined by comparison with a matrix free solution spiked with internal standard immediately prior to injection. Average recoveries of the internal standards in the samples were between 22% (PFBA) and 112% (PFDoDA) (see Table S5). Despite the low recovery of PFBA, the use of an isotope labeled PFBA surrogate standard provided for satisfactory data quality as evidenced by good method repeatability.

Limits of quantification (LoQs) (Table S6) were calculated on the basis of the lowest validated calibration standard (signal:noise ratio ≥10). They were derived from the amount injected back calculated to an extract volume of 1 mL and divided by the average extracted sample quantity. A method blank (beginning with simulated extraction without matrix) was prepared with every batch of samples; these blanks showed no quantifiable contamination. Solvent blanks were injected every ten injections to check for contamination of the LC system and for memory effects, but no contamination or memory effects were observed during the study.

All PFAA concentrations from the non-spiked lysimeters (in plant parts as well as in soil or pore water) were subtracted from the concentrations in the spiked lysimeters. This corrected for any impact of atmospheric deposition or other sources besides soil on PFAA levels in the plants. Resulting concentrations below the LoQ were neglected.

Since PFOS is the only compound for which branched isomers were included in the standards used for the calibration curve, branched isomers could only be quantified for PFOS. All reported PFOS concentrations are sum concentrations of non-branched and branched isomers.

Results and discussion

Radish and lettuce plants grown in the highest exposure level soil were markedly smaller at the time of harvest than those growing in the lower exposure levels, suggesting that the PFAAs had phytotoxic effects (see Figure S2 and Felizeter et al. (Felizeter et al. 2020)). Results for radish and lettuce from the highest exposure level were therefore not used in the data interpretation. Pea and maize plants showed no visible signs of phytotoxicity.

PFAA fate in soil

After spiking, the concentrations in soil were generally within the intended concentration range (Table S7). At harvest, concentrations in soil of the shortest chain PFAAs, the C4-C6 PFCAs and PFBS, were almost reduced to background concentrations through the whole soil column (<3% of the initial mass remaining, Table S8). A large fraction of the short chain PFAAs was recovered in the drainage water, illustrating the transient nature of surface soil contamination with these substances (McLachlan et al. 2019). From this perspective, lysimeter studies can provide a more realistic simulation of plant uptake than laboratory hydroponic or pot experiments with constant exposure concentrations. In contrast to the short chain PFAAs, 80-90% of the spiked PFDoDA, PFTrDA and PFTeDA were found in the soil at the harvest date. The mid-chain length compounds (PFOA, PFNA and PFOS) generally had the highest concentrations in soil pore water (Table S9).

The behaviour of PFAAs in the lysimeter soil was analyzed in another paper (McLachlan et al. 2019). We showed that leaching was the dominant process for the loss of the short-chain PFAAs from the soil. Furthermore, leaching occurred at a faster rate than expected from calculated KD values, and this accelerated leaching was greater when the initial spiked soil concentration level was higher. We attributed the accelerated leaching to interactions between the PFAAs related to competition for sorption sites in the soil. Analysis of the leachate collected from the lysimeters suggested that leaching occurred primarily at the beginning of the experiment. Additionally, lower rainfall towards the end of the experiment resulted in more stable conditions. The evidence indicates that soil concentrations were more stable towards the end of the experiment.

Concentration factors

To evaluate the plant uptake of the PFAAs, concentration factors were calculated for the different plant tissues on the basis of the sampled exposure media, soil and pore water (ng g^{-1} ww plant tissue / ng g^{-1} dw soil, or ng g^{-1} ww plant tissue / ng mL^{-1} pore water, respectively). Soil and pore water concentrations were only available for the start and the end of the experiment. We used the soil and pore water concentrations at the end of the experiment (i.e., date of harvest) for the calculation of concentration factors because the bulk of transpiration and hence pore water uptake occurred during the latter part of the growth period. Soil concentrations were judged to have been more stable during this period, but it is nevertheless possible that the concentration factors were overestimated for the shorter chained PFAAs for which the concentration in soil decreased over the experiment (see above). As the soil sampled from the radish lysimeters was from the top 1-2 cm only (see above) and not representative for the root zone, the concentration in these samples was not used. The PFAA concentrations in surface soil at harvest were comparable between crops for most PFAAs, except for the concentrations of the shorter chained PFAAs which tended to decrease over time. Since lettuce was the first crop sampled after radish, the concentrations in soil from the lettuce lysimeters were used to evaluate the radish results.

Effect of exposure concentration

The PFAA concentrations in the different tissues for each of the different plants and exposure concentrations are reported in Tables S10-S13. The uptake of PFAAs by plants has generally been observed to be linearly correlated with exposure concentration (Stahl et al. 2009; Lechner et al. 2011; Felizeter et al. 2012; Blaine et al. 2013; Blaine et al. 2014b; Felizeter et al. 2014; Krippner et al. 2014). However, in hydroponic studies we observed lower root concentration factors for C7-C14 PFCAs and PFOS at 10 ng mL^{-1} compared to lower exposure concentrations (Felizeter et al. 2012; Felizeter et al. 2014). Although we had higher pore water concentrations in the present study (Table S9), there was no consistent pattern of lower root concentration factors at higher exposure levels for these PFAAs (Tables S14-S15). This indicates that the non-linear root – pore water isotherms observed under hydroponic conditions cannot be extrapolated to soil systems. We therefore averaged the concentration factors from all exposure levels for our evaluation. The geometric mean was used because concentration factors are logarithmically distributed. Concentration factors for each exposure level can be found in Tables S14-S19. It was not possible to retrieve any pore water from the pea lysimeters at the time of harvest, so concentrations in pore water from the lettuce lysimeters were used for calculating pore water-based concentration factors for pea.

Differences in the log concentration factor were statistically assessed using the T-test (two tailed, two sample unequal variance, significance threshold $p<0.05$) in Excel (Microsoft). However, due to the limited number of data points (max. 4 per species and compound) the explanatory power of the statistical analysis is limited.

Accumulation in edible plant parts

Of primary interest for human exposure is accumulation in edible plant parts. Edible plant part concentration factors referenced to soil (ECF_S) were calculated for radish bulbs, lettuce leaves, peas and maize kernels (Figure 1, Table S16). Very large variability was observed between the different PFAAs, with ECFS ranging by as much as seven orders of magnitude for a given edible part. In

addition, there were large differences between different edible parts for a given PFAA, in some cases in excess of three orders of magnitude. Within this great variability there was also evidence of systematic structure. In particular, ECFS tended to decrease with PFAA chain length.

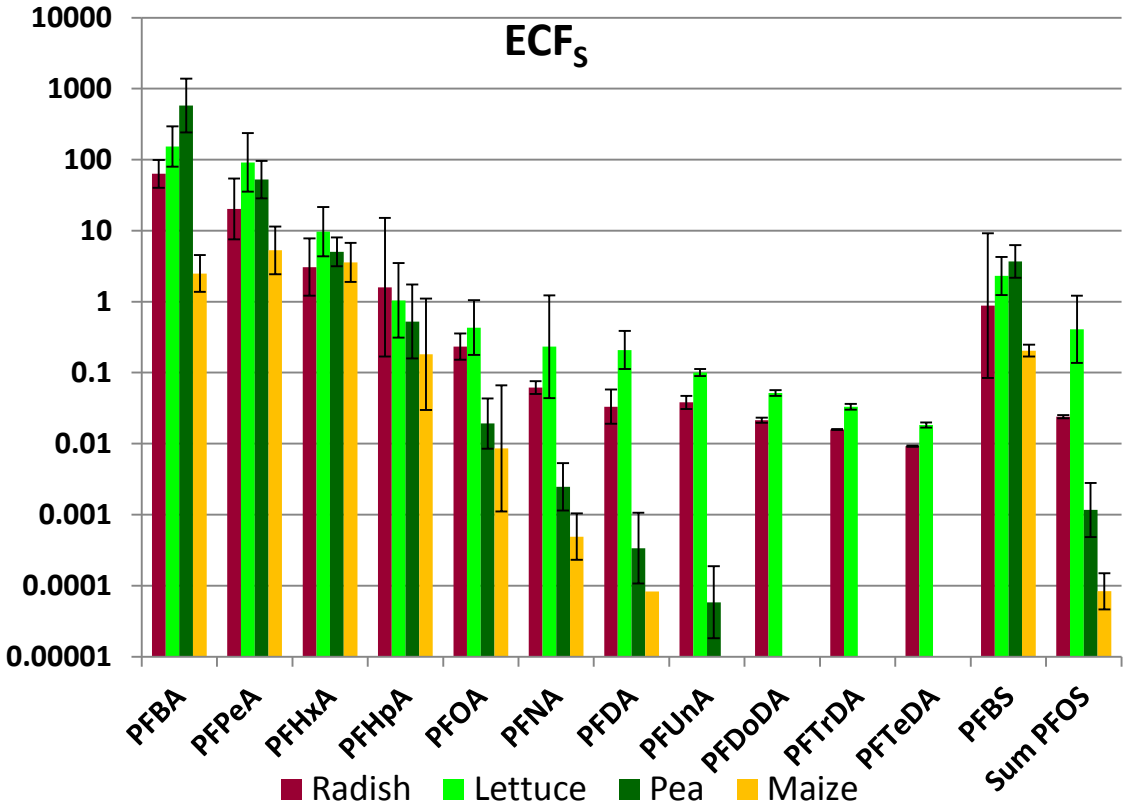

Figure 1: Edible part concentration factor based on concentration in soil (ECF_S, kg soil dry weight per kg edible part fresh weight). The average and standard deviation from experiments conducted at different exposure levels are shown, assuming a log-normal distribution. In cases with no error bars, ECF_S could be determined in just one exposure level.

In order to explore the factors determining PFAA transfer to edible plant parts, we use a mechanistically-based framework. In this framework, uptake occurs from soil into the root, and this is the rate limiting step for accumulation in the plant. From there the transpiration stream transports the PFAAs through the roots and the stem to the leaves, where they accumulate due to evaporation of the transpired water. Transport into plant fruits originates in the leaves, where PFAAs enter the phloem and flow to fruit tissues. In alignment with this framework, we first examine the PFAA uptake into the whole plant. We then consider what fraction of this uptake is retained in the roots and the stem. Finally, we study the relationship between concentrations in fruits and leaves before assembling a mathematical model to describe the observations.

Whole plant concentration factors

Whole plant concentrations C_P were calculated by summing the PFAA quantities in the different plant parts and dividing by the total plant biomass. C_P was used to determine whole plant concentration factor referenced to soil (PCF_S, Figure 2, Table S17). The between species variability in PCF_S was low; significant differences between species exceeding a factor two were observed only nine times: PFBA

(between lettuce and pea), PFHxA (pea/corn), PFNA (radish/corn), PFDoDA (radish/all others) and PFBS (pea/all others) (Figure 2). This is a quite remarkable finding. We had expected PCF_S to depend on plant variables such as the cumulative amount of water transpired per unit plant biomass as well as species-specific differences in the barrier limiting PFAA transport across the root endodermis into the transpiration stream. Figure 2 suggests that these factors did not differ greatly between the plants studied here.

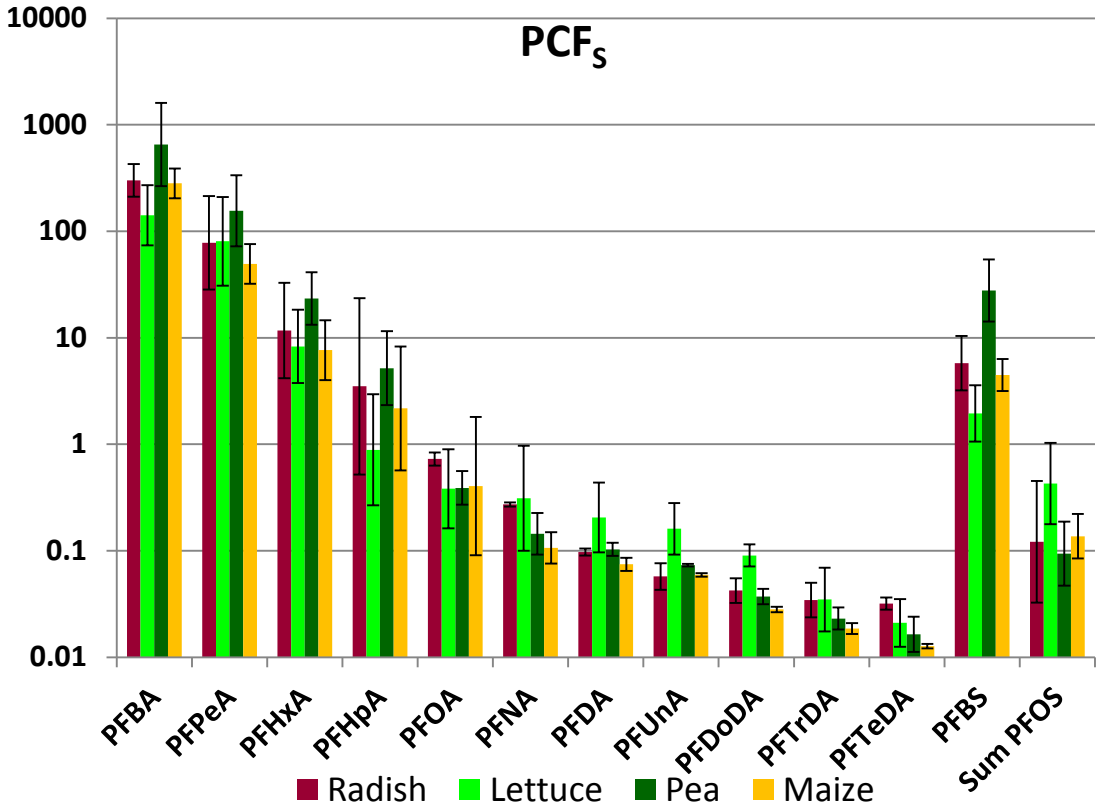

Figure 2: Whole plant concentration factor based on concentration in soil (PCF_S, kg soil dry weight per kg plant fresh weight). The average and standard deviation from experiments conducted at different exposure levels are shown, assuming a log-normal distribution.

The inter-chemical variability in PCF_S was large, ranging up to 4.5 orders of magnitude, but nevertheless considerably less than the inter-chemical variability in ECF_S (Figure 2). There was a very consistent trend of decreasing PCF_S with increasing chain length for the PFCAs. PCF_S for the PFSAs corresponded to PCF_S for the PFCA with the same or a slightly longer perfluorinated chain; PCF_S for PFBS was similar to PFHxA, while PCF_S for PFOS was similar to PFNA/PFDA. Increasing chain length corresponds to increasing tendency of the PFAAs to sorb to the soil solids (McLachlan et al. 2019). This suggests that the strong inverse relationship between PCF_S and chain length may have been a result of PFAA sequestration to soil solids reducing availability for uptake.

The whole plant concentration factor referenced to pore water (PCF_{PW}) eliminates the influence of sorption to soil solids on the bioaccumulation metric. PCF_{PW} shows a much lower inter-chemical variability than PCF_S (Figure 3, Table S18). PCF_{PW} ranged over 1.5-2 orders of magnitude for a given species compared to 4 orders of magnitude for PCF_S. The variability between species was once again

small, with significant differences observed for only five pairs: PFNA (lettuce/corn and pea/corn), PFBS (lettuce/pea and pea/corn) and PFOS (pea/corn). There was a pronounced chain length trend in mean PCF_{PW}, with similar PCF_{PW} for short and long chain lengths and decreasing PCF_{PW} towards intermediate chain lengths with a minimum in the vicinity of PFOA. The trends were overlain by pronounced PCF_{PW} variability between the exposure levels, so that there were few significant differences between PFAAs for radish and lettuce. However, for pea and maize the concentrations of PFOA (maize only) and PFOA (pea and maize) were significantly lower than most of the other PFAAs (Table S19). The trends suggest that the uptake of the intermediate chain length PFAAs across the root endodermis is restricted compared to the other PFAAs. However, PCF_{PW} for the shorter chain PFAA may be overestimated due to the uncertainty in the concentrations in pore water (see above), and PCF_{PW} for the longer chain PFAAs may contain a contribution from sorption to the root surface or contamination of foliage with soil particles (see below). Similar to PCF_S, PCF_{PW} for the PFSAs corresponded to PCF_{PW} for the PFCA with a one CF_2 unit longer perfluoroalkyl chain; PFBS was similar to PFHxA, while PFOS was similar to PFDA. The strong reduction in variability of PCF_{PW} (Figure 3) compared to PCF_S (Figure 2) demonstrates the dominant role that sorption to soil plays in regulating plant uptake of PFAAs.

