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Toxicokinetics of Seven Perfluoroalkyl Sulfonic and Carboxylic Acids in Pigs Fed a Contaminated Diet

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Supporting Information

ABSTRACT: The transfer of a mixture of perfluoroalkyl acids (PFAAs) from contaminated feed into the edible tissues of 24 fattening pigs was investigated. Four perfluoroalkyl sulfonic (PFSAs) and three perfluoroalkyl carboxylic acids (PFCAs) were quantifiable in feed, plasma, edible tissues, and urine. As percentages of unexcreted PFAA, the substances accumulated in plasma (up to 51%), fat, and muscle tissues (collectively, meat 40–49%), liver (under 7%), and kidney (under 2%) for most substances. An exception was perfluorooctanesulfonic acid (PFOS), with lower affinity for plasma (23%) and higher for liver (35%). A toxicokinetic model is developed to quantify the absorption, distribution, and excretion of PFAAs and to calculate elimination half-lives. Perfluorohexanoic acid (PFHxA), a PFCA, had the shortest half-life at 4.1 days. PFSAs are eliminated more slowly (e.g., half-life of 634 days for PFOS). PFAAs in pigs exhibit longer elimination half-lives than in most organisms reported in the literature, but still shorter than in humans.

KEYWORDS: *pharmacokinetics, persistent organic pollutants, risk assessment, carry-over experiment, Sus scrofa domesticus*

INTRODUCTION

The transfer of a series of perfluoroalkyl acids (PFAAs)[1] from feed to tissues was studied in fattening pigs (*Sus scrofa domesticus* of the German Landrace breed). PFAAs are perfluoroalkyl substances (PFAS) with a wide range of unique properties and applications.[2] They are very useful in industry as surface active agents.[3] Unfortunately, they are also persistent in the environment, with bioaccumulative as well as toxic properties (PBT). Because of their high chemical stability and solubility in environmental media, PFAAs have the capacity to undergo long-range transport. PFAAs can accumulate in living organisms,[4] including humans. The toxic effects[5,6] of PFAAs can be exemplified by perfluorooctanoic acid (PFOA). An elevated human exposure study after a contamination event in the Mid-Ohio Valley communities has linked PFOA to hypercholesterolemia, ulcerative colitis, thyroid disease, pre-eclampsia, and kidney and testicular cancers.[7] The eight-carbon PFOA and perfluorooctanesulfonic acid (PFOS) are the best toxicologically researched PFAAs, with an established tolerable daily intake (TDI) by the European Food Safety Authority (EFSA).[8] For the rest of the PFAAs, no TDI has yet been established.[9] For the aforementioned reasons, and because of its historical industrial significance, PFOS has become restricted under the Stockholm Convention on Persistent Organic Pollutants. The restriction of other PFAS is under discussion. PFAAs are the most relevant perfluorinated substances for kinetic transfer studies, because PFAAs are considered terminal products of the (bio)transformation of perfluorinated precursors,[10−12] such as polyfluoroalkyl-diester phosphate surfactants (DiPAPs).[13] Rats have been shown to biotransform DiPAPs to PFAAs.[11] The importance of PFAA precursors for human exposure is only now becoming apparent.[4,12,14] Humans become exposed to PFAAs through several routes, such as dust inhalation, drinking water,[15] and food consumption,[16] including breast milk,[17] fish, seafood, and meat and meat products.[9,16] PFAAs are known to biomagnify in the food chain, becoming absorbed and concentrated in plant[18] tissues, animal[1,19] tissues, eggs[20] and milk.[21−24] Livestock become exposed through drinking water[24] and animal feed.[18] In turn, plants used as feed have been shown to take up PFAAs from the soil and accumulate it in vegetative and storage organs.[18] Pork is thus a potential route of exposure for humans. Whereas the biomagnification and elimination half-lives of PFAAs have been more extensively studied in laboratory animals[1] and humans,[14,17,25] the knowledge gaps for farm animals are only now attempted to be closed by this study on pigs and similar ones in ruminants[21,22,24] and pigs.[19] In this feed-to-food contaminant carry-over experiment and subsequent toxicokinetic modeling on pigs, we survey the behavior of seven PFSAs and PFCAs regarding the animals' absorption, tissue distribution, and excretion. We present and discuss measurements on edible tissues and excretions of fattening pigs fed a contaminated diet of known composition. On the basis of

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those measurements, we propose a toxicokinetic model for the absorption, distribution, and excretion of seven PFAAs in pigs. The behavior of PFAAs in pigs is also of interest because pigs are touted a biomedical model[26] of human physiology. Additionally, understanding the toxicokinetics in domestic pigs may help relate the body burden of wild boars (a related subspecies) to the environmental ubiquity of PFAAs. Our main focus is understanding PFAA kinetics from feed to porcine livestock and food products for consumer risk assessment and protection.