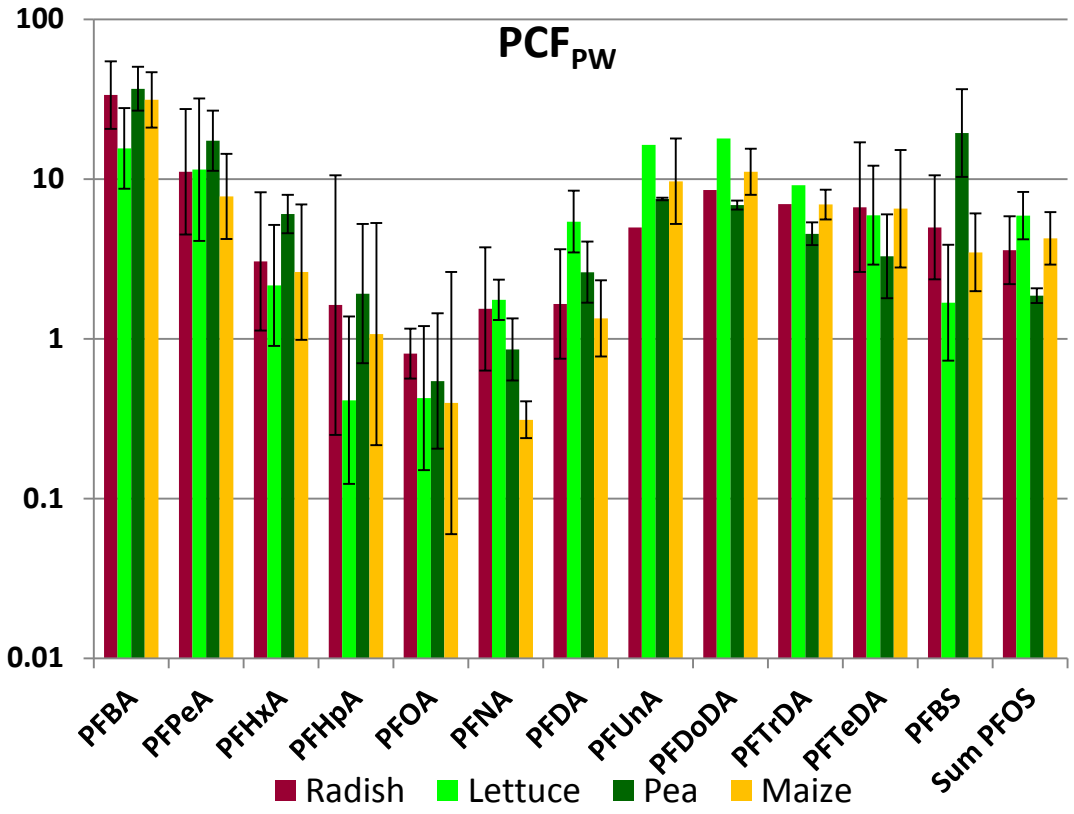

Figure 3: Whole plant concentration factor based on concentration in pore water (PCF_{PW}, L pore water per kg plant fresh weight). The average and standard deviation from experiments conducted at different exposure levels are shown, assuming a log-normal distribution.

One significant departure from the species similarity in PCF_{PW} is seen for PFHpA, which was 4-5 times lower in lettuce than in the other four species. PCF_{PW} was also lower in lettuce for PFHxA, PFOA and PFBS. This is consistent with measured transpiration stream concentration factors, which showed a

pronounced V-shaped dependence with chain-length with a minimum at PFHpA that was more pronounced for lettuce than for other plant species (Felizeter et al. 2012; Felizeter et al. 2014). A second significant departure is seen for PFNA in maize. In maize, the minimum for PCF_{PW} was shifted to the right from PFOA to PFNA.

Retention in roots

Once having entered the roots from the soil, some portion of the chemical will be retained in the roots and some portion may be transported to the aerial plant parts with the transpiration stream. We assessed this using a root retention factor (RRF), defined as the fraction of the chemical mass in the whole plant at harvest that was present in the roots. RRF ranged from 99.5% (PFDoDA in maize) to 1% ((PFBA in maize) (Figure 4 (normal scale) and Figure S3 (log scale), Table S20). For a given PFAA the RRF was not significantly different for 50 out of 78 sample pairs (Table S21), indicating that plant specific properties did not have a dominant influence on root retention. No species had an RRF that was greater than another species for all PFAAs. For a given plant species, RRF generally increased with chain length up to n_{CF} = 10, after which it remained approximately constant. This observation is consistent with the increase in sorption tendency of PFAAs with increasing n_{CF} (McLachlan et al. 2019) the longer chained PFAA sorb more strongly to root tissue after they are taken up, resulting in a smaller fraction being available for further transport with the transpiration stream. Once again, the RRFs for the PFSAs corresponded to the RRF for the PFCA with the same or a slightly longer perfluorinated chain.

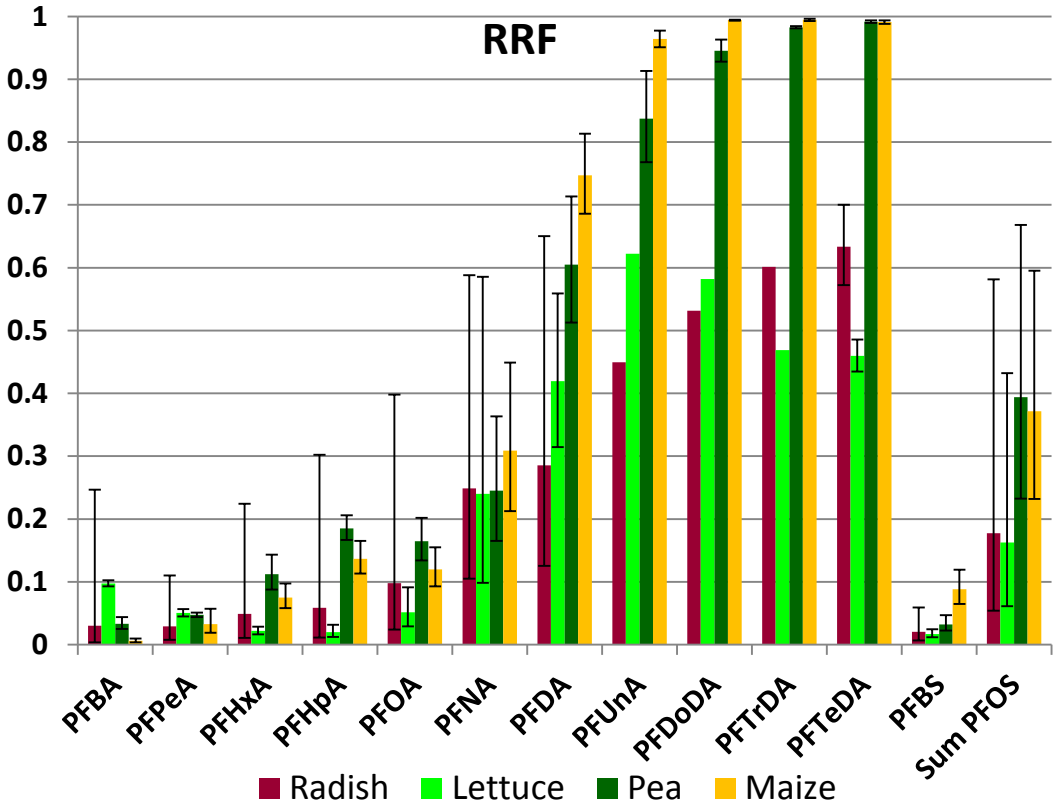

Figure 4: Root retention factor (RRF), equal to the PFAA mass in the roots as a fraction of the PFAA mass in the whole plant. The average and standard deviation from experiments conducted at different exposure levels are shown, assuming a log-normal distribution.

There are several notable exceptions to these trends. One is that RRF of the longer chained PFAAs ($n_{CF} \geq 10$) is consistently lower for radish and lettuce than for pea and maize, in most cases significantly (Figure 4, Table S21). The latter two plants have RRFs of 99% for the longest chained PFAAs, while for radish and lettuce it is only 50-60%. Radish and lettuce also have a large fraction of the longest chained PFAAs in the leaves. This may be due to contamination of the leaves with soil particles by processes such as rain splash. A leaf/soil concentration factor of 0.1 – 0.25 kg dry soil kg^{-1} leaf dry weight (roughly equivalent to 0.01 – 0.025 kg dry soil kg^{-1} leaf wet weight) in leaves growing close to the soil has been observed for other organic contaminants that are not taken up appreciably via the roots (Prinz et al. 1991). Hence the lower RRFs for these plants may not reflect lower root retention, but instead be an artifact arising from contributions of another uptake pathway to plant contamination.

A second exception is the significantly lower RRF for PFHxA, PFHpA, PFOA and PFBS in lettuce compared to pea and maize. As noted above, PCF_{PW} of these substances was also lower in lettuce compared to the other species, presumably due to more restricted uptake across the root endodermis. However, we could identify no mechanistic link that would explain a positive relationship between uptake efficiency across the root epidermis and retention in root tissue. At this time we can offer no explanation for the weaker retention of these particular PFAAs in lettuce roots.

PFBA builds a third exception to the general trends in RRF. The differences in RRF between species exceed an order of magnitude. This may be due to the temporal variability in PFBA exposure. Due to its low K_D, PFBA was rapidly leached out of the soil. In addition, concentrations in pore water would be expected to increase as soil dries out since PFBA in pore water is not buffered by a sorbed fraction. Levels in roots will tend to reflect more recent exposure while levels in foliage represent cumulative exposure, so consequently a dynamic exposure situation can result in changing RRF values over time.

Retention in stems

For pea and corn it was also possible to evaluate retention in stems. A stem retention factor (SRF) was calculated as the fraction of the PFAA mass in the above-ground plant parts that was present in the stem. Like the RRF, SRF increased with PFAA chain length, reflecting the increasing tendency of the PFAAs to sorb to plant tissue with increasing chain length (Figure S4). For the shorter chain PFAAs ($n_{CF} \leq 7$) there was little variation in SRF with chain length and there was a marked difference between the two species (~0.15 in pea and 0.05 in maize). However, the stem played a minor role in the storage of these PFAAs in pea and maize.

Distribution between leaves and edible plant parts

The bulk of the PFAAs in aerial plant parts was stored in the leaves (see Figure S5). This was expected, as PFAAs would be expected to accumulate at the location where water is lost from the plant. We anticipate that the leaves are the primary source of PFAAs in fruits because the molecular building blocks for the fruit are synthesized largely in leaves and then transported via the phloem to the fruit (Hopkins et al. 2008). Edible plant part/leaf concentration factors (ELCFs) were calculated as the quotient of PFAA concentration in the edible plant part (radish bulbs, peas and maize kernels) and in the respective leaf or foliage sample (Figure 5, Table S22). ELCFs for pea and maize were similar, with only PFBA and PFBS being significantly different (lower for maize). For these crops the ELCF generally decreased with increasing n_{CF}. Interestingly, the plant parts enclosing peas and maize

displayed a different accumulation pattern. The maize husk/leaf and maize cob/leaf concentration factors were relatively independent of n_{CF}, with log values of -1.47 ± 0.16 and -1.96 ± 0.32, respectively, and pea pod/leaf concentration factors were independent of n_{CF} for $n_{CF} \geq 6$ (Figures S6 and S7). Concentration factors calculated from PFAA measurements in corn leaves, husks, cobs and kernels made by Liu et al. were similar to our results with the exception of PFOS, which shows a unique behaviour in their study (Figure S8). Liu et al. also studied soybean, and the pod/leaf and soybean/leaf concentration factors in their study were similar to our observations for pea pods and peas (Figure S9) (Liu et al. 2019b). Blaine et al. measured PFAAs in pea shoots (stem + leaves) and fruit, and the derived pea pod/leaf concentration factors agreed quite well with ours (Figure S9)(Blaine et al. 2014a).

The lack of chain length dependence of the concentration factors for husks, cobs and pods suggest that the primary source of PFAAs to these tissues may be the same as the primary source to the leaves, namely the transpiration stream. The low values of the concentration factors (generally 0.01-0.04) could suggest much lower cumulative transpiration flow to these tissues than to the leaves. The pronounced chain length dependence for the peas, beans and kernels suggest that they have a different primary PFAA source. The chain length differentiation may arise during loading of the phloem. If there is no barrier preventing equilibration of PFAAs between the phloem contents and the freely dissolved fraction in the plant part where the phloem is loaded, then the phloem contents would contain a PFAA pattern that represents the freely dissolved fraction in the plant part, modified by any sorption occurring to solids in the phloem contents. If the sorption capacity of the phloem contents is lower than for the leaf as a whole, then the longer chained PFAAs will be more weakly represented in the phloem.

A pronounced chain length dependence of fruit/leaf concentration factors has been observed for other plants. For instance, we measured a log-linear relationship between the tomato fruit/leaf concentration factor and chain length in a hydroponic study, the concentration factor decreasing by three orders of magnitude from PFPeA to PFUnA, and similar trends were observed for tomato in two soil-based studies (see Figure S10)(Blaine et al. 2014a; Felizeter et al. 2014). However, not all crops show this pronounced chain length selection in PFAA transfer to edible plant parts. Little chain length dependence was observed in wheat grain/leaf (straw) concentration factors (Wen et al. 2014), suggesting that other phenomena are governing transfer of PFAAs to grains.

In contrast to the other edible plant parts, the ELCF for radish showed no pronounced chain length dependence, varying around 0.1 for n_{CF} of 3-8 (Figure 5). Similar values of ELCF were calculated from the measurements of Blaine et al. and Liu et al. (Blaine et al. 2014a; Liu et al. 2019b) with no chain length dependence for n_{CF} of 3-8 (Figure S11). Radish bulbs are in between the roots and the foliage of the radish plant, so it is likely that PFAAs also reached the bulbs via the transpiration stream (xylem) from the roots. The fraction of the total plant residue that was present in the bulb was similar for the different PFAAs, ranging between 0.16 and 0.40 with the exception of PFBA (0.11) and PFOS (0.06).

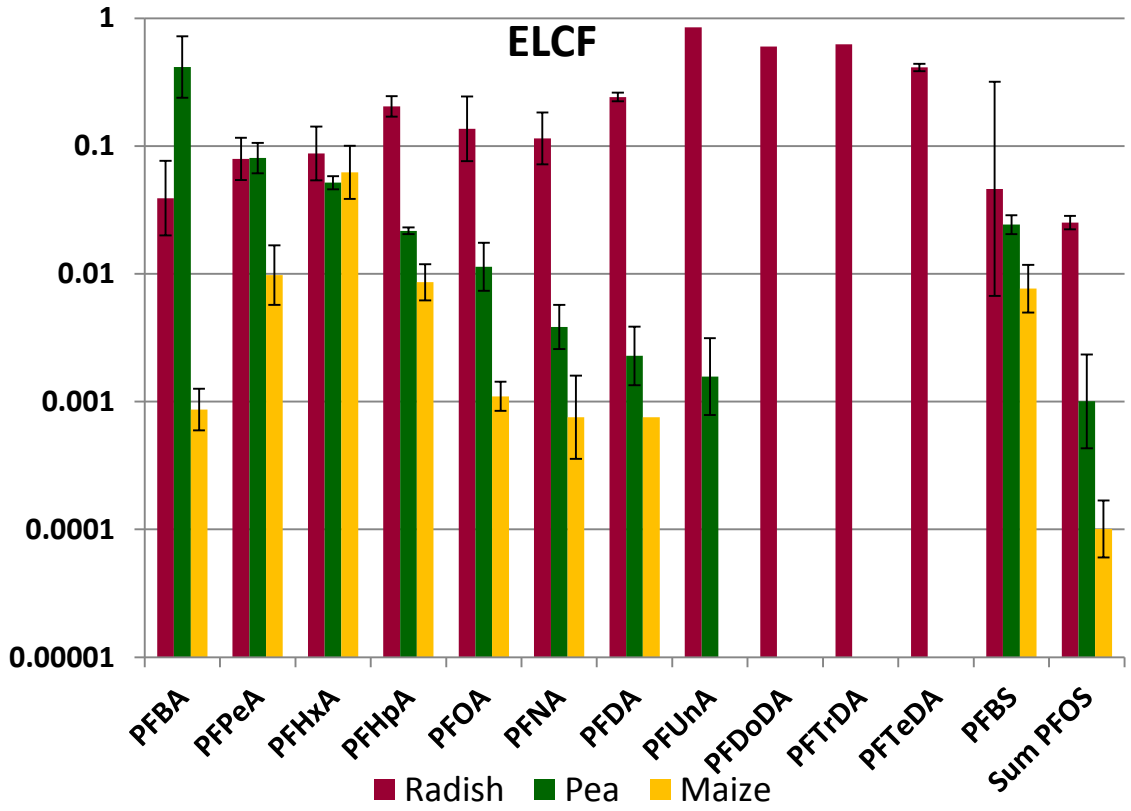

Figure 5: Edible part to leaf concentration factor (ELCF, kg leaf fresh weight per kg edible part fresh weight). The average and standard deviation from experiments conducted at different exposure levels are shown, assuming a log-normal distribution.

A quantitative model of PFAA uptake into crops

The experimental results assembled here, with concentrations of 13 PFAAs in different tissues of four crop species exposed at four different levels, provided a large, internally consistent data set with which to explore the influence of different parameters on PFAA uptake in plants. We used this data to develop a simple quantitative empirical model of PFAA accumulation in roots, leaves, fruits and radish bulbs, taking into consideration the limitations in the data arising from the experimental design and information available in the literature.

Uptake in the whole plant

PFAA uptake into the plant is treated as the sum or two processes, uptake from soil via the roots and uptake from soil directly to foliage. The former is estimated using a function of PCF_{PW} versus n_{CF} based on Figure 3, with constant PCF_{PW} for shorter and longer chain lengths and a V-shaped minimum centered at n_{CF} = 7 (Table 2). Here and in the remainder of the model, an extra unit is added to n_{CF} of the PFSAs to account for their stronger sorption compared to the PFCA of equivalent n_{CF}. Uptake from soil directly to foliage is estimated using a soil-to-leaf concentration factor (SLCF) of 0.02 kg dry soil kg^{-1} leaf wet weight and applied only to plants growing close to the soil surface (here radish and lettuce).

$$N_P = N_{PR} + N_{PL} \quad (1)$$

$$N_{PR} = m_P PCF_{PW} C_{PW} \quad (2)$$

$$N_{PL} = m_L SLCF\ C_S \quad (3)$$

where N_P is the quantity of PFAA in the plant (mol), N_{PR} is the quantity of PFAA in the plant due to uptake via roots (mol), N_{PL} is the quantity of PFAA in the plant due to uptake via leaves (mol), m_P is the mass of the plant (kg wet weight), m_L is the mass of the leaves (kg wet weight), C_{PW} is the PFAA concentration in pore water (mol L^{-1}) and C_S in the PFAA concentration in soil (mol kg^{-1} dry soil).

Table 2: Whole plant/pore water concentration factors PCF_{PW} (L kg^{-1} wet weight) as a function of perfluorinated chain length n_{CF} used in the model. When modeling PFSAs, one unit was added to n_{CF}.

n_{CF}	PCF_{PW}
3	6
4	6
5	4.1
6	2.7
7	0.9
8	1.6
9	2
10	6
11	6
12	6
13	6

Concentration in roots

The PFAA concentration in roots C_R (mol kg^{-1} wet weight) is calculated according to:

$$C_R = \frac{N_{PR} RRF}{m_R} \quad (4)$$

where m_R is the mass of the roots (kg wet weight) and RRF is defined by:

$$\log RRF = 0.21 n_{CF} - 2.24 \qquad (\max RRF = 1) \quad (5)$$

where this equation was determined from the data in Figure S3 neglecting PFBA in all species and PFHxA, PFHpA and PFOA in lettuce.

Concentration in leaves

The PFAA concentration in leaves C_L (mol kg^{-1} wet weight) is calculated according to:

$$C_L = \frac{(1-RRF) N_{PR} + N_{PL}}{m_L} \quad (6)$$

where m_L is the mass of the leaf (kg wet weight). This approach assumes that all of the residues not retained in the roots are retained in the leaves. This is clearly a simplification, but most of the residues were retained in the leaves for almost all substances (Figure S4). Hence, we judge this to be

a reasonable approach to estimate C_L. Furthermore, the model cannot predict C_L for $n_{CF} > 10$ since RRF =1. However, the concentrations of these long-chained PFAAs in leaves are very low and unlikely to be of relevance for exposure assessment.

Concentration in fruit

The PFAA concentration in fruit C_F (mol kg^{-1} wet weight) is calculated according to:

$$C_F = FLCFC_L \quad (7)$$

where FLCF, the fruit/leaf concentration factor (kg wet weight kg^{-1} wet weight), is defined using a relationship derived from Figure 5:

$$\log FLCF = -0.38n_{CF} + 0.33 \quad (8)$$

Concentrations in bulbs

The PFAA concentration in bulbs C_B (mol kg^{-1} wet weight) was derived from the radish bulb data and is calculated according to:

$$C_B = \frac{0.24N_{PR}}{m_B} \quad (9)$$

where m_B is the mass of the bulb (kg wet weight). This model assumes that 24% of the PFAA taken up from the soil is stored in the bulb.

Model evaluation

The ability of this simple model to describe the experimental observations was tested by calculating C_R, C_L, C_F and C_B from measured C_{PW} and C_S using the above equations and comparing the modeled values with the measured values for each PFAA at each exposure level in each species. Good agreement was obtained, with most of the modeled concentrations lying within a factor 3 (C_L and C_B) or factor 4 (C_R and C_F) of the measured concentrations (Figure 6). The simple model explains a very large portion of the variability in plant/soil concentration factors, which covered up to 7 orders of magnitude (Figure 1). This indicates that the basic structure of the model, uptake into the roots followed by transport via the transpiration stream and accumulation in the leaves, captures the central features of PFAA behaviour. Furthermore, the processes described in the model give an indication of the key factors controlling PFAA transfer from soil to plant parts. These are: i) decreasing bioavailability in soil with increasing chain length (captured by using the concentration in pore water instead of soil as the driver for uptake); ii) increasing retention of PFAAs in the roots (and hence decreasing transfer to the leaves) with increasing chain length (Eq. 5); iii) decreasing transfer from leaves to fruits with increasing chain length (Eq. 8). Each of these three processes is governed by the sorption properties of the PFAAs. Since they act sequentially on the transfer of PFAAs from soil to fruit, there is a multiplicative effect that leads to the very strong influence of chain length on ECF_S seen in Figure 1.

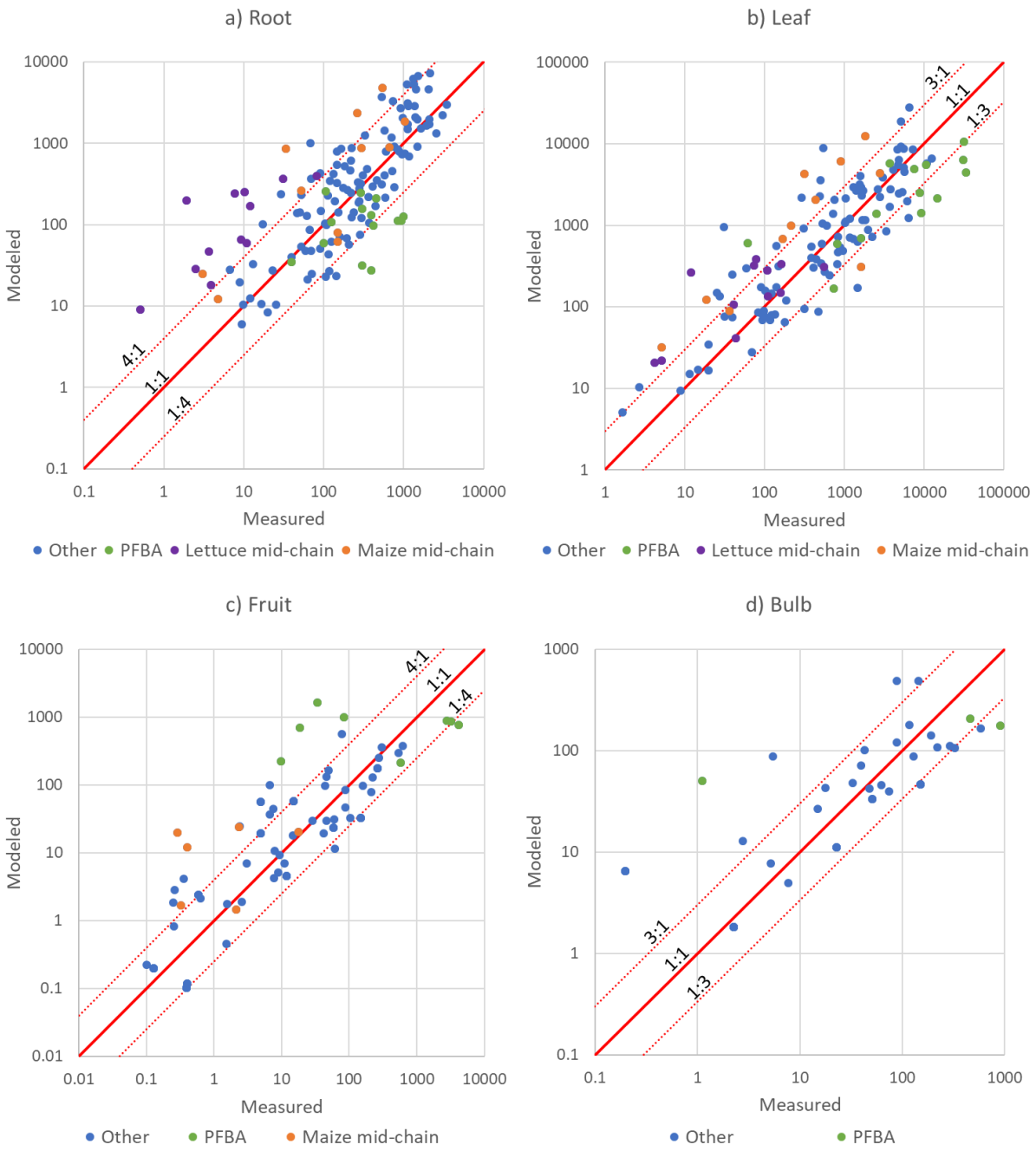

Figure 6: X-Y plots of modeled versus measured PFAA concentration in: a) roots; b) leaves; c) fruit; d) radish bulb.

An independent evaluation of fruit-leaf model (Eq. 8) was performed by forecasting the concentrations in tomatoes and soybeans from concentration in tomato and soybean leaves using data from studies in the literature (Felizeter et al. 2014; Liu et al. 2019b). The agreement was better than a factor 4 for most of the 33 data points (Figure S11), but still poorer than the agreements for fruits from this study which were modeled from the PFAA concentration in pore water (Figure 6c). We recall that there was no chain length dependence in wheat grain/leaf transfer factors (Wen et al. 2014) and hence the model cannot be expected to perform well for all fruits. Measurement of the leaf to fruit transfer of PFAAs for a broader spectrum of plant species is required.

The nature of the data points showing poor model-measurement agreement provides insight into which features of PFAA accumulation behaviour are not captured well by the model. A considerable number of the poorly fitting data were for PFHxA, PFHpA, PFOA or PFBS in lettuce or for PFOA, PFNA or PFDA in maize (Figure 6). For these cases, the poor agreement can be attributed to the shift in the minimum for PCF_{PW} from PFOA to PFHpA in lettuce and from PFOA to PFNA in maize (Figure 3). For lettuce the deviation is amplified by lower RRF for the same substances (Fig. 4). In our model we have assumed that the dip in PCF_{PW} for mid-chain length PFAAs is the same for all species. This is clearly an oversimplification. Better understanding of this dip in PCF_{PW} and its variability between species is one key for improving our ability to describe PFAA accumulation in plants.

PFBA also generally showed poorer agreement than the other PFAAs (Figure 6). This may be due to the particularly high uncertainty in exposure for this substance. Most of the other data points outside of the dotted lines were not grouped according to chemical or species, but rather reflected the experimental variability. We believe that the major source of experimental variability was the uncertainty in the concentrations in pore water during the growth period. In future work, more effort should be devoted to measuring the exposure over time.

Perspectives

In general, we found the highest concentrations in the leaves and the roots of the plants and the lowest in the fruit, regardless of the species. Hence leafy and root vegetables pose the highest risk for dietary exposure followed by fruit-bearing crops. This was also concluded by other studies, e.g., Blaine et al. (Blaine et al. 2014a). The concentrations in the leaves depend on the concentrations in the pore water, which reflect the bioavailable fraction in the soil.

When confronted with an agricultural soil with elevated PFAA concentrations, the type of crop posing the greatest risk for exposure is dependent on which PFAAs are present in the soil. If long chained PFAAs are present, root vegetables like potatoes, carrots or radishes or crops for which the edible part can be in direct contact with the soil pose the highest risk for exposure, while the fruits of fruit-bearing crops will remain largely unaffected. If only short chain PFAAs are present in the soil then the differences between crops are smaller (see Figure 1) and it may be difficult to identify a crop that will have lower concentrations in the edible part. In this case crops that are used for animal feed may be preferential, as a significant portion of the PFAA will be removed from the human food chain due to incomplete absorption or metabolism in the livestock (Vestergren et al. 2013).

The chain length dependence of soil to crop transfer is particularly relevant in the context of recent and ongoing changes in PFAA production. Manufacturers switched their production from C8-compounds towards shorter chain compounds (Ahearn 2019) due to phase-out actions driven by adverse health effects and bioaccumulation in humans and wildlife (Houde et al. 2011; Grandjean et al. 2014). From a bioaccumulation point of view this change is questionable as ECF_S values are much higher for C4 than for C8 PFAAs (up to 30,000 times higher for pea, see Figure 1). The shorter residence time of short chain PFAAs in soil means that crops will not be impacted for as long a period of time. However, the higher mobility of the short chain PFAAs in soil will lead to groundwater becoming contaminated faster and with higher concentrations. Farmers also often use groundwater from their own fields for irrigation. In such cases a contamination cycle will result, prolonging the contamination of the respective fields.

To address concerns arising from the presence of PFAAs in agricultural soil, it is first necessary to determine the PFAA concentrations in porewater. The concentrations in crops can then be predicted using models. The simple models presented here are a first step in this direction, but more sophisticated models should be developed. More extensive empirical observations from controlled field studies are required to this end, whereby particular care needs to be paid to accounting for variable exposure concentrations of more mobile PFAS. Finally, while this study has shown the strong influence of chain length on PFAA behaviour, many replacement PFAS are not PFCAs or PFSAs but instead contain other structural modifications that may also influence their uptake from soil into edible plant parts.

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Supporting Information

Table S1: List of chemicals used, their purity and suppliers.

Chemical	Purity	Supplier
MPFAC-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
MPFAS-Mix (internal standard)		Wellington Laboratories, Ontario, Canada
M5PFPeA (internal standard)		Wellington Laboratories, Ontario, Canada
M4PFHpA (internal standard)		Wellington Laboratories, Ontario, Canada
PFAC-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFAS/FOSA-Mix (calibration standard)		Wellington Laboratories, Ontario, Canada
PFBA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFPeA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHxA	≥97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFHpA	99%	Sigma Aldrich, Zwijndrecht, Netherlands
PFOA	96%	Sigma Aldrich, Zwijndrecht, Netherlands
PFNA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDA	98%	Sigma Aldrich, Zwijndrecht, Netherlands
PFUnA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFDoDA	95%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTrDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
PFTeDA	97%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFBS	≥98%	Sigma Aldrich, Zwijndrecht, Netherlands
K-PFOS	≥98%[§]	Sigma Aldrich, Zwijndrecht, Netherlands
Ammonium acetate	≥99,999%	Sigma Aldrich, Zwijndrecht, Netherlands
Methanol	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands
Water	ULC/MS-grade	Biosolve, Valkenswaard, Netherlands

[§] Mixture of linear and branched isomers.

Table S2: List of the analytes, their abbreviations and molecular formulas, the 13C-labelled internal standards used for their quantification, and the mass transitions used in the MS/MS analysis of the analytes.

Abbreviation	Compound	Transition 1	Transition 2	Quantification by internal Standard	Molecular Formula
PFBA	Perfluoro-n-butanoic acid	213 → 169	-	$^{13}C_4$ PFBA	$CF_3(CF_2)_2COOH$
PFPeA	Perfluoro-n-pentanoic acid	263 → 219	-	$^{13}C_5$ PFPeA	$CF_3(CF_2)_3COOH$
PFHxA	Perfluoro-n-hexanoic acid	313 → 269	313 → 119	$^{13}C_2$ PFHxA	$CF_3(CF_2)_4COOH$
PFHpA	Perfluoro-n-heptanoic acid	363 → 319	363 → 169	$^{13}C_4$ PFHpA	$CF_3(CF_2)_5COOH$
PFOA	Perfluoro-n-octanoic acid	413 → 369	413 → 169	$^{13}C_8$ PFOA	$CF_3(CF_2)_6COOH$
PFNA	Perfluoro-n-nonanoic acid	463 → 419	463 → 219	$^{13}C_9$ PFNA	$CF_3(CF_2)_7COOH$
PFDA	Perfluoro-n-decanoic acid	513 → 469	513 → 269	$^{13}C_6$ PFDA	$CF_3(CF_2)_8COOH$
PFUnA	Perfluoro-n-undecanoic acid	563 → 519	563 → 269	$^{13}C_7$ PFUnA	$CF_3(CF_2)_9COOH$
PFDoDA	Perfluoro-n-dodecanoic acid	613 → 569	613 → 319	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{10}COOH$
PFTrDA	Perfluoro-n-tridecanoic acid	663 → 619	663 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{11}COOH$
PFTeDA	Perfluoro-n-tetradecanoic acid	713 → 669	713 → 369	$^{13}C_2$ PFDoDA	$CF_3(CF_2)_{12}COOH$
PFBS	Perfluorobutane sulfonate	299 → 80	299 → 99	$^{18}O_2$ PFHxS	$CF_3(CF_2)_3SO_3$
PFOS	Perfluorooctane sulfonate	499 → 80	499 → 99	$^{13}C_8$ PFOS	$CF_3(CF_2)_7SO_3$
$^{13}C_4$ PFBA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	217 → 172	-		
$^{13}C_5$ PFPeA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	268 → 223	-		
$^{13}C_2$ PFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	315 → 270	315 → 119		
$^{13}C_4$ PFHpA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	367 → 323	367 → 169		
$^{13}C_8$ PFOA	Perfluoro-n-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanoic acid	421 → 376	421 → 172		
$^{13}C_9$ PFNA	Perfluoro-n-[1,2,3,4,5,6,7,8,9-$^{13}C_9$]nonanoic acid	472 → 427	472 → 223		
$^{13}C_6$ PFDA	Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	519 → 474	519 → 219		
$^{13}C_7$ PFUnA	Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	570 → 525	570 → 270		
$^{13}C_2$ PFDoDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	615 → 570	615 → 369		
$^{18}O_2$ PFHxS	Perfluoro-1-hexane[$^{18}O_2$]sulfonate	403 → 84	403 → 103		
$^{13}C_8$ PFOS	Perfluoro-1-[1,2,3,4,5,6,7,8-$^{13}C_8$]octanesulfonate	507 → 80	507 → 99		

Figure S1: Lysimeter set-up at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Schmallenberg, Germany

Table S3: Water inputs to the lysimeters (L per lysimeter)

Date	Precipitation	Watering radish	Watering lettuce	Watering pea	Watering maize
2011-06-21	2.8	5	5	5	5
2011-06-22	18.4				
2011-06-23	1.3				
2011-06-24	2.7				
2011-06-25	2.4				
2011-06-26	1.3				
2011-06-27	0				
2011-06-28	0				
2011-06-29	6.7				
2011-06-30	0.3				
2011-07-01	0.4				
2011-07-02	0				
2011-07-03	0				
2011-07-04	0				
2011-07-05	0		3		6
2011-07-06	0				3
2011-07-07	0	3	3	3	6
2011-07-08	4.8				
2011-07-09	0				
2011-07-10	0.1	3	3	3	6
2011-07-11	0				
2011-07-12	0	3	3	3	6
2011-07-13	1.9				
2011-07-14	0.7				
2011-07-15	2.8				
2011-07-16	0.8				
2011-07-17	13				
2011-07-18	2.9				
2011-07-19	0				
2011-07-20	2.6				
2011-07-21	18.9				
2011-07-22	0.1				
2011-07-23	0.5				
2011-07-24	11.5				

2011-07-25	3.1				
2011-07-26	12.5				
2011-07-27	13.7				
2011-07-28	21.6				
2011-07-29	0.1				
2011-07-30	0.5				
2011-07-31	0				
2011-08-01	0				
2011-08-02	0				
2011-08-03	15.3				
2011-08-04	4.4				
2011-08-05	0.8				
2011-08-06	7.4				
2011-08-07	0.6				
2011-08-08	15.1				
2011-08-09	8.2				
2011-08-10	0.2				
2011-08-11	0				
2011-08-12	11.5				
2011-08-13	6				
2011-08-14	12				
2011-08-15	0.1				
2011-08-16	0.3				
2011-08-17	0.1				
2011-08-18	28.9				
2011-08-19	2.4				
2011-08-20	0				
2011-08-21	2.1				
2011-08-22	1.3				
2011-08-23	0.5				
2011-08-24	0.6				
2011-08-25	0.1				
2011-08-26	11.8				
2011-08-27	9.6				
2011-08-28	1.6				
2011-08-29	0				
2011-08-30	0.1				
2011-08-31	0				
2011-09-01	0				
2011-09-02	0				
2011-09-03	0				
2011-09-04	16.7				
2011-09-05	0.5				
2011-09-06	1.2				
2011-09-07	6.6				
2011-09-08	17.4				
2011-09-09	0.3				
2011-09-10	0				
2011-09-11	10.4				
2011-09-12	0.1				
2011-09-13	0				
2011-09-14	0				
2011-09-15	0				
2011-09-16	0				
2011-09-17	0.2				
2011-09-18	1.6				
2011-09-19	0.2				
2011-09-20	0				
2011-09-21	0				
2011-09-22	0.1				

2011-09-23	0				
2011-09-24	0				
2011-09-25	0				
2011-09-26	0				
2011-09-27	0				
2011-09-28	0				
2011-09-29	0				
2011-09-30	0				
2011-10-01	0				
2011-10-02	0				
2011-10-03	0				
2011-10-04	0				
2011-10-05	0				
2011-10-06	5.2				
2011-10-07	8.5				
2011-10-08	2.5				
2011-10-09	2.3				
2011-10-10	3				
2011-10-11	12.3				
2011-10-12	19.2				
2011-10-13	0.1				
2011-10-14	0				
2011-10-15	0				
2011-10-16	0				
2011-10-17	0				
2011-10-18	2.5				
2011-10-19	4				

Table S4: Dates of the planting and harvesting.