MATERIALS AND METHODS

Chemicals. Native and ^{13}C-labeled PFAAs were purchased from Wellington Laboratories, Guelph, ON, Canada. Methanol absolute, acetonitrile, formic acid (99%), and ammonium acetate (all ULC-MS) were purchased from Biosolve, Valkenswaard, The Netherlands. Sodium acetate anhydrous (p.a.), potassium hydroxide (p.a.), ammonia (25% p.a.), and acetic acid (100% p.a.) were obtained from Merck, Darmstadt, Germany. Pepsine (from porcine gastric mucosa), Lipase Type VII (L 1754), and Protease Type XIV (P 5147) were purchased from Sigma-Aldrich, Steinheim, Germany. Glycerol (99%) was obtained from ACROS Organics, Geel, Belgium. Water was double distilled using distillation unit 2001/2 of the Gesellschaft für Lebensmittel-Forschung.

Animals, Housing, and Feeding. Three groups of fattening pigs (German Landrace breed) consisting of 10 gilts (female pigs), 10 barrows (castrated male pigs), and 10 young boars (uncastrated prepubescent male pigs) were purchased from a commercial breeding farm (Agrargenossenschaft Groß Machnow, Germany) and transported to the experimental station of the Federal Institute for Risk Assessment (BfR). The animal experiment was authorized by the Landesamt für Gesundheit and Soziales in Berlin with approval G0043/10, complying with the German Animal Welfare Act (Tierschutzgesetz) and supervised by the BfR institutional animal welfare officer. The fattening pigs with initial body weight (BW) of 83 ± 12 kg (mean ± sample standard deviation) were separately housed in pens of eight experimental animals and two control animals with free access to water and restrictive feeding (2 kg/day and animal). Room temperature was 21 °C, and humidity was kept constant at approximately 68% throughout the entire feeding study. Health status of the fattening pigs was checked daily. The BW of each animal was recorded once a week. Upon arrival, all fattening pigs received a commercial cereal-based nonpelleted feed for 14 days, which was gradually exchanged during the first 7 days for the pelleted control diet with approximately 17% hay (adaption period). After the adaption period, fattening pigs were fed either PFAA-contaminated feed (3 × 8 experimental animals) or PFAA-free feed (3 × 2 control animals) for 21 days. Discussions refer to experimental animals, except where control animals are explicitly mentioned.

Contaminated Feed Cultivation and Preparation. PFAA-contaminated hay and barley were cultivated and harvested from a PFAA-contaminated region in Lower Saxony, Germany. The agricultural patch was polluted from the use of a soil improver provided by a recycling company that laced it with industrial waste containing high concentrations of PFAS.[27] To counteract an uneven contaminant distribution in the soil, the final feed was homogenized. PFAA-contaminated barley and hay used for the PFAA feed were determined to be substantially equivalent to PFAA-free varieties of barley and hay with regard to nutritional value. The control feed and the PFAA feed fulfilled the recommendations for nutrient and energy requirements of fattening pigs of the Society of Nutrition Physiology (GfE).[28] Nutrition data are shown in the Supporting Information (Table S1).