Date	Action	Samples taken
21.06.2011	Start of experiment; Seeding radish and pea; Planting seedlings of maize and lettuce	Soil samples of the upper and lower layer of all spiked lysimeters, as well as from unspiked soil
09.08.2011	Harvest of radish	6 radishes per lysimeter were taken and divided into roots, bulbs and foliage. Surface soil samples of radish lysimeters were taken.
01.09.2011	Harvest of lettuce	4 lettuce plants were taken from each lysimeter and divided into roots and foliage. Soil samples from 2 different depths (0-15, 15-30, 30-45 and 45-60cm) were taken from each lettuce lysimeter. Porewater was extracted from each sub-sample.
04.10.2011	Harvest of pea	All pea plants from each lysimeter were sampled, samples of roots, stem, leaves, pods and peas were taken. Soil samples from 2 depths (0-15 and 15-30cm) were taken. No pore water could be extracted.
19.10.2011	Harvest of maize End of experiment	3 maize plants were sampled from each lysimeter, samples of roots, stem, leaves, cobs, kernels and hull-leaves were taken. Soil samples from 2 different depths (0-15, 15-30, 30-45 and 45-60cm) were taken from each corn lysimeter. Pore water was extracted from each subsample.

Table S5: Recoveries (in %) of internal mass-labeled standards. The recovery was determined in each sample by comparing the standard signal in the sample to the signal in matrix-free solutions which had been spiked with the same quantity of internal standard immediately prior to analysis. The quantity of standard employed varied with matrix and exposure level. The bold entries are the mean recoveries (in %), while the non-bold entries are the respective relative standard deviations.

		13C PFBA	13C PFPeA	13C PFHxA	13C PFHpA	13C PFOA	13C PFNA	13C PFDA	13C PFUnA	13C PFDoDA	18O PFHxS	13C PFOS
Soil		**103%**	**91%**	**92%**	**94%**	**92%**	**93%**	**106%**	**109%**	**112%**	**105%**	**101%**
		13%	12%	10%	9%	6%	11%	8%	12%	13%	4%	9%
Radish	Root	**17%**	**62%**	**91%**	**95%**	**87%**	**86%**	**80%**	**80%**	**100%**	**95%**	**89%**
		2%	4%	4%	5%	3%	11%	10%	11%	4%	3%	10%
	Bulb	**27%**	**79%**	**91%**	**109%**	**79%**	**63%**	**89%**	**87%**	**80%**	**83%**	**62%**
		11%	14%	17%	9%	11%	5%	10%	10%	9%	5%	8%
	Foliage	**36%**	**72%**	**90%**	**102%**	**91%**	**73%**	**93%**	**82%**	**65%**	**84%**	**72%**
		14%	8%	9%	7%	8%	9%	8%	8%	9%	14%	7%
Lettuce	Root	**23%**	**75%**	**98%**	**81%**	**67%**	**86%**	**58%**	**50%**	**85%**	**102%**	**43%**
		8%	10%	9%	10%	6%	10%	10%	9%	6%	14%	7%
	Foliage	**22%**	**95%**	**71%**	**71%**	**63%**	**50%**	**58%**	**53%**	**91%**	**94%**	**52%**
		9%	13%	12%	14%	14%	13%	13%	14%	14%	10%	15%
Pea	Root	**26%**	**78%**	**87%**	**100%**	**104%**	**73%**	**90%**	**117%**	**129%**	**93%**	**80%**
		4%	8%	5%	8%	8%	8%	10%	6%	9%	9%	10%
	Stem	**19%**	**85%**	**95%**	**104%**	**104%**	**81%**	**102%**	**103%**	**110%**	**99%**	**78%**
		4%	9%	10%	8%	11%	10%	10%	12%	9%	11%	12%
	Leaves	**8%**	**45%**	**120%**	**93%**	**95%**	**61%**	**79%**	**83%**	**79%**	**91%**	**61%**
		1%	2%	19%	3%	9%	7%	12%	16%	16%	3%	6%
	Pods	**23%**	**75%**	**134%**	**107%**	**112%**	**83%**	**104%**	**96%**	**80%**	**102%**	**68%**
		1%	3%	26%	6%	7%	3%	11%	13%	10%	7%	3%
	Peas	**20%**	**71%**	**102%**	**94%**	**99%**	**67%**	**105%**	**108%**	**86%**	**82%**	**72%**
		2%	3%	11%	13%	10%	8%	10%	10%	10%	10%	8%
Maize	Root	**27%**	**98%**	**119%**	**90%**	**91%**	**97%**	**108%**	**108%**	**113%**	**90%**	**80%**
		6%	8%	6%	7%	11%	12%	8%	14%	13%	9%	6%
	Stem	**23%**	**89%**	**114%**	**106%**	**97%**	**78%**	**99%**	**90%**	**95%**	**89%**	**72%**
		6%	11%	6%	10%	11%	11%	14%	10%	9%	7%	10%
	Leaves	**14%**	**49%**	**89%**	**98%**	**87%**	**61%**	**94%**	**100%**	**81%**	**71%**	**71%**
		1%	7%	6%	7%	7%	11%	15%	10%	10%	10%	11%
	Cobs	**42%**	**107%**	**137%**	**93%**	**96%**	**102%**	**98%**	**89%**	**101%**	**93%**	**89%**
		5%	8%	6%	7%	9%	8%	11%	8%	10%	6%	12%
	Hull-Leaves	**35%**	**96%**	**123%**	**95%**	**93%**	**85%**	**87%**	**80%**	**80%**	**94%**	**79%**
		11%	10%	7%	11%	10%	9%	8%	6%	9%	11%	10%
	Kernels	**22%**	**92%**	**96%**	**67%**	**75%**	**84%**	**96%**	**92%**	**86%**	**85%**	**78%**
		2%	7%	14%	9%	9%	10%	6%	8%	7%	8%	7%

Table S6: Limits of Quantification (LoQ) in ng g^{-1} fresh weight (plant samples), in ng g^{-1} dry weight (soil) and ng mL^{-1} (pore water).

		PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	L-PFBS	L-PFOS	Br-PFOS	Sum PFOS
Radish	Roots	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.177	0.145	0.039	0.184
	Bulb	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.018	0.014	0.004	0.018
	Foliage	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.028	0.023	0.006	0.029
Lettuce	Roots	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.022	0.018	0.005	0.023
	Foliage	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.017	0.014	0.004	0.017
Pea	Roots	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.177	0.145	0.039	0.184
	Stem	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.035	0.030	0.024	0.006	0.031
	Leaves	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.035	0.029	0.008	0.037
	Pods	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.019	0.016	0.013	0.004	0.017
	Peas	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.021	0.018	0.014	0.004	0.018
Maize	Roots	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.022	0.018	0.005	0.023
	Stem	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.022	0.018	0.005	0.023
	Leaves	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.035	0.029	0.008	0.037
	Cobs	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.033	0.027	0.007	0.034
	Hull-Leaves	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.053	0.044	0.036	0.010	0.046
	Kernels	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.011	0.009	0.002	0.011
Soil		0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.089	0.072	0.019	0.092
Pore-water		0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.089	0.072	0.019	0.092

Figure S2: Comparison of a radish harvested from an unspiked lysimeter (left) with a radish harvested from the highest exposure level (right) on the same date.

Table S7: Concentrations in soil at the start of the experiment in ng g^{-1} dry weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS	Water content (mL g^{-1} dw)
Radish														
Level 1	96.4	109.4	110.3	104.0	105.5	86.4	89.1	115.8	88.4	119.3	83.2	110.2	107.4	0.014
Level 2	981.3	984.4	837.1	1083	1146	1098	952.8	1089	988.6	1015	938.1	999.34	906.5	0.034
Level 3	4929	4358	3218	5286	5044	4455	5089	4722	4857	5123	4977	4693	5257	0.027
Level 4	7287	9961	4590	10400	9735	9572	10198	10530	10122	10339	8969	9877	10581	0.032
Lettuce														
Level 1	105.3	99.0	105.0	98.9	97.8	98.2	103.8	94.9	87.3	115.2	87.9	97.4	108.3	0.022
Level 2	952.7	972.5	916.2	1033	1116	1066	973.3	1054	1019	1017	978.9	1015	1008	0.004
Level 3	4626	4511	3170	4950	4937	4737	5096	4640	4717	4905	5131	4757	5244	0.041
Level 4	7769	9321	4832	10063	9990	9999	10239	10102	10212	9888	8790	9607	9834	0.028
Pea														
Level 1	100.3	99.3	98.3	87.6	89.2	97.6	99.0	81.9	85.0	102.1	85.9	92.0	103.9	0.006
Level 2	914.5	929.5	748.4	1040	1042	1066	946.7	1030	1003	953.7	909.5	990.3	1036	0.026
Level 3	3984	4252	2349	4473	5095	4971	5042	4591	4331	4885	4980	4793	5138	0.008
Level 4	6406	9087	4269	9897	10143	10030	10396	9417	10072	8876	8183	9250	9354	0.027
Maize														
Level 1	119.2	88.2	106.4	105.2	98.8	110.7	123.2	87.1	88.6	124.2	94.6	90.1	113.7	0.005
Level 2	962.1	1004	1163	976.3	1161	1035	1020	1043	1065	1083	1089	1055	1080	0.014
Level 3	4966	4921	3942	5091	4671	4786	5156	4608	4963	4709	5437	4785	5335	0.017
Level 4	9613	8916	5638	9892	10092	10397	10125	10359	10441	10447	9217	9695	9566	0.020

Table S8: Concentrations in soil at the time of the harvest in ng g^{-1} dry weight.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS	Water content (mL g^{-1} dw)
Radish														
Level 1	<LoQ	0.27	0.42	0.62	0.34	1.63	21.3	38.7	47.9	80.0	52.6	0.51	16.6	0.163
Level 2	5.23	6.11	4.07	6.08	4.93	16.2	273.0	444.6	729.9	838.6	837.1	5.56	123.7	0.169
Level 3	6.70	7.33	6.78	10.6	24.8	71.0	1727	2129	3439	3819	4108	17.1	716.0	0.168
Level 4	18.4	9.89	5.31	9.60	28.6	158.1	3177	5559	7384	7951	7885	14.1	1373	0.155
Lettuce														
Level 1	2.57	2.09	2.57	44.9	30.9	46.2	51.7	60.0	62.7	93.9	72.9	3.39	78.2	0.146
Level 2	10.1	8.40	7.76	46.2	210.8	450.0	516.1	629.9	735.0	770.5	848.5	17.1	712.3	0.136
Level 3	10.4	11.4	20.1	60.8	583.9	2150	2494	2883	3245	3928	4259	38.1	3801	0.132
Level 4	10.8	14.2	31.8	132.9	1683	3973	5072	6037	7098	7779	7299	62.6	7075	0.140
Pea														
Level 1	0.56	0.93	1.85	26.5	41.0	53.7	62.8	67.3	65.7	77.6	79.8	2.79	83.3	0.054
Level 2	1.95	3.51	6.51	35.1	365.8	597.9	679.5	835.8	713.6	795.5	808.6	9.33	836.2	0.071
Level 3	15.8	20.9	33.6	76.6	1394	2852	3591	4206	3357	4218	4478	82.8	4317	0.042
Level 4	11.3	16.1	32.5	121.0	2035	5980	6962	8237	7454	7180	7674	67.2	7271	0.038
Maize														
Level 1	3.06	2.48	2.19	9.42	35.0	22.7	36.7	42.1	57.2	96.7	74.9	3.01	66.2	0.135
Level 2	21.4	23.2	26.6	114.8	312.7	258.2	353.4	590.5	639.5	849.6	918.5	31.2	616.4	0.127
Level 3	12.5	10.8	11.9	22.0	159.2	1386	1897	2651	3279	3847	4868	20.0	3850	0.135
Level 4	16.6	18.5	27.6	79.1	347.5	2813	3978	5826	6324	8547	8919	65.7	6513	0.134

Table S9: Concentrations in pore water at the time of the harvest in ng mL^{-1}.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1	<LoQ	0.42	0.40	0.37	<LoQ	0.21	0.14	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.25
Level 2	36.3	30.8	11.7	10.5	3.32	2.82	4.65	1.85	2.23	4.94	7.44	6.65	3.22
Level 3	39.2	31.4	12.5	16.1	10.6	8.61	28.3	15.3	12.0	14.5	19.1	13.8	19.6
Level 4	60.1	27.0	3.81	4.85	5.59	4.37	31.4	56.4	59.5	81.4	117.4	3.9	18.2
Lettuce													
Level 1	24.0	12.7	9.09	93.9	24.8	3.28	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	4.53	<LoQ
Level 2	99.2	67.9	29.6	113.2	322.7	86.4	16.0	<LoQ	<LoQ	<LoQ	2.39	23.3	35.2
Level 3	84.8	80.0	84.5	118.0	346.7	875.6	258.7	42.0	19.2	22.0	34.5	32.7	402.7
Level 4	96.2	101.8	127.4	368.6	3994	1777	293.1	81.5	47.8	50.7	74.8	175.5	547.9
Maize													
Level 1	30.5	14.9	5.18	13.2	21.5	3.73	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	2.95	0.73
Level 2	224.7	187.3	118.2	470.4	742.9	79.9	12.2	1.78	0.12	<LoQ	0.69	68.4	21.2
Level 3	94.9	55.8	23.4	33.2	54.3	722.9	134.9	23.1	8.61	9.62	13.3	23.6	175.8
Level 4	136.2	119.8	96.8	154.6	766.2	1457	279.9	51.2	20.7	23.4	32.3	70.4	400.8

Table S10: Concentration in radish plant compartments in ng g^{-1} ww

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Roots													
Level 1	99.8	89.7	39.7	89.7	124.6	106.6	51.8	32.5	25.3	27.6	30.4	8.97	18.9
Level 2	288.8	198.9	61.6	182.7	146.4	217.8	113.1	245.1	248.6	293.8	271.6	17.4	221.3
Level 3	451.4	292.0	68.7	146.6	165.4	1310	533.9	1141	1110	1129	910.7	50.1	1109
Level 4	499.7	193.0	18.5	67.9	144.4	2734	1225	5373	2406	2345	2176	27.2	1939
Bulb													
Level 1	1.10	15.0	2.75	5.40	5.17	2.26	0.90	<LoQ	<LoQ	<LoQ	<LoQ	0.20	<LoQ
Level 2	462.3	189.0	47.6	323.9	42.7	32.7	22.9	20.7	14.8	12.3	7.68	50.5	17.7
Level 3	902.9	584.9	88.3	292.4	219.0	142.6	117.9	128.0	73.6	61.9	39.8	150.1	87.6
Level 4	1505	654.8	73.8	302.7	452.4	282.0	238.7	260.0	174.6	113.7	85.9	191.7	169.4
Leaves													
Level 1	60.9	147.7	25.4	30.7	31.1	11.5	3.46	<LoQ	<LoQ	<LoQ	<LoQ	39.4	2.73
Level 2	8743	3690	946.6	1690	602.2	386.1	94.1	26.3	25.8	19.5	19.6	441.7	645.6
Level 3	14495	6127	716.1	1163	1012	1578	528.8	140.0	117.7	99.6	92.0	879.2	3792
Level 4	18098	8577	774.6	1122	1427	4035	1288	393.7	226.2	206.3	215.5	745.9	7369

Table S11: Concentrations in lettuce plant compartments in ng g^{-1} ww

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Roots													
Level 1	389.2	141.9	3.88	1.94	2.50	16.4	28.4	30.5	25.8	8.83	5.08	0.51	16.2
Level 2	929.8	225.4	10.9	7.68	31.0	176.3	366.7	486.0	267.6	107.0	67.1	3.67	308.7
Level 3	420.0	91.3	12.0	10.1	81.7	1376	2096	2552	1160	601.4	327.5	9.24	1433
Level 4	158.1	28.3	5.95	7.77	61.8	1173	2569	4140	2096	1194	612	4.01	2410
Leaves													
Level 1	732.4	474.0	43.2	12.0	5.11	1.64	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	4.14	<LoQ
Level 2	1621	833.0	109.1	73.1	108.4	185.8	69.4	58.2	35.6	27.1	14.7	40.8	134.2
Level 3	810.3	388.1	78.3	162.3	552.5	1837	806.2	313.9	179.8	121.1	82.8	159.2	3337
Level 4	292.1	97.9	35.3	83.5	819.8	4184	2156	619.2	355.6	251.7	150.5	16.5	6571

Table S123: Concentrations in pea plant compartments in ng g^{-1} ww

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Roots													
Level 1	302.4	117.2	63.19	52.6	12.9	12.1	27.1	31.0	12.8	8.94	5.13	25.1	14.8
Level 2	392.4	237.4	192.8	280.2	131.5	145.3	297.8	354.6	173.4	121.2	108.4	52.4	216.1
Level 3	851.5	442.0	278.2	767.3	716.2	740.2	1477	1655	884.0	759.4	577.0	286.5	1087
Level 4	982.9	588.3	412.1	1473	1339	1501	3069	3452	2079	1395	1127	572.9	2138
Stem													
Level 1	623.9	200.8	44.6	19.1	11.4	6.53	4.89	0.89	0.16	<LoQ	<LoQ	24.9	2.42
Level 2	1380	583.8	147.9	186.4	84.2	70.6	51.5	28.4	5.17	1.31	0.58	186.9	51.6
Level 3	3795	1318	508.8	464.1	557.8	533.9	303.9	144.1	33.6	7.70	2.80	732.7	499.0
Level 4	4859	1613	537.0	497.4	743.5	960.4	618.1	347.9	55.9	9.94	5.24	964.4	1210
Leaves													
Level 1	2520	2216	508.7	287.7	90.6	19.9	6.84	0.93	0.41	<LoQ	<LoQ	1454	7.89
Level 2	3700	2998	1031	1406	487.1	307.1	121.5	44.7	7.73	0.84	<LoQ	1988	139.2
Level 3	7469	5665	1549	2647	4828	5106	1641	411.1	32.1	5.17	0.39	6402	4831
Level 4	10565	10693	4084	7262	6510	5062	1731	519.4	23.7	12.1	5.92	12529	5578
Pods													
Level 1	1511	265.7	18.1	8.19	1.25	0.27	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	41.0	<LoQ
Level 2	2949	675.9	76.8	65.4	28.3	12.9	4.89	1.23	0.32	<LoQ	<LoQ	165.7	14.3
Level 3	4495	1130	187.7	148.1	155.6	109.4	30.7	11.7	1.93	0.37	<LoQ	510.5	141.1
Level 4	4305	1561	450.3	398.4	193.4	61.9	23.4	3.62	0.56	0.07	<LoQ	807.7	76.7
Peas													
Level 1	573.9	88.1	9.45	2.39	0.26	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	11.9	<LoQ
Level 2	2816	279.3	46.5	28.6	7.92	1.56	0.40	0.11	<LoQ	<LoQ	<LoQ	58.5	1.52
Level 3	4198	540.0	87.5	60.7	61.8	14.8	2.58	0.40	<LoQ	<LoQ	<LoQ	147.0	8.83
Level 4	3187	628.4	220.6	158.4	45.7	6.7	0.6	0.13	<LoQ	<LoQ	<LoQ	265.6	3.08

Table S13: Concentrations in maize plant compartments in ng g^{-1} ww

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Roots													
Level 1	39.1	23.0	9.86	6.68	3.03	4.71	15.8	15.7	11.9	10.3	6.00	9.38	19.7
Level 2	105.3	120.4	29.1	68.3	33.5	52.7	150.0	206.7	115.4	99.5	71.0	46.6	218.1
Level 3	125.6	104.6	69.6	157.6	149.7	264.8	658.9	944.0	525.7	464.0	348.4	58.6	973.5
Level 4	301.4	361.5	136.5	266.5	293.4	538.3	1023	1923	1042	837.0	704.4	152.1	2031
Stem													
Level 1	40.6	14.3	2.00	0.77	0.38	0.20	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	6.05	0.42
Level 2	175.1	101.9	22.0	18.0	10.0	9.16	5.64	2.01	<LoQ	<LoQ	<LoQ	53.1	52.0
Level 3	187.5	138.5	46.1	91.9	83.6	72.3	42.7	17.9	1.84	0.60	0.50	73.3	257.9
Level 4	479.6	305.2	86.9	99.5	115.3	104.4	73.8	34.9	3.64	0.61	0.20	144.7	471.8
Leaves													
Level 1	9209	1296	102.0	39.6	18.6	5.12	0.77	<LoQ	<LoQ	<LoQ	<LoQ	97.8	8.77
Level 2	31329	4577	503.0	537.9	313.8	168.9	36.0	2.68	0.81	0.69	0.94	751.1	568.6
Level 3	33174	5363	1167	1448	1613	895.7	214.3	27.3	2.05	3.66	4.60	822.5	2766
Level 4	30552	4749	1290	1621	2782	1831	435.0	59.3	2.94	3.60	5.32	1028	5558
Hull-Leaves													
Level 1	146.0	19.9	3.19	0.67	0.24	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	3.73	0.19
Level 2	571.5	122.5	14.8	20.5	9.62	7.37	1.39	<LoQ	<LoQ	<LoQ	<LoQ	33.2	13.2
Level 3	464.3	121.5	19.3	48.7	63.2	42.7	18.3	1.33	<LoQ	<LoQ	<LoQ	33.5	100.1
Level 4	798.9	227.9	31.8	125.2	136.3	86.9	32.9	2.05	<LoQ	<LoQ	<LoQ	79.9	202.7
Cobs													
Level 1	22.5	3.39	1.86	0.20	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.49	<LoQ
Level 2	78.4	34.8	20.2	8.83	3.34	1.91	0.35	<LoQ	<LoQ	<LoQ	<LoQ	4.12	1.94
Level 3	44.2	25.4	19.0	17.6	22.5	19.3	6.85	0.55	<LoQ	<LoQ	<LoQ	5.19	32.8
Level 4	139.0	113.1	56.9	62.0	60.6	34.4	11.7	1.13	<LoQ	<LoQ	<LoQ	9.08	36.4
Kernels													
Level 1	9.92	7.59	7.71	0.26	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.58	<LoQ
Level 2	33.6	78.1	44.4	6.66	0.29	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	4.97	0.10
Level 3	18.6	49.9	42.1	11.1	2.13	0.40	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	4.91	0.25
Level 4	84.3	301.9	214.8	104.1	17.8	2.35	0.33	<LoQ	<LoQ	<LoQ	<LoQ	14.9	0.36

Table S14: Root concentration factors based on soil concentrations (RCF_S; concentration in the roots divided by the concentration in the soil. Units: kg soil dry weight per kg root fresh weight). Level 4 was not considered for radish and lettuce due to phytotoxic effects.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1	38.9	43.0	15.4	2.00	4.03	2.31	1.00	0.54	0.40	0.29	0.42	2.65	0.24
Level 2	28.7	23.7	7.94	3.96	0.69	0.48	0.22	0.39	0.34	0.38	0.32	1.02	0.31
Level 3	43.4	25.6	3.41	2.41	0.28	0.61	0.21	0.40	0.34	0.29	0.21	1.32	0.29
Lettuce													
Level 1	152	68.0	1.51	0.043	0.081	0.36	0.55	0.51	0.41	0.094	0.070	0.15	0.21
Level 2	92.4	26.8	1.41	0.17	0.15	0.39	0.71	0.77	0.36	0.14	0.079	0.21	0.43
Level 3	40.4	8.00	0.60	0.17	0.14	0.64	0.84	0.89	0.36	0.15	0.077	0.24	0.38
Pea													
Level 1			34.1		0.31	0.23	0.43	0.46	0.20	0.12	0.064	9.02	0.18
Level 2	201	67.6	29.6	7.99	0.36	0.24	0.44	0.42	0.24	0.15	0.13	5.61	0.26
Level 3	54.0	21.2	8.29	10.0	0.51	0.26	0.41	0.39	0.26	0.18	0.13	3.46	0.25
Level 4	87.0	36.5	12.7	12.2	0.66	0.25	0.44	0.42	0.28	0.19	0.15	8.53	0.29
Maize													
Level 1	12.8	9.25	4.50	0.71	0.09	0.21	0.43	0.37	0.21	0.11	0.080	3.12	0.30
Level 2	4.93	5.20	1.09	0.60	0.11	0.20	0.42	0.35	0.18	0.12	0.077	1.49	0.35
Level 3	10.1	9.64	5.87	7.15	0.94	0.19	0.35	0.36	0.16	0.12	0.072	2.93	0.25
Level 4	18.2	19.5	4.95	3.37	0.84	0.19	0.26	0.33	0.16	0.10	0.079	2.31	0.31

Table S15: Root concentration factors based on pore water concentrations (RCF_{PW}; concentration in the roots divided by the concentration in the pore water. Units: L pore water per kg root fresh weight). Level 4 was not considered for radish and lettuce due to phytotoxic effects. No values could be calculated for pea because no pore water could be retrieved at the time of harvest.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1	4.16	7.06	4.36	0.96	5.03	32.6						1.98	
Level 2	2.91	2.93	2.08	1.61	0.45	2.52	7.05				114	0.75	6.28
Level 3	5.32	3.65	0.81	1.24	0.48	1.50	2.06	27.2	57.9	51.4	26.4	1.53	2.75
Lettuce													
Level 1	16.2	11.2	0.43	0.021	0.10	5.02						0.11	
Level 2	9.37	3.32	0.37	0.068	0.10	2.04	22.9				28.1	0.16	8.76
Level 3	4.95	1.14	0.14	0.086	0.24	1.57	8.10	60.8	60.5	27.4	9.50	0.28	3.56
Maize													
Level 1	1.28	1.55	1.90	0.51	0.14	1.26						3.18	
Level 2	0.47	0.64	0.25	0.15	0.045	0.66						0.68	10.3
Level 3	1.32	1.88	2.97	4.74	2.76	0.37	4.89	40.8	61.1	48.2	26.3	2.48	5.54
Level 4	2.21	3.02	1.41	1.72	0.38	0.37	3.65	37.5	50.3	35.7	21.8	2.16	5.07

Table S16: Edible part concentration factor based on soil concentrations (ECF_S; concentration in the edible parts divided by the concentration in the soil. Units: kg dry soil per kg edible part fresh weight). The edible parts were defined as: bulbs (radish), foliage (lettuce), peas (pea) and kernels (maize). Level 4 was not considered for radish and lettuce due to phytotoxic effects.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1	0.43	7.2	1.1	0.12	0.17	0.049	0.017					0.059	
Level 2	46	23	6.1	7.0	0.20	0.073	0.044	0.033	0.020	0.016	0.0091	2.9	0.025
Level 3	87	51	4.4	4.8	0.38	0.066	0.047	0.044	0.023	0.016	0.0093	3.9	0.023
Lettuce													
Level 1	285	227	16.8	0.27	0.17	0.036						1.2	
Level 2	161	99	14.1	1.6	0.51	0.41	0.13	0.092	0.048	0.035	0.017	2.4	0.19
Level 3	78	34	3.9	2.7	0.95	0.85	0.32	0.11	0.055	0.031	0.019	4.2	0.88
Pea													
Level 1	1029	95	5.1	0.09	0.01							4.3	
Level 2	1445	80	7.1	0.82	0.02	0.0026	0.00059	0.00014				6.3	0.0018
Level 3	266	26	2.6	0.79	0.04	0.0052	0.00072	0.000094				1.8	0.0020
Level 4	282	39.0	6.8	1.3	0.02	0.0011	0.000090	0.000015				4.0	0.00042
Maize													
Level 1	3.2	3.1	3.5	0.028								0.19	
Level 2	1.6	3.4	1.7	0.058	0.00092							0.16	0.00016
Level 3	1.5	4.6	3.5	0.51	0.013	0.00029						0.25	0.000064
Level 4	5.1	16.3	7.8	1.3	0.051	0.00083	0.000082					0.23	0.000055

Table S17: Whole plant concentration factors based on soil concentrations (PCF_S; concentration in the whole plant divided by the concentration in the soil. Units: kg soil dry weight per kg plant fresh weight). Level 4 was not considered for radish and lettuce due to phytotoxic effects.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1		25	4.2	0.39	0.66	0.27	0.105	0.042	0.031	0.023	0.032	2.9	0.027
Level 2	234	119	33	13.6	0.85	0.29	0.090	0.062	0.048	0.046	0.036	8.1	0.25
Level 3	385	161	11.5	7.9	0.68	0.26	0.098	0.072	0.050	0.039	0.028	8.2	0.27
Lettuce													
Level 1	263	201	14.3	0.23	0.151	0.089	0.092	0.085	0.069	0.016	0.012	1.04	
Level 2	150	87	12.0	1.347	0.45	0.41	0.23	0.21	0.101	0.053	0.028	2.0	0.23
Level 3	72	30	3.3	2.255	0.81	0.82	0.41	0.24	0.106	0.051	0.029	3.5	0.79
Pea													
Level 1	1644	388	41	1.61	0.33	0.094	0.088	0.071	0.029	0.017	0.0093	60	0.041
Level 2	1184	193	29	6.9	0.25	0.114	0.097	0.074	0.038	0.022	0.0194	32	0.070
Level 3	255	62	10.7	6.7	0.54	0.26	0.123	0.074	0.041	0.027	0.0188	11.8	0.178
Level 4	366	124	23	9.7	0.51	0.154	0.106	0.075	0.042	0.029	0.021	26	0.153
Maize													
Level 1	389	70	7.7	0.68	0.083	0.064	0.074					5.2	0.067
Level 2	190	28	3.2	0.76	0.156	0.122	0.086	0.059	0.030	0.020	0.013	3.8	0.193
Level 3	342	69	15.1	10.7	1.61	0.130	0.078	0.062	0.027	0.020	0.012	6.7	0.150
Level 4	248	44	9.1	4.0	1.30	0.127	0.062	0.057	0.027	0.016	0.013	3.0	0.178

Table S18: Whole plant concentration factors based on pore water concentrations (PCF_{PW}; concentration in the whole plant divided by the concentration in the pore water. Units: L pore water per kg plant fresh weight). Level 4 was not considered for radish and lettuce due to phytotoxic effects. Values for radish and pea were calculated using concentrations in pore water from the lettuce lysimeters.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1		4.0	1.19	0.189	0.82	3.8						2.2	
Level 2	24	14.7	8.6	5.6	0.56	1.49	2.9				12.9	5.9	5.1
Level 3	47	23	2.7	4.1	1.15	0.64	0.95	5.0	8.5	7.0	3.4	9.5	2.5
Lettuce													
Level 1	28	33	4.0	0.110	0.19	1.25						0.78	
Level 2	15.2	10.8	3.1	0.55	0.30	2.1	7.4				9.8	1.48	4.6
Level 3	8.8	4.2	0.80	1.16	1.37	2.0	3.9	16.4	17.9	9.2	3.6	4.1	7.5
Pea													
Level 1	38	28	8.3	0.45	0.54	1.54						37	
Level 2	23	10.0	6.5	2.1	0.29	0.79	4.1				6.6	12.7	1.65
Level 3	47	16.2	4.3	4.4	2.2	0.85	1.71	7.4	7.2	5.1	2.4	30	1.91
Level 4	43	19.6	5.8	3.2	0.26	0.52	2.5	7.6	6.6	4.0	2.2	10.1	2.0
Maize													
Level 1	39	11.6	3.3	0.48	0.134	0.39						5.3	6.2
Level 2	18.1	3.5	0.72	0.187	0.066	0.39	2.5	19.7	16.0		17.2	1.73	5.6
Level 3	45	13.3	7.6	7.1	4.7	0.25	1.10	7.1	10.2	8.1	4.4	5.6	3.3
Level 4	30	6.8	2.6	2.0	0.59	0.25	0.88	6.5	8.4	6.0	3.6	2.8	2.9

Table S19: P-values from the T-test (two tailed, two sample unequal variance) comparing PCF_{PW} between PFAAs.
a) Radish

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
PFBA	1.000	0.175	0.039	0.097	0.017	0.015	0.062				0.202	0.042	0.044
PFPeA		1.000	0.172	0.211	0.025	0.054	0.103				0.600	0.304	0.169
PFHxA			1.000	0.642	0.135	0.426	0.506				0.451	0.535	0.824
PFHpA				1.000	0.586	0.966	0.990				0.347	0.416	0.547
PFOA					1.000	0.338	0.405				0.166	0.034	0.081
PFNA						1.000	0.931				0.211	0.157	0.264
PFDA							1.000				0.251	0.249	0.379
PFUnA													
PFDoDA													
PFTrDA													
PFTeDA											1.000	0.749	0.516
PFBS												1.000	0.595
Sum PFOS													1.000

Significant values ($p < 0.05$) are highlighted in red.

Table S19: P-values from the T-test (two tailed, two sample unequal variance) comparing PCF_{PW} between PFAAs.
b) Lettuce

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
PFBA	1.000	0.684	0.038	0.020	0.012	0.011	0.113				0.259	0.024	0.101
PFPeA		1.000	0.100	0.023	0.017	0.077	0.350				0.461	0.069	0.387
PFHxA			1.000	0.133	0.108	0.726	0.221				0.262	0.739	0.178
PFHpA				1.000	0.976	0.167	0.051				0.053	0.181	0.051
PFOA					1.000	0.133	0.036				0.045	0.151	0.036
PFNA						1.000	0.117				0.223	0.942	0.055
PFDA							1.000				0.894	0.136	0.850
PFUnA													
PFDoDA													
PFTrDA													
PFTeDA											1.000	0.183	0.993
PFBS												1.000	0.110
Sum PFOS													1.000

Significant values ($p < 0.05$) are highlighted in red.

Table S19: P-values from the T-test (two tailed, two sample unequal variance) comparing PCF_{PW} between PFAAs.
c) Pea

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
PFBA	1.000	0.035	0.000	0.007	0.002	0.000	0.002	0.002	0.001	0.001	0.010	0.139	0.000
PFPeA		1.000	0.009	0.015	0.003	0.000	0.004	0.030	0.021	0.006	0.020	0.783	0.001
PFHxA			1.000	0.102	0.012	0.001	0.058	0.220	0.435	0.196	0.214	0.027	0.001
PFHpA				1.000	0.122	0.215	0.608	0.073	0.084	0.184	0.419	0.011	0.957
PFOA					1.000	0.442	0.042	0.013	0.013	0.020	0.031	0.001	0.085
PFNA						1.000	0.025	0.002	0.002	0.003	0.037	0.000	0.036
PFDA							1.000	0.053	0.059	0.153	0.628	0.004	0.311
PFUnA								1.000	0.290	0.141	0.142	0.057	0.001
PFDoDA									1.000	0.138	0.166	0.045	0.000
PFTrDA										1.000	0.457	0.015	0.038
PFTeDA											1.000	0.015	0.243
PFBS												1.000	0.004
Sum PFOS													1.000

Significant values ($p < 0.05$) are highlighted in red.