Sampling of Feed, Tissues, and Excretions. For PFAA analysis in feed, pooled samples were taken to obtain five representative feed samples for each week (2 weeks of adaption, 3 weeks of experimental feeding). Samples of blood plasma were taken of each experimental animal toward the end of the adaption period (day −4), twice a week during PFAA feeding (days 4, 8, 11, 15, and 18), and at the day of slaughter (22). Blood from control animals was taken at the end of the adaption period as well as at the day of slaughter. The blood was collected by puncturing the *vena jugularis* in a lithium−heparin monovette and centrifuged to obtain the plasma, pipetted off, and stored frozen (−20 °C) until analysis in PFAA-free polypropylene vessels. Urine was collected with varying regularity during feeding, weighing, or blood sampling. After the 21 days of the feeding period, the pigs had an average BW of 103 ± 11 kg and were fasted for 20 h before being slaughtered at the BfR slaughterhouse according to approved methods. Pigs were immediately killed by blood removal after stunning. To prevent cross-contamination between control and experimental animals, controls were slaughtered first. Liver and kidneys were carefully removed, weighed, and individually frozen in PFAA-free bags at −20 °C. The bladder was removed, and the urine it contained was emptied into polypropylene vessels. Samples of muscle tissue from the *musculus longissimus dorsi* (further termed dorsal muscle tissue), *musculus obliquus externus/internus abdominalis*, and *transversus abdominis* (further termed ventral muscle tissue) and the fat tissue covering the dorsal muscle tissue were frozen at −20 °C. Subsequent sample preparation and analysis were performed by the Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL). To avoid interferences between PFAA and biomolecules, matrix-specific sample preparations were performed. Plasma and urine was treated with half-concentrated formic acid.[29] Feed samples were extracted with methanol, and an aliquot was diluted with water (VDLUFA-Method). Liver, kidney, and muscle tissue meat samples were hydrolyzed with pepsine.[30] Sample solutions were purified and concentrated with solid phase extraction on an OasisWAX column[31] (60 mg/3 mL).

Analytical Determination. A total of 12 compounds was analyzed using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). These included the following seven PFCAs, perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA); and the following five PFSAs, perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS), PFOS, and perfluorodecanesulfonic acid (PFDS). The limit of detection (LOD) was 0.2 μg/kg for solid samples and 0.2 μg/L for liquid samples for all PFAAs except PFDS, with an LOD of 0.5 μg/kg for solid samples and 0.5 μg/L for liquid samples. It was not possible to identify perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPA) unequivocally because both substances show only one mass transition in multiple reaction monitoring mode (MRM) during HPLC-MS/MS analysis. Due to this fact, the substances were withdrawn from the substance spectra. PFPS, PFNS, PFUnS, and PFDoS were also excluded because standard substances were not commercially available. See Table S2 in the Supporting Information for a complete list of relevant substances for this study.

Analytical Measurement. The purified solutions were measured using HPLC-MS/MS, run in negative ion MRM mode. The separation was performed on an Agilent 1200 SL HPLC system. MS/MS detection was performed with an Agilent 6460 triple-quadrupole mass spectrometer equipped with an electrospray interface (ESI) operating in the negative ion mode. The MRM settings are published elsewhere.[32] The relative standard deviations were around 20% for concentrations near the limit of quantification (LOQ) and 10−15% for higher concentrations.

Quantification and Isomer Separation. Quantification was performed with isotope-labeled standards and a seven-point calibration curve. The LOD was defined as the signal-to-noise ratio of 3:1 of the qualifier ion. The LOQ is defined as the concentration at which a substance is identified unequivocally and quantified with a relative standard deviation of 20% or lower. In the case of PFOS, it could be ensured that only the linear isomer was quantified. Due to the fact that standard substances of branched PFHxA, PFHpA, PFBS, PFHpS, and PFHxS were not commercially available, it was not possible to ascertain that all of the branched substances are separated completely

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from the linear ones. Only peaks with the same retention time as the respective linear substances were integrated. The ratios of the two most intense MRM transitions of the integrated PFHpS and PFHxS are the same as in the linear standard substance. Due to the finding that the transitions of the branched isomers differ from the transitions of the linear substances in the case of PFOS, this is an indication that the uncertainty caused by the branched isomers is small for PFHpS and PFHxS. The PFCA concentrations in the analyzed matrices were very low, so that a differentiation between the linear and the branched substances was not possible. It is described in the literature that 78% of the PFOA manufactured by the 3M Co. using electrochemical fluorination is linear.[33] The ratios of the two most intense MRM transitions of the integrated PFCA were the same as in the linear standard substances. Thus, it can be assumed that also in the case of PFCAs, the potential uncertainty caused by the branched isomers is small. The analytical method is described in more detail in the Supporting Information and by Ehlers.[30]