Table S19: P-values from the T-test (two tailed, two sample unequal variance) comparing PCF_{PW} between PFAAs.
d) Maize

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
PFBA	1.000	0.012	0.009	0.021	0.017	0.000	0.002	0.058	0.014	0.005	0.068	0.001	0.000
PFPeA		1.000	0.116	0.084	0.045	0.001	0.012	0.665	0.372	0.751	0.776	0.101	0.154
PFHxA			1.000	0.385	0.142	0.019	0.305	0.083	0.053	0.139	0.246	0.636	0.407
PFHpA				1.000	0.453	0.221	0.807	0.065	0.058	0.100	0.116	0.242	0.183
PFOA					1.000	0.816	0.294	0.037	0.036	0.055	0.053	0.101	0.084
PFNA						1.000	0.029	0.005	0.000	0.001	0.019	0.001	0.000
PFDA							1.000	0.015	0.008	0.023	0.063	0.080	0.044
PFUnA								1.000	0.753	0.459	0.553	0.084	0.130
PFDoDA									1.000	0.150	0.395	0.020	0.017
PFTrDA										1.000	0.915	0.097	0.122
PFTeDA											1.000	0.340	0.484
PFBS												1.000	0.576
Sum PFOS													1.000

Significant values ($p < 0.05$) are highlighted in red.

Table S20: Root retention factors (RRF_S; mass in the roots as a fraction of the PFAA mass in the whole plant). Level 4 was not considered for radish and lettuce due to phytotoxic effects.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1	0.34	0.134	0.28	0.39	0.47	0.66	0.74					0.070	0.70
Level 2	0.009	0.015	0.019	0.022	0.063	0.130	0.188	0.48	0.54	0.64	0.68	0.010	0.095
Level 3	0.009	0.012	0.023	0.023	0.032	0.179	0.168	0.42	0.52	0.57	0.59	0.012	0.084
Lettuce													
Level 1	0.096	0.056	0.018	0.031	0.089	0.67						0.024	
Level 2	0.103	0.051	0.020	0.021	0.054	0.159	0.51	0.63	0.60	0.44	0.48	0.018	0.32
Level 3	0.094	0.045	0.030	0.012	0.029	0.130	0.34	0.62	0.56	0.50	0.44	0.011	0.082
Pea													
Level 1	0.048	0.047	0.121	0.178	0.138	0.35	0.71	0.94	0.96			0.022	0.68
Level 2	0.025	0.051	0.146	0.169	0.21	0.31	0.65	0.84	0.93	0.98		0.026	0.55
Level 3	0.031	0.049	0.112	0.22	0.139	0.143	0.48	0.77	0.93	0.98	0.99	0.042	0.22
Level 4	0.034	0.043	0.081	0.182	0.186	0.24	0.60	0.81	0.96	0.98	0.99	0.047	0.29
Maize													
Level 1	0.0055	0.022	0.097	0.174	0.174	0.54	0.97					0.099	0.75
Level 2	0.0043	0.031	0.057	0.129	0.114	0.28	0.82	0.98	0.99	0.99	0.99	0.065	0.31
Level 3	0.0049	0.023	0.064	0.110	0.097	0.24	0.74	0.96	0.99	0.99	0.99	0.073	0.28
Level 4	0.0122	0.073	0.090	0.140	0.107	0.25	0.69	0.96	0.99	1.00	0.99	0.127	0.29

Table S21: Edible part to leaf concentration factor (ELCF, kg leaf fresh weight per kg edible part fresh weight). Level 4 was not considered for radish due to phytotoxic effects.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoDA	PFTrDA	PFTeDA	PFBS	PFOS
Radish													
Level 1	0.018	0.102	0.108	0.176	0.166	0.196	0.26					0.005	
Level 2	0.053	0.051	0.050	0.192	0.071	0.085	0.24	0.79	0.57	0.63	0.39	0.114	0.027
Level 3	0.062	0.095	0.123	0.25	0.22	0.090	0.22	0.91	0.63	0.62	0.43	0.171	0.023
Pea													
Level 1	0.23												
Level 2	0.76	0.093	0.045	0.020	0.0163	0.0051	0.0033	0.0026				0.029	
Level 3	0.56	0.095	0.056	0.023	0.0128	0.0029	0.00157	0.00096				0.023	0.00183
Level 4	0.30	0.059	0.054	0.022	0.0070							0.021	0.00055
Maize													
Level 1	0.00108	0.0059	0.076	0.0066								0.0060	
Level 2	0.00107	0.0171	0.088	0.0124	0.00091							0.0066	0.000177
Level 3	0.00056	0.0093	0.036	0.0077	0.00132	0.00044						0.0060	0.000089
Level 4						0.00128	0.00075					0.0145	0.000065

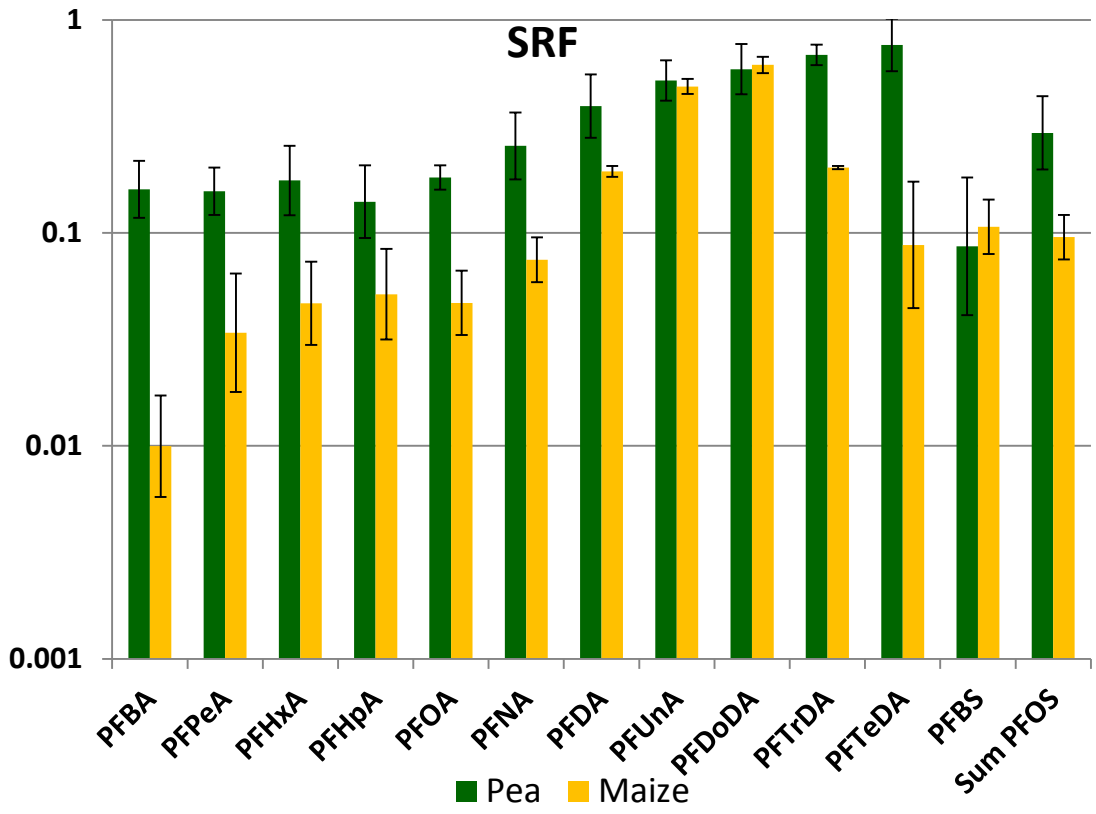


Figure S3: Stem retention factor (SRF), equal to the PFAA mass in the stem as a fraction of the PFAA mass in the above-ground plant parts. The average and standard deviation from experiments conducted at different exposure levels are shown, assuming a log-normal distribution.

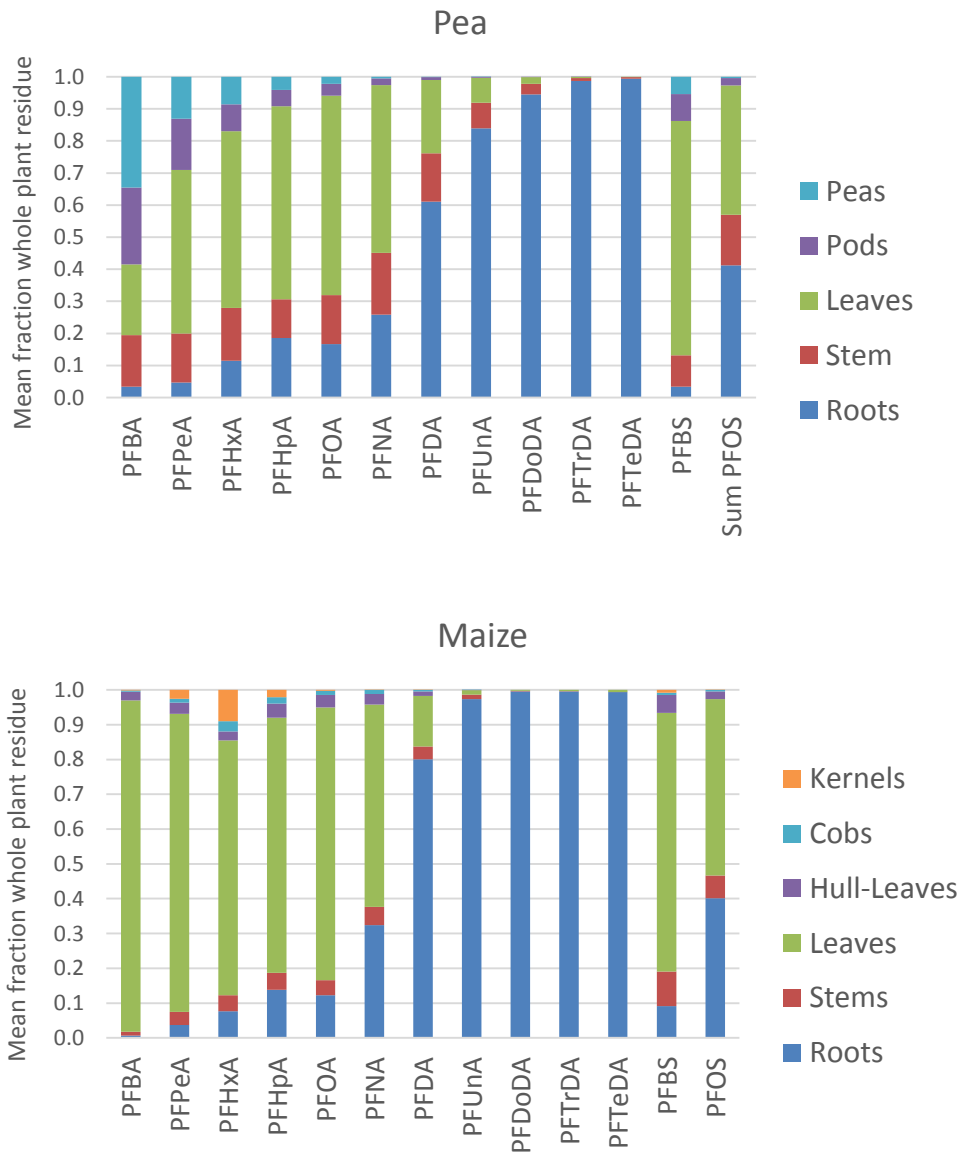


Figure S4: Distribution of the PFAAs between different plant parts in a) pea and b) maize.

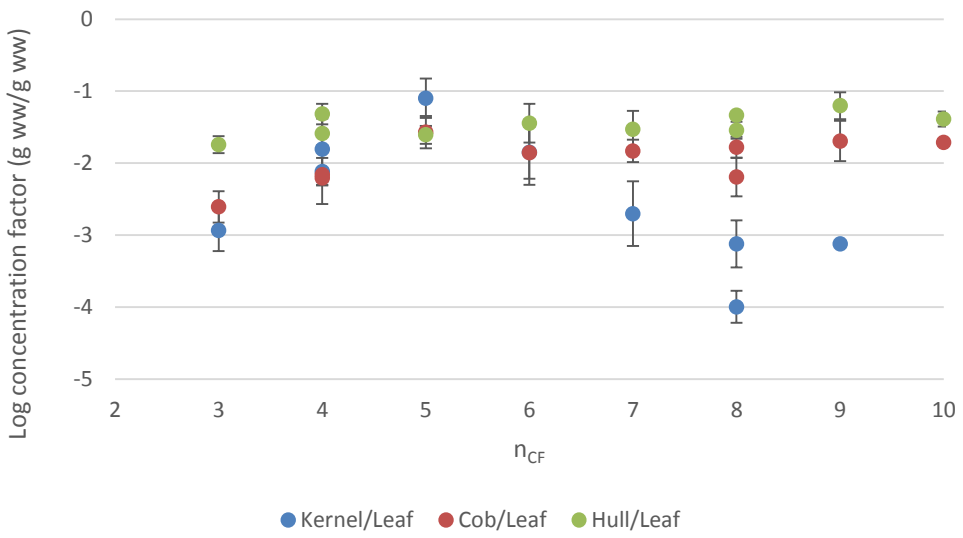


Figure S5: Concentration factors between different maize parts and maize leaves from this study (kg fresh weight per kg fresh weight) plotted against the number of fluorinated carbons (n_{CF}). The mean and standard deviation of the logged concentration factors are shown.

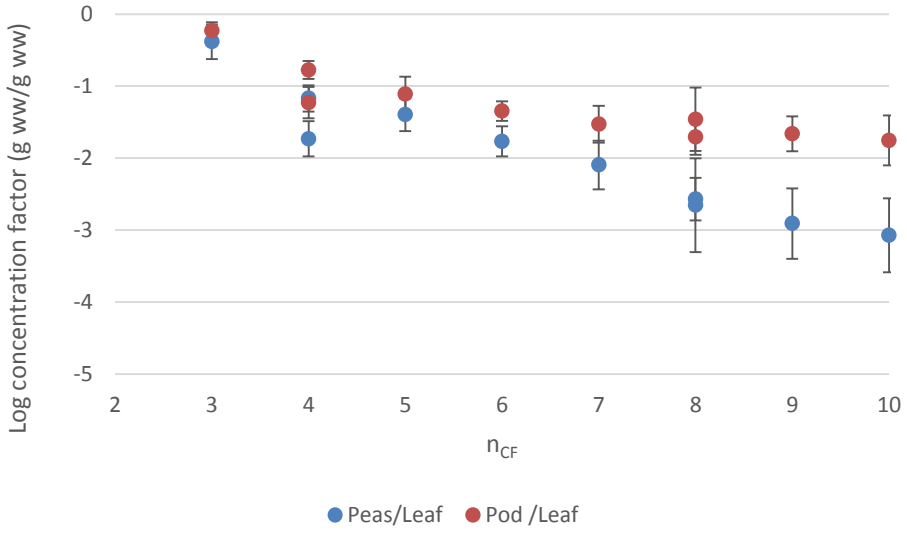


Figure S6: Concentration factors between different pea parts and pea leaves from this study (kg fresh weight per kg fresh weight) plotted against the number of fluorinated carbons (n_{CF}). The mean and standard deviation of the logged concentration factors are shown.

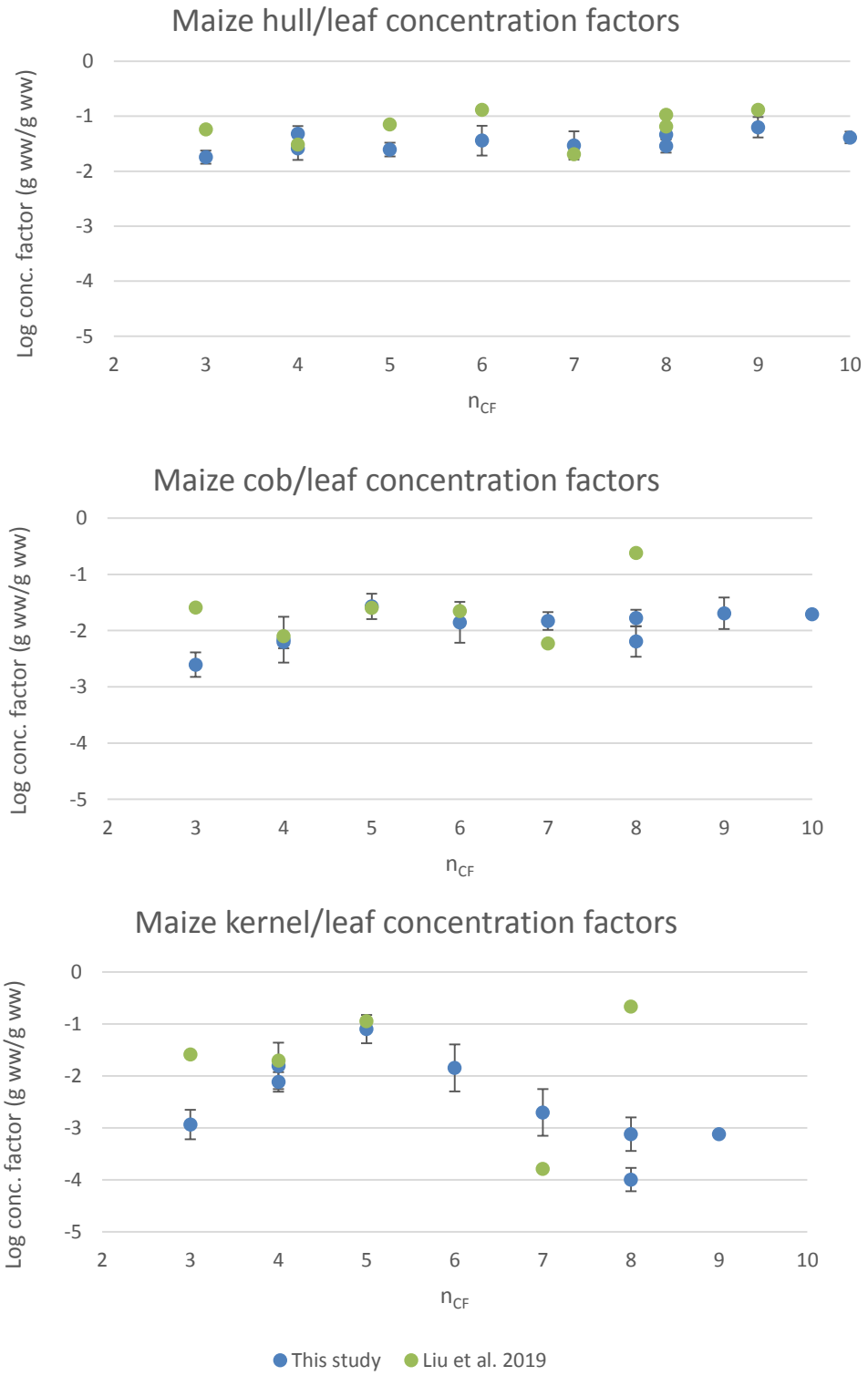
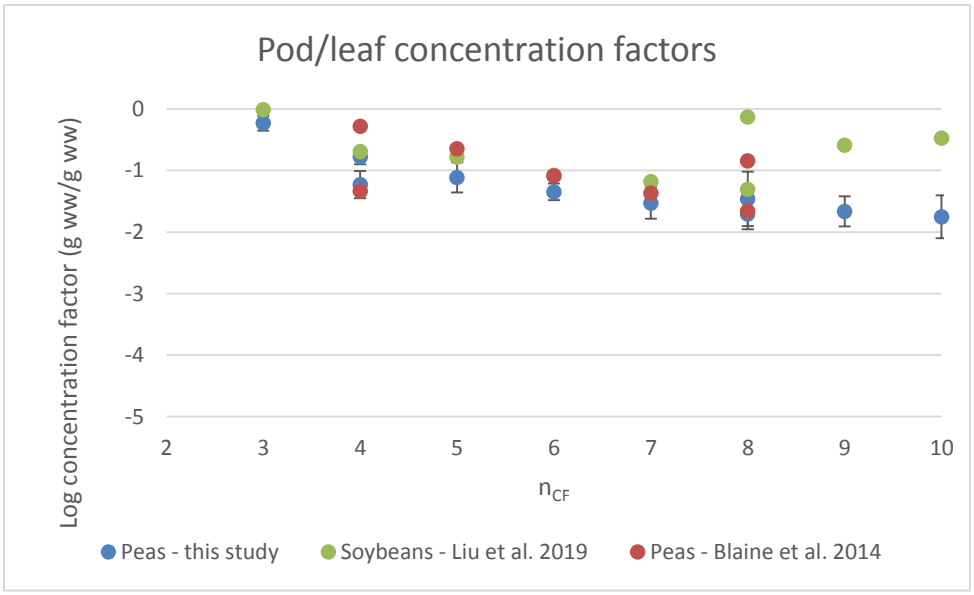


Figure S7: Comparison of concentration factors for maize from this study with those of Liu et al. (2019) plotted against the number of fluorinated carbons (n_{CF}). The concentration factors for this study are wet weight based, while those for Liu et al. are dry weight based. For this study the mean and standard deviation of the logged concentration factors are shown.



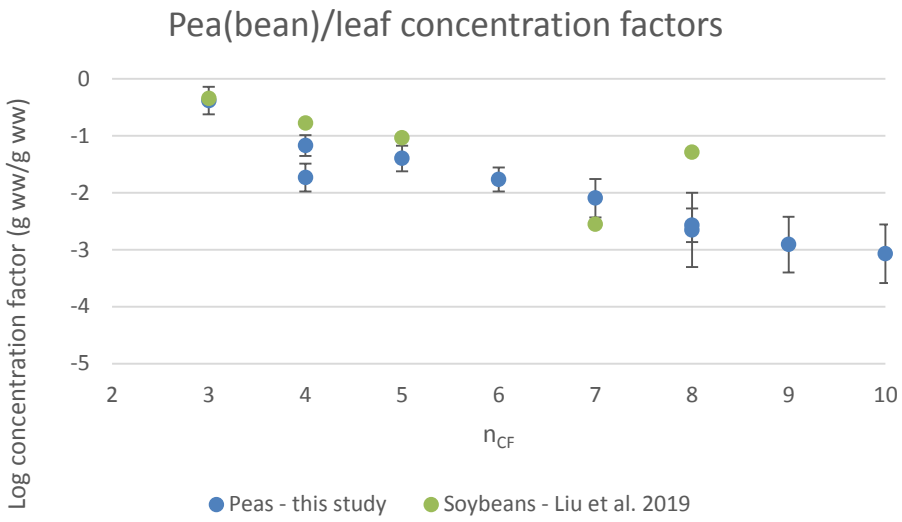


Figure S8: Comparison of concentration factors for peas from this study with peas from Blaine et al. (2014) and soybeans from Liu et al. (2019) plotted against the number of fluorinated carbons (n_{CF}). The data from Blaine et al. provided fruit/shoot concentration factors which were converted to pod/leaf concentration factors using the pea/pod and shoot/leaf fractions from this study. The concentration factors for this study and Blaine et al. are wet weight based, while those for Liu et al. are dry weight based. For this study the mean and standard deviation of the logged concentration factors are shown.

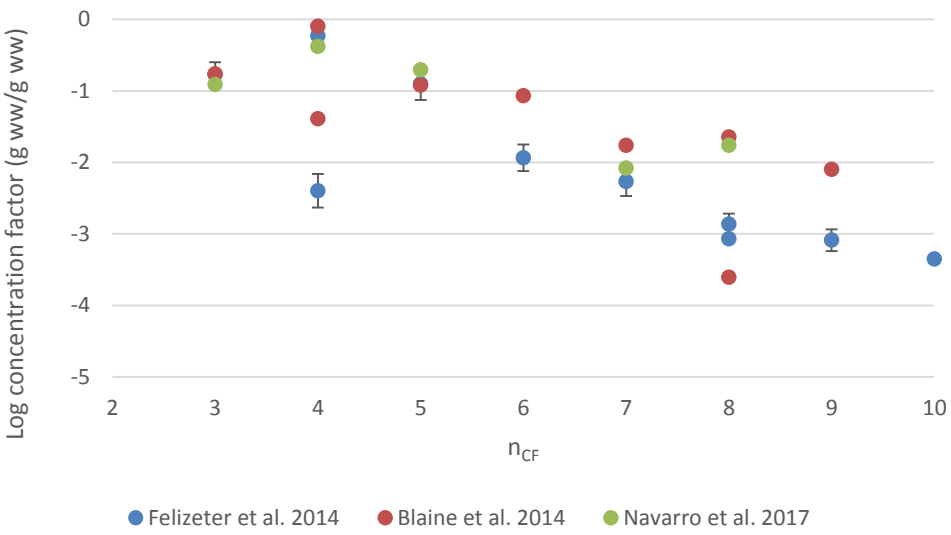


Figure S9: Tomato fruit/leaf concentration factors from 3 studies in the literature plotted against the number of fluorinated carbons (n_{CF}). The data from Blaine et al. are fruit/foliage concentration factors (not fruit/leaf). The concentration factors for Navarro et al. were converted from a dry weight to a wet weight basis using water content data from Blaine et al. For Felizeter et al. the mean and standard deviation of the logged concentration factors are shown.

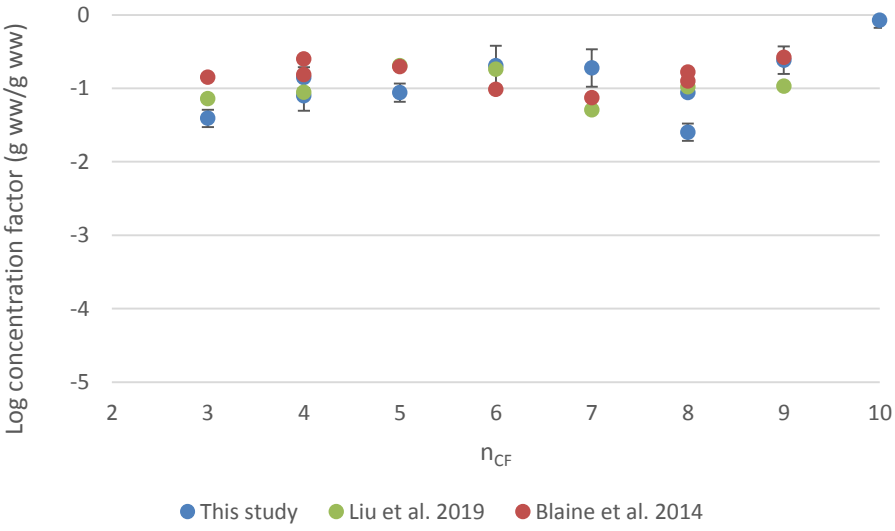


Figure S10: Comparison of radish bulb/shoot concentration factors from this study, Liu et al. (2019) and Blaine et al. (2014). The concentration factors for this study and Blaine et al. are wet weight based, while those for Liu et al. are dry weight based. For this study the mean and standard deviation of the logged concentration factors are shown.

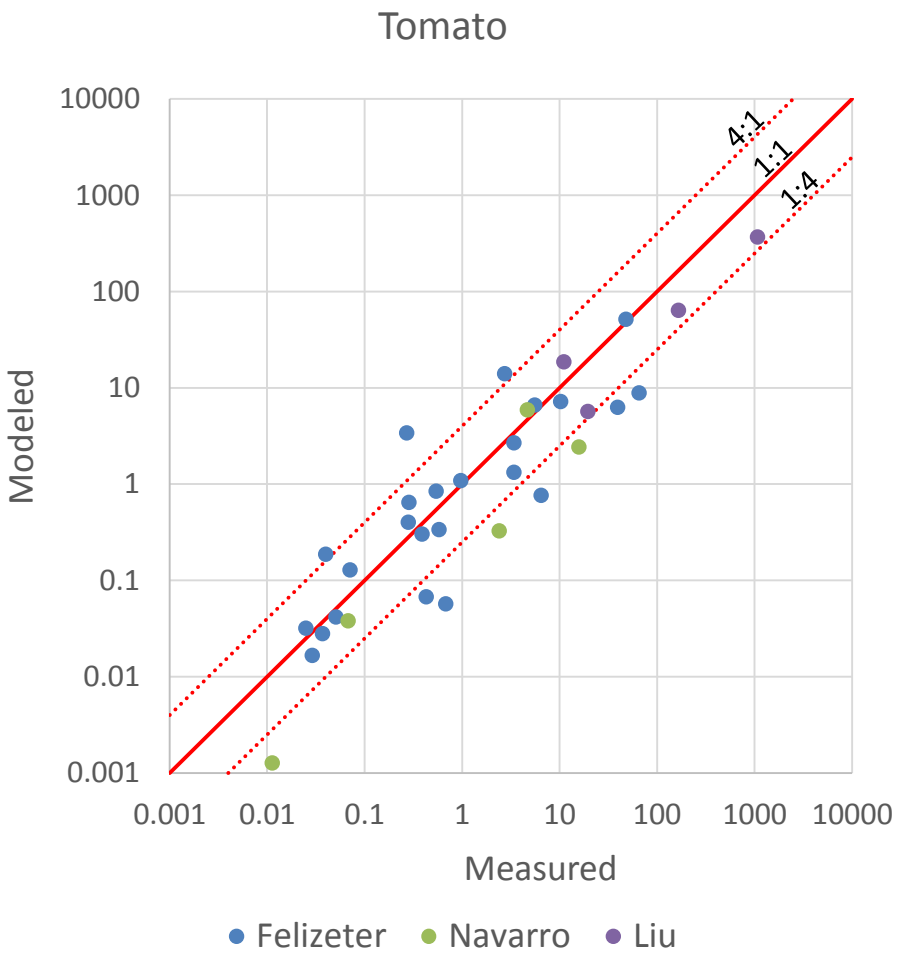


Figure S11: X-Y plots of modeled versus measured PFAA concentration in tomato plants based on data from Felizeter et al. (2014), Navarro et al. (2017) and Liu et al. (2019).

Chapter 7

SYNTHESIS

The work of this thesis contributed to the scientific understanding of the uptake of PFAAs by plants. Uptake rates of a wide range of PFAAs by several plants were calculated for roots and other plant parts. The applicability of the results of hydroponic experiments to field conditions was tested and possible uptake mechanisms discussed. The initial hypothesis that PFAAs are taken up by the plants passively with the water and are distributed with the plant's vascular system driven by transpiration was only partly correct, because already the uptake into the root interior is increasingly inhibited with increasing carbon chain length. However, PFAAs reaching the vascular system of the plants (i.e., the xylem) end up mainly in the leaves of the plants, from where the main water loss in the plants occurs through evaporation. Interestingly, PFAA distribution with the phloem towards the fruits of the plants is much lower than distribution with the xylem flow. This results in much lower PFAA concentrations in the edible parts of fruit bearing crops than in leafy vegetables like cabbage or lettuce. This is also valid for root vegetables (like radish or carrot) and tubers (like potatoe), but for these also sorption of PFAAs to the surface influences the concentration, resulting in higher concentrations of the longer chain compounds in the roots and tubers than in fruits. What vegetables pose the highest risk for human exposure depends on the chain length of the compounds, the amount ingested by the costumer or the fraction of the total diet. The results presented in this thesis show that root vegetables pose the highest risk for human exposure to long chained PFAAs, while leafy vegetables pose the highest risk for human exposure to short and mid-chain PFAAs. Fruits pose a relatively low risk, but they can still contain a considerable amount of short chain PFAAs and can contribute to the human dietary exposure (D'Hollander et al. 2010a; Klenow et al. 2013).

The field experiment, where four crops were grown in spiked soil, generally confirmed the findings of the greenhouse experiments, with the exception that root uptake, i.e., sorption to roots, was much lower than in the greenhouse. Possible reasons are i) the sorption capacity of the roots is influenced by the presence of the soil, perhaps because of the many other solutes originating from the soil that compete for the sorption sites in the root surface tissue, ii) Root exudates that might not have been present in the hydroponic experiment, could lower the sorption capacity by either competition for sorption sites or by a washing-off effect.

Uptake factors for foliage and other vegetative plant parts calculated on the basis of measured pore-water concentrations were, on the other hand, comparable to the uptake factors from the hydroponic experiments, which indicates that in both cases the uptake was governed by the same processes. The PFAAs are transferred from the water to the foliage via uptake across the Casparian strip into the vascular root tissue and transpiration through the roots to the foliage.

The field experiment showed that even though short chain compounds get quickly washed out of the soil column, concentrations in the foliage and above ground edible parts were still highest for the short chain compounds (PFAAs with <C7), while long chain compounds (PFCAs >C11) mainly remain in the soil. Conclusively, contamination events with short chain compounds during the growth phase (e.g., by irrigation with contaminated water or by contaminated fertilizer) will lead to significant levels in plants and groundwater, while contamination events with long chain PFAAs will only affect the roots/bulbs of plants and have no or only little impact on the groundwater.

Overall, the following conclusions can be drawn from the work of this thesis:

- Adsorption of PFAAs to the root surface is the predominant uptake mechanism for plant roots with the exception of short chain PFAAs, where intake into root tissue can be of higher or equal importance.
- Translocation of PFAAs from roots to shoots does not follow existing models describing translocation as a function of hydrophobicity.
- The length of the perfluorinated carbon chain and the associated hydrophobicity of the compound was the variable that influenced plant uptake of PFAAs most.
- In contrast to PFAA accumulation in animals, plants can accumulate considerable amounts of short chain PFAAs in their leaves and fruits.
- PFAA uptake rates into foliage and other vegetative parts of the plants from hydroponic experiments can be compared to field conditions as long as pore-water concentrations are used for the comparison.
- Soil has a large effect on the availability and hence root uptake of PFAAs by plants due to strong sorption of most PFAAs to the soil.
- Relatively low concentrations of short chain PFAAs in soil can lead to high concentrations in leaves, even though these are washed out of the soil quickly.
- Plant uptake via the roots is a viable pathway for PFAAs to enter the human food chain.
- While there are systematic differences between plant species, uptake rates are broadly similar for all tested plant species.

Prior to this work it was believed that the bioaccumulation potential of PFAAs was positively correlated to the length of the fluorinated carbon chain, with higher bioaccumulation with increasing chain length. This assumption was mainly based on studies with fish and some invertebrates. Findings of PFAAs in human blood and breast milk supported this assumption. However, the present work showed that vegetative plant parts accumulate shorter chain PFAAs to a larger extent than longer chain PFAAs, showing that short chain PFAAs are not per se less bioaccumulative and a lesser risk for human exposure. In fact, the field experiment showed that short chain PFAAs pose a higher risk for aquifers, as short chain compounds are washed out of the soil column more quickly and in larger amounts than longer chain compounds, as well as for crop production, as short chain PFAAs are taken up much more than longer chain PFAAs. The rapid leaching however can lead to a cycle of contamination, because farmers may use their own water wells for irrigation. Thus, the compounds that leached into the groundwater will be put on the fields again with the irrigation water.

Furthermore, the toxicological effects of short chain compounds are still relatively unknown, which is reflected in the fact that there are still no tolerable daily intake rates (TDI) for short chain PFAAs. So there is an urgent need for more toxicological data about PFAAs of different chain lengths, especially for short chain PFAAs, to ensure food safety and thus human helath safety. Currently only a tolerable weekly intake rate exists for the sum of PFOA, PFNA, PFHxS and PFOS. For drinking water legislation in the EU is already one step further with a new quality standard of 0.1 µg/L for the sum of 20 PFAS to be implemented in 2026 (EU 2020). Furthermore, a toxic equivalency factor approach has been recently suggested for a mixture of PFAS by Bil et al. (2020). This approach could be used for setting future quality standards in the environment or in food items.

Because PFAA production has shifted towards short chain compounds due to the potentially wrong assumption that they pose a lesser risk to human and animal health, and because filtering techniques in e.g., wastewater treatment plants are less efficient for short chain compounds than for other

PFAAs, there is reason for concern. The amount of short chain PFAAs entering the environment is likely to increase dramatically, as it is also suspected that a larger amount of short chain PFAAs is needed to achieve the same industrial and commercial effects as the longer chained compounds they are replacing.