Body Weight of Pigs as a Function of Time. The pigs were weighed two times during the 14 day adaptation period, three times during the 21 day PFAA-contaminated diet experiment, and once more before being slaughtered on day 22 for a total of six BW measurements $M_k^{\text{exptl}}(t)$ per pig. To interpolate between those measurements, the BW function

$$M_k(t) = \omega_{\text{A},k} + \omega_{\text{B},k}(t+\theta)^{\omega_{\text{C},k}} \quad (1)$$

was fitted to the experimental weights $M_k^{\text{exptl}}(t)$ using the nonlinear least-squares Levenberg–Marquardt algorithm[34] with fitted constants ω. Equation 1 is the empirical Janoschek growth equation,[35,36] valid only for the short experimental period of 35 days. Note that other growth equations should be used for longer periods of fattening time. The offset $\theta = 14$ days represents the adaptation period, so that $t = 1, 2, 3, .., 21$ days represent the experimental period in which PFAA-contaminated feed was fed. See Figure S1 and Table S3 in the Supporting Information for the three fitted ω constants for each individual pig (k).

Estimation of the Mass of Tissues. Several sources of data[37−40] were compounded to provide a consistent picture of the weight of each part of the pig (blood, edible and inedible tissues). Blood was estimated as $p_B = 6.5\%$ of BW.[40] Hematocrit (proportion of red blood cells and other cells to plasma) was taken[40] as 39%. From these two values, we calculate the mass of blood plasma (P) as $m_{\text{P},k}(t) = 0.03965 M_k(t)$. The carcass is composed of edible parts of a slaughtered pig excluding blood, kidney, liver, and others deemed unfit for human consumption in some cultures. The weight of the carcass of pig k is then estimated as $(1 - p_B)M_k(t) - m_{\text{L},k} - m_{\text{K},k}$, where $m_{\text{L},k}$ is the measured weight of liver (L) and $m_{\text{K},k}$ is the measured weight of kidney (K). The amount of muscle tissue and fat tissue is calculated as a fraction of the weight of the carcass. From Table 6.2 of ref 37 we use the estimates valid for a pig between 90 and 100 kg, which indicate 62.6% of carcass is muscle tissue and 18.9% of carcass is fat tissue (F). Because we separately analyzed the PFAA content for samples of dorsal and ventral muscle tissues, we further differentiate between them. From Figure 6.1 of ref 37 we assign 51.5% of muscles as dorsal muscle tissues (D: chop, filet, and ham) and 48.5% of muscles as ventral muscle tissues (V: belly, jowl, blade shoulder, blade, knuckle, and dewlap). The mass of each tissue is given by

$$m_{X,k}(t) = p_X[(1 - p_B)M_k - m_{\text{L},k} - m_{\text{K},k}], \text{ with } X = \text{F, D, V} \quad (2)$$

and constants $p_F = 0.189$, $p_D = 0.626 \times 0.515$, and $p_V = 0.626 \times 0.485$. Other parts of the carcass not analyzed in this study represent 18.6% of its weight (bones, tendons, and skin, Table 6.2 of ref 37). Other parts of the body excluded from the carcass, such as the head, are neglected.

Urine Output. A pig urinates[39] between 5 and 30 mL/kg BW. The total urine output as a function of time is between $m_{\text{U},k}(t) = 0.005 \times M_k(t)$ and $0.030 \times M_k(t)$ in L/day. We are forced to estimate the urine output per pig because it was not practicable to quantify the liquid volume per day in the experiment. The urine concentration $C_{\text{U},j,k}^{\text{exptl}}(t)$ of each PFAA substance j in pig k was however experimentally determined every few days.

Mathematical Model of Toxicokinetics. We propose a two-compartment, first-order toxicokinetic model of the pig for each of the seven PFAA substances. The blood plasma represents the model's central compartment (compartment 1). The edible tissues taken together represent compartment 2 (Figure 1). To simplify notation,

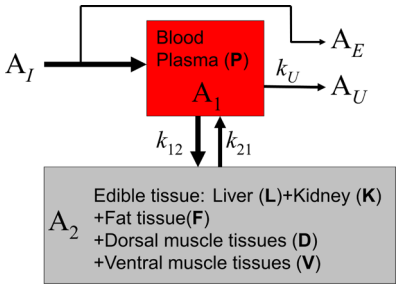

Figure 1. Graphical representation of the two-compartment model. The amount of PFAA in feed is designated as the intake A_I. The amount of PFAA in the central compartment is A_1, whereas the amount in edible tissue is designated A_2. The kinetic constants k_{12} and k_{21} quantify the speed of exchange. A_E is the amount found in feces, considered a fraction f_E of intake A_I, which bypasses A_1 unabsorbed. A_U is the amount excreted in urine, and k_U is the urinal elimination constant. Such a model is fitted independently for each substance and pig.

we will in most cases omit the subscripts for substance (j) and pig (k). The dynamic behavior of the amount of substance present A_1 and A_2 is described by two coupled ordinary differential equations:

$$\frac{dA_1(t)}{dt} = A_1(t) - A_E(t) - k_{12}A_1(t) + k_{21}A_2(t) - k_U A_1(t) \quad (3a)$$

$$\frac{dA_2(t)}{dt} = k_{12}A_1(t) - k_{21}A_2(t) \quad (3b)$$

The kinetic constants k_{12} and k_{21} quantify the speed of substance exchange between compartments 1 and 2, whereas k_u is the urinal excretion rate constant for each substance. These three kinetic constants are the fitted model parameters. $A_E(t)$ is the amount excreted in feces as a function of time t. $A_1(t)$ is the PFAA intake per unit of time or, in other words, the amount ingested by a pig, calculated from measurements

$$A_1(t) = C_I^{\text{exptl}}(t) m_I^{\text{exptl}}(t) \quad (4)$$

where $C_I^{\text{exptl}}(t)$ is the experimentally analyzed feed concentration of PFAA. The feed concentration was held nearly constant for each substance in the carry-over experiment. The second term, $m_I^{\text{exptl}}(t)$, is the average amount of feed intake per day for each pig. To provide estimates for the model parameters k_{12},k_{21}, and k_u, we integrate a form of eqs 3 to best fit the experimentally observed A_1^{exptl} and A_2^{exptl} as described under Numerical Method To Fit the Model to Experiment. Time series of seven data points were experimentally measured for the concentration of all seven PFAAs in blood plasma (P), from which we calculate the amount of each substance in compartment 1

$$A_1^{\text{exptl}}(t) = C_P^{\text{exptl}}(t) m_P(t) \quad (5)$$

where $C_P^{\text{exptl}}(t)$ is the experimental plasma concentration and $m_P(t)$ is the mass of the blood plasma. Because only one data point per pig and substance was measured after slaughter for each edible tissue, we group liver (L), kidney (K), fat (F), dorsal muscle tissues (D), and ventral muscle tissues (V) into the peripheral or edible tissue compartment (compartment 2). The total amount of PFAA in edible tissue compartment 2 at slaughter date $t_{\text{end}} = 22$ days is

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$$A_2^{\text{exptl}}(t_{\text{end}}) = \sum_{X=\text{L,K,F,D,V}} C_X^{\text{exptl}}(t_{\text{end}}) m_X(t_{\text{end}}) \quad (6)$$

The amount in edible tissues at the beginning of the experiment t_0 = 0 days is confirmed experimentally to be negligible, $A_2^{\text{exptl}}(t_0) = 0$. The amount excreted in feces is assumed proportional to the amount ingested, so $A_E(t) = f_E A_I(t)$, where the constant f_E for each substance is obtained from previously published experimental data for sheep[22] (Table 1). This simply means that the amount excreted in feces is estimated as an unabsorbed proportion (outer arrow A_E in Figure 1) of the ingested amount. The assumption was made that pigs behave like sheep just in this respect. The fractions in Table 1 are small, so they do not affect the mass balance greatly, but are used for completeness. Furthermore, the amount excreted in urine is a result of the model through the fitted k_U. We assume that there is no metabolic interaction between the substances, so that the models for each of the seven PFAAs are independent from each other. See Figure 1 for a graphical representation of the model.

Table 1. Fractions of Ingested PFAA Deemed Unabsorbed and Excreted in Feces of Sheep from Data in Reference 22 and Table 63 of Reference 30[a]

substance	fraction excreted in feces f_E (%)	substance	fraction excreted in feces f_E (%)
PFHpS	0.65	PFBS	4.30
PFHxS	1.35	PFHxA	4.35
PFHpA	3.20	PFOS	8.45
PFOA	3.95		

[a]These data were used to estimate the feces contribution in the mass balance for pigs of Figure 2.

Excretion in Urine. For each pig, the PFAA concentration of urine $C_U^{\text{exptl}}(t)$ was analyzed for up to four samples spread over the total 22 days. On average, 2.5 urine samples per pig were measured, plus 1 sample after slaughter (from bladder contents). These data were put on equal footing among pigs by performing a least-squares linear fit of the urinal PFAA concentration of the form $C_U^{\text{exptl}}(t) = \beta\, t$. This functional form was chosen by inspecting the apparent linear rise of the experimental blood plasma concentration $A_1^{\text{exptl}}(t)$ for most substances and pigs (Figure 3). Consistent with a linear rise in plasma concentration is a linear rise in urine concentration $C_U(t)$ for first-order kinetics, disregarding the variation in liquid amount. Using the linear fit for $C_U^{\text{exptl}}(t)$ above, the total cumulative urine excretion from experiment is integrated as

$$A_U^{\text{exptl, total}} = \int C_U^{\text{exptl}}(t) m_U(t)\, dt \quad (7)$$

Likewise, the cumulative amount excreted in urine for the model is obtained by integrating

$$A_U^{\text{total}} = \int k_U A_1(t)\, dt \quad (8)$$

Excretion via Feces. The PFAA amounts excreted in feces were not analyzed, because they were presumed to be relatively small with respect to the total cumulative PFAA dose. This assumption is confirmed by the mass balance, because most of the PFAA dose is accounted for by tissues and urine without resorting to the feces (Figure 2). It has been shown experimentally that anionic salts of PFOA and PFOS are secreted by the bile into the intestinal lumen.[41] It is hypothesized that PFOA and PFOS are recycled within enterohepatic circulation,[42] so that material already absorbed is not excreted into feces. Because we did not measure the PFAA content of feces but nevertheless wanted to have an estimate of the amounts excreted, we resorted to extrapolating the results obtained from measurements in sheep feces (ref 22 and Table 63 of ref 30). From the cumulative PFAA amounts that were ingested by sheep, we calculate the fractions of PFAA that remained unabsorbed and were excreted (Table 1).

Normalization of Mass Balance Equations. The mass balance performed using the pig physiological parameters, measured weights, and experimental PFAA concentration data is close to an average of 100% (Figure 2) of the cumulative ingested PFAA, calculated as the total amount of substance j ingested by pig k in the 22 days of the experiment. As suggested in ref 43, we opt for normalizing the data, so that the sum of accumulated and excreted PFAA mass becomes exactly

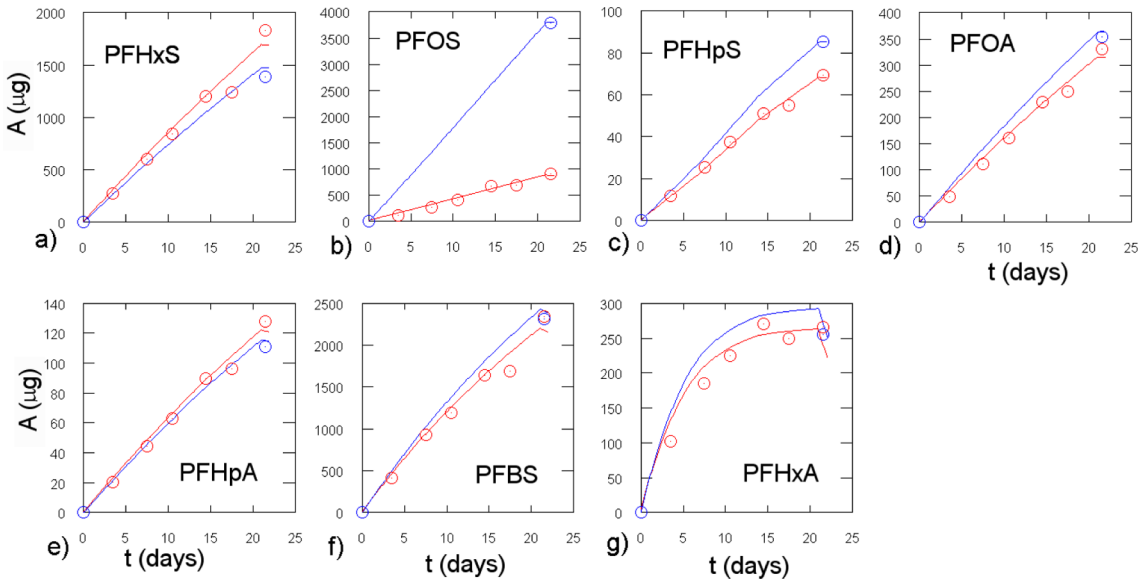

Figure 3. Dynamic behavior of the amount A of seven PFAA substances in an exemplary pig (gilt 8, one of 24 pigs, chosen for its similarity to the mean behavior). Circles correspond to experimental data (mediated by the pig physiological and growth estimates), and lines are the model solution of eq 10 using best-fit kinetic parameters. The substances are ordered from slowest (a) to fastest (g) urinary excretion k_U. The color red stands for the amount A_1 in blood plasma or central compartment. The color blue stands for the amount A_2 in the edible tissue compartment. Only the data for PFHxA suggest approach to steady state between days 1 and 21. Chart b stands out because most of the PFOS partitioned to edible tissue, in contrast to the rest of the PFAAs (panels a and c–g). On day 22, animals were fasted before slaughter (so the curves on that day show only excretion as a downward trend).

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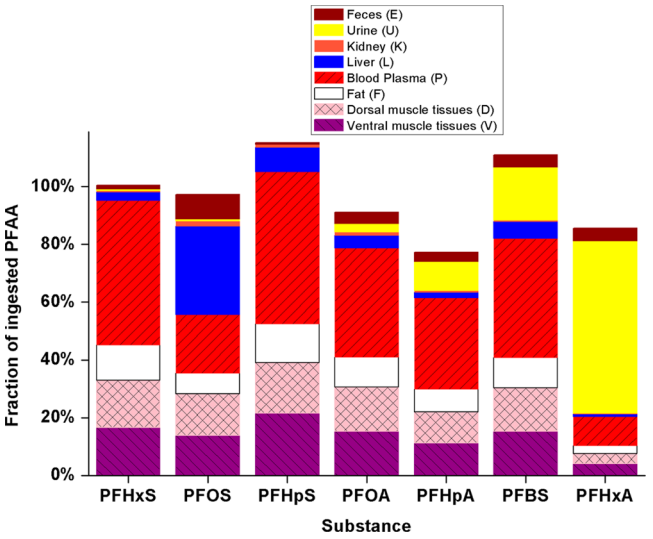

Figure 2. Mass balance for the seven PFAAs shows the ratio of substance in each tissue and excretion to the total cumulative PFAA ingested in feed. The substances are ordered from lowest percentage excreted (left) to highest percentage excreted in 21 days (right). By comparing the height of each bar, one gains insight into the relative affinity of each substance for the given matrices. The mass balance shown accounts on average for 97% of the PFAAs ingested and has not been normalized by eq 9.

equal to 100% of the total ingested and absorbed PFAA for each substance and each pig individually. The normalization factor for the mass balance equations is the ratio of PFAA found in tissues and excretions to PFAA ingested from feed, so that

$$\alpha = \frac{A_1^{\text{exptl}}(t_{\text{end}}) + A_2^{\text{exptl}}(t_{\text{end}}) + A_U^{\text{exptl, total}}}{\sum_{t=1}^{22} A_I^{\text{exptl}}}\left(\frac{1}{1-f_E}\right) \quad (9)$$

The normalization is performed by multiplying the first two terms of the right-hand side of eq 3a with the factor α in eq 9 and recognizing that $A_E(t) = f_E A_I(t)$. After rearranging, we obtain the set of differential equations

$$\frac{dA_1(t)}{dt} = \alpha(1-f_E)A_1(t) - k_{12}A_1(t) + k_{21}A_2(t) - k_U A_1(t) \quad (10a)$$

$$\frac{dA_2(t)}{dt} = k_{12}A_1(t) + k_{21}A_2(t) \quad