However, TDIs do not help customers or authorities to decide whether or not food items are of concern for human health. TDIs are related to the person itself and not to the food item. A more helpful way would be maximum residue levels (MRLs) as they exist for example for pesticides. If the MRL is exceeded for a certain food item, then it is not allowed to be sold on the market and thus a (relative) safety for end customers is achieved. This would require that PFAAs are included in regular food monitoring analysis.

Despite these concerns, the actual exposure through plant food is rather low as has been shown amongst others by PERFOOD's European Food Survey in combination with PERFOOD's risk assessment (see Chapter 6). The data on short chain PFAA levels in crops and food items in the peer reviewed literature are rare and show most of the time concentrations below the limit of quantification/detection. This is, however, due to the fact that short chain PFAAs were not included in most of the measurements, or the detection limits were too high. The PERFOOD European food survey was a big step forward in this matter, as short chain compounds were included with low detection limits.

Unless there are accidental spills or field applications with contaminated matter (illegal or legal), the risk of exposure through plants is relatively low as soil concentrations are generally much lower than in the conducted experiments. The legal application of sewage sludge, or so-called biosolids, can lead to uptake in plants and is a pathway for animal and human exposure to PFAS (Bolan et al. 2021). While in many western countries the use of sewage sludge in agriculture is restricted or completely forbidden, in many countries in the world the use of these biosolids is legal and unrestricted and can cause serious contamination of agricultural products as well as general secondary contamination of the environment (Scher et al. 2018). Furthermore, in so-called hot spot areas (e.g.,, near perfluoro-chemical production facilities or firefighting training grounds) PFAA concentrations in the environment can be elevated. In these areas drinking water concentrations should be monitored thoroughly and frequently and crop production should not include root and leafy vegetables, in order to reduce the risk of exposure.

A case of field contamination has occurred in the south-German area of Baden Baden, where approximately 4 mio m^2 of agricultural fields are contaminated with PFAAs. The contamination probably occurred between 2005 and 2008 when compost with high PFAA concentrations was applied to the fields and was only noticed in 2012 when elevated PFAA concentrations were measured in a drinking water plant in that area. Two water supply companies were closed as a result. The compost was partly made from waste from a paper factory, which was likely the source of the PFAA contamination. German media have referred to this as the largest environmental scandal ever and the costs for remediation of the contaminated area are estimated to be around 1 billion euros. Due to the high costs and the large area affected a complete remediation is considered to be impossible (see e.g., http://www.faz.net/aktuell/wissen/medizin-ernaehrung/umweltskandal-woher-kam-das-zeug-bloss-14418841.html in german).

Contamination incidents such as the Baden-Baden case and another one that occurred in the Sauerland region in Germany (Wilhelm et al. 2008), but also legal application of biosolids and leachates from landfills (Eschauzier et al. 2013) demonstrate that it is of the utmost importance to know whether or not crops grown on those contaminated fields pose a risk to human health and for the environment. Because remediation of contaminated fields is very time consuming and costly, the interest in this knowledge is not only of scientific nature. Farmers and authorities need this knowledge to decide if contaminated fields are still fit for agricultural use. A field contaminated with long chain PFAAs could still be fit for agricultural use when only certain crops are grown on it, e.g., leafy or fruit bearing crops. A field contaminated with short chain PFAAs on the other hand could still be fit for agricultural use when only root vegetables are grown on it.

One of the major findings of this thesis is that short chain PFAAs accumulate in plants. Before the start of the PERFOOD project it was believed that short chain PFAAs do not accumulate and that the accumulation potential of PFAAs increases with increasing chain length. The work presented in this thesis, as well as some other recent studies reviewed by Lesmeister et al. (2021), have shown that this assumption does not apply for plants. Unfortunately, this assumption led the manufacturers of PFAAs to switch production towards shorter chain compounds. The possibility of plant accumulation is sadly overlooked by most risk assessment studies and also in the evaluation and authorization of chemicals. The results of this thesis as well as food market studies (Ericson et al. 2008; Herzke et al. 2013; Klenow et al. 2013) show that plant accumulation should be included in regulation and risk assessment by the authorities as it can play an important role in the human exposure as well as in the fate of these chemicals in the environment.

Epilogue

Author Contributions

Chapter 2: UPTAKE OF PERFLUORINATED ALKYL ACIDS BY HYDROPONICALLY GROWN LETTUCE (Lactuca sativa)

Felizeter, S.T.: Conceived the idea and designed the methodology, executed the experiment, collected, analyzed and interpreted the data, led the writing of the manuscript

McLachlan, M.S. : Interpreted the data, assisted in writing and editing

De Voogt, P.: Conceived the idea and designed the methodology, interpreted the data, gave comments and editorial help

Chapter 3: ROOT UPTAKE AND TRANSLOCATION OF PERFLUORINATED ALKYL ACIDS BY THREE HYDROPONICALLY GROWN CROPS

Felizeter, S.T.: Conceived the idea and designed the methodology, executed the experiment, collected, analyzed and interpreted the data, led the writing of the manuscript

McLachlan, M.S. : Interpreted the data, assisted in writing and editing

De Voogt, P.: Conceived the idea and designed the methodology, interpreted the data, gave comments and editorial help

Chapter 4: FATE OF A PERFLUOROALKYL ACID MIXTURE IN AN AGRICULTURAL SOIL STUDIED IN LYSIMETERS

McLachlan, M.S.: Interpreted the data, led the writing of the manuscript

Felizeter, S.: Conceived the idea and designed the methodology, executed the experiment, collected, analyzed and interpreted the data, gave comments

Klein, M.: helped with modelling the data, gave comments

Kotthoff, M.: Gave comments

De Voogt, P.: Conceived the idea and designed the methodology, interpreted the data, gave comments and editorial help

Chapter 5: INFLUENCE OF SOIL ON THE UPTAKE OF PERFLUOROALKYL ACIDS BY LETTUCE: A COMPARISON BETWEEN A HYDROPONIC STUDY AND A FIELD STUDY

Felizeter, S.T.: Conceived the idea and designed the methodology, executed the experiment, collected, analyzed and interpreted the data, led the writing of the manuscript

Jürling, H.: Helped executing the experiment on site

Kotthoff, M.: Gave Comments

De Voogt, P.: Conceived the idea and designed the methodology, interpreted the data, gave comments and editorial help

McLachlan, M.S. : Interpreted the data, assisted in writing and editing

Chapter 6: UPTAKE OF PERFLUORINATED ALKYL ACIDS BY CROPS: RESULTS FROM A FIELD STUDY

Felizeter, S.T.: Conceived the idea and designed the methodology, executed the experiment, collected, analyzed and interpreted the data, led the writing of the manuscript

Jürling, H.: Helped executing the experiment on site

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De Voogt, P.: Conceived the idea and designed the methodology, interpreted the data, gave comments and editorial help

McLachlan, M.S. : Interpreted the data, did the modelling, wrote the modelling part and assisted in writing and editing

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Summary

Perfluorinated alkyl acids (PFAAs) are a group of chemicals that consist of a fully fluorinated carbon chain and a functional group (e.g., carboxylate). While these chemicals have properties that are needed for a lot of commercial and industrial applications, they are also persistent in the environment, bioaccumulative and are suspected to have adverse effects on human and animal health. Human exposure to these chemicals happens mainly via dietary intake, but how these chemicals enter the human food chain is poorly known. Prior to the work in this thesis, it was believed that drinking water and sea food were the main sources for human exposure, but the possibility of vegetal uptake was mainly overlooked. Furthermore, it was assumed that the paradigm "the longer the perfluorinated carbon chain the higher is the bioaccumulation potential" is generally valid for these chemicals.

In the experiments of this thesis the uptake of PFAAs in several crops was studied in two different experimental setups: in the greenhouse with hydroponic solutions and in the field with soil in lysimeters. The crops tested in the greenhouse were lettuce, tomato, zucchini and cabbage, and in the field experiment lettuce, corn, radish and peas were tested. These crops were chosen because of their different edible parts and affiliation to different vegetal family groups (e.g., brassicae, poaceae etc.).
Root concentration factors (RCF, ratio of the concentration in the roots to the concentration in the surrounding medium) were above 1 for all compounds for all plants in the hydroponic experiments with a relatively similar pattern over chain length for all 4 plant species investigated. The work of this thesis showed that root accumulation of PFAAs is mainly influenced by sorption to lipophilic root solids on the root surface. This confirmed the hypothesis that PFAAs behave more like neutral compounds rather than ionic ones, because for ionic compounds adsorption to lipophilic root solids should be very low.

While short chain PFAAs only slightly accumulate in the roots, long chain compounds had root uptake factors of up to 700. A direct correlation between root concentration factor and PFAA chain length was found for tomato, zucchini and cabbage. Lettuce showed a distinctive minimum in RCF for Perfluorohexanoic acid (PFHxA) that was not present for tomato, zucchini and cabbage, indicating that for the short chain compounds Perfluorbutanoic acid (PFBA), Perfluoropentanoic acid (PFPeA) and Perfluorobutane sulfonate (PFBS) actual uptake into the root tissue is dominant over sorption to the root surface for lettuce.

The transfer from roots to the vegetative parts of the plants showed a PFAA chain length relationship that was opposite of that for RCF, with short chain compounds having the highest concentrations in all vegetative plant parts and long chain compounds being present only in negligible concentrations or not detected at all.

Furthermore, transpiration stream concentration factors (TSCF, ratio between concentration in transpiration stream and concentration in external solution) for all compounds except PFAAs >C11 were more similar for tomato, zucchini and cabbage than for lettuce. While all 4 species had similar patterns of the TSCF vs. chain length relationship, the extremes were much more pronounced for lettuce. The existing model for the correlation between TSCF and hydrophobicity parameters by Briggs et al. (Briggs et al. 1982), namely a Gaussian bell shape, did not apply for PFAAs. For lettuce it

was even shown that the PFAAs with the hydrophobicity that according to Briggs et al. should result in the highest TSCFs actually resulted in the lowest TSCFs. For the other three species tested a similar trend was observed, but it was not as pronounced as for lettuce. PFAAs also did not follow an updated model with a sigmoidal shape proposed by Dettenmaier et al (Dettenmaier et al. 2009).

The transfer factors calculated for the different plant parts showed sometimes large differences between the plant species of up to an order of magnitude but were in general similar for all plant species. The long chain PFCAs were translocated much less than all other investigated compounds in all species. The functional group of the compounds did not play a significant role in the uptake, as PFCAs only got slightly better translocated by the plants than the respective homologues of the PFSAs.

The field experiments showed that results from hydroponic studies can be compared to field conditions as long as pore water concentrations are used instead of soil concentrations. The exception here is root concentration factors that were much lower in the field experiment.

Furthermore, it was shown that short chain compounds get washed out of the soil column relatively quickly with precipitation water, while long chain compounds remain sorbed to the soil. Thus, soil contamination with short chain compounds will lead to elevated concentrations in groundwater.

Overall, uptake of PFAAs by plants is a viable route to get in the human food chain, especially for short chain compounds and crops grown in hot spot areas, e.g., near perfluorochemical production sites. These crops should be monitored for their PFAA concentrations before they get on the market.

Samenvatting

Geperfluoreerde alkyl zuren (in het Engels 'perfluorinated alkyl acids', PFAAs) behoren tot een groep van organische chemicaliën waarbij een functionele groep aanwezig is (bijvoorbeeld een carboxylaat) en de koolstofketen verder volledig is gefluoreerd. Deze chemicaliën hebben de eigenschap om vuil-, vet- en water-afstotend te zijn, waardoor ze in veel commerciële en industriële toepassingen worden gebruikt. Deze eigenschappen zorgen er echter ook voor dat deze chemicaliën persistent kunnen zijn in het milieu, bioaccumuleren (ophopen in de voedselketen) en schadelijke effecten kunnen veroorzaken voor mens en milieu. Mensen zijn voornamelijk blootgesteld via voedsel, maar we begrijpen nog niet goed hoe deze chemicaliën in het voedsel terechtkomen. Voordat dit proefschrift was gepubliceerd, was de aanname dat drinkwater en zeevruchten de belangrijkste bronnen zijn voor blootstelling van de mens aan PFAAs. De mogelijkheid van opname via groenten was nog niet of nauwelijks bestudeerd. Ook werd aangenomen dat hoe langer de geperfluoreerde keten is, hoe hoger de mate van bioaccumulatie zal zijn.

In het onderzoek voor dit proefschrift zijn experimenten gedaan met verschillende gewassen. Hierbij is gekeken naar de opname van PFAAs in twee verschillende opstellingen, nl. in kassen met gewassen welke groeiden op een hydrocultuur, en in het veld waarbij gewassen groeiden in grond in zogenaamde lysimeters. In de kassen zijn sla, tomaat, courgettes en kool getest. In het veld sla, mais, radijs en erwten. Deze groenten zijn gekozen vanwege hun verschillen in eetbare delen (denk bijvoorbeeld aan blad of wortel) en vanwege de taxonomische variatie (verschillende families, bv. brassicae, poaceae, enz.).

De zogeheten 'root concentration factors, RCF' (wortel concentratiefactor, de verhouding van de concentratie in de wortel tot de concentratie in het medium, de omgeving) waren groter dan 1 voor alle geteste PFAAs in de hydrocultuurexperimenten. Ook werd bij deze experimenten een vergelijkbaar patroon van opname in de wortels waargenomen voor alle ketenlengtes, bij alle vier de groenten. Dit werk laat zien dat opname van PFAAs in de wortels voornamelijk afhangt van sorptie aan lipofiele vaste deeltjes ('root solids') op het worteloppervlak. Het bevestigt de hypothese dat PFAAs zich meer als neutrale stoffen gedragen, dan als ionische verbindingen, omdat ionische verbindingen maar matig aan de lipofiele vaste deeltjes op de wortel zouden kunnen adsorberen.

PFAAs met lange koolstofketens toonden opnamefactoren tot aan 700, terwijl PFAAs met korte ketens maar een beetje werden opgenomen in de wortels. Een directe correlatie tussen de concentratiefactor en PFAA-ketenlengte werd gevonden voor tomaten, courgette en kool. Bij sla werd een duidelijk minimum gevonden in de concentratiefactor voor perfluorhexaanzuur (perfluorohexanoic acid, PFHxA) terwijl dit niet werd waargenomen bij tomaten, courgettes en kool. Dit geeft aan dat, bij sla, de stoffen met korte ketens (perfluorbutaanzuur, perfluorbutanoic acid, PFBA; perfluorpentaanzuur, perfluoropentanoic acid, PFPeA; en perfluorbutaan sulfonaat, perfluorobutane sulfonate, PFBS) daadwerkelijk vooral opgenomen worden in het wortelweefsel, en minder sorberen aan het worteloppervlak.

In de vegetatieve delen van de plant waren de concentraties van de PFAAs met korte ketens het hoogst, terwijl de concentraties van de PFAAs met een lange keten daarverwaarloosbaar klein of geheel niet detecteerbaar waren. Deze relatie laat precies de omgekeerde trend zien van die welke werd gevonden bij de wortelconcentratiefactor.

Verder is in dit proefschrift gekeken naar de zogeheten 'transpiration stream concentration factors, TSCF' (transpiratie concentratiefactor, verhouding tussen de concentratie in het transpiratievocht van een plant en de concentratie in mediumoplossing, de omgeving). Het bleek dat voor alle stoffen met een ketenlengte korter dan C11 de TSCF vergelijkbaar is voor tomaat, courgette en kool maar minder voor sla. Voor alle vier de groenten zijn overeenkomstige patronen waargenomen tussen de TSCF en ketenlengte, maar de extremen waren groter voor sla. Het door Briggs voorgestelde model voor de relatie tussen de TSCF en hydrofobiciteitsparameters (Briggs et al. 1982, de Gaussische klokvorm) bleek niet toepasbaar op de PFAAs. In de experimenten met sla werd zelfs gedemonstreerd dat de PFAAs die volgens het Briggs et al. model de hoogste TSCF zou hebben, feitelijk de laagste TSCFs hadden. Voor de andere drie groenten werd eenzelfde trend waargenomen, maar niet zo duidelijk als die voor sla. De waargenomen trend in de transpiratie concentratiefactoren van de PFAAs was evenmin te voorspellen met een aangepast model gepubliceerd door Dettenmaier et al. (Dettenmaier et al. 2009, sigmoïdale vorm).

De overdrachtfactoren die werden berekend voor de verschillende plantendelen scheelden soms veel, tot aan een orde grootte, tussen plantensoorten, maar in het algemeen kwamen ze redelijk overeen met elkaar. De geperfluoreerde carbonzuren (PFCAs, 'perfluorinated carboxylic acids') met lange ketens verplaatsten zich veel minder dan de andere stoffen en dit gold voor alle geteste gewassen. De functionele groep van de stoffen bleek geen grote rol te spelen in de opname, want de geperfluoreerde carbonzuren verplaatsen zich maar iets beter dan de homologe perfluorsulfonzuren (PFSAs, 'perfluoro sulfonic acids').

De veldstudie liet vergelijkbare resultaten zien met de hydrocultuurexperimenten, zolang de poriewaterconcentraties gebruikt werden voor de berekeningen, in plaats van de bodemconcentraties. De wortel concentratiefactoren van de veldexperimenten waren wel veel lager dan die van de hydrocultuurexperimenten.

Verder bleek dat de stoffen met een korte ketenlengte relatief sneller uit de bodemkolom spoelen met neerslag dan die met een lange ketenlengte, deze laatste blijven sterker gesorbeerd aan de bodem. Hierdoor zal bodemverontreiniging met PFAAs leiden tot verhoogde grondwaterconcentraties voor de verbindingen met korte ketens.

De opname van PFAAs door planten blijkt een aannemelijke route waarlangs deze stoffen in de menselijke voedselketen terechtkomen, vooral voor verbindingen met korte ketens en voor gewassen die worden geteeld in hotspotgebieden, b.v. in de buurt van perfluorochemische productielocaties. Deze gewassen moeten worden gecontroleerd op hun PFAA-concentraties voordat ze op de markt komen.

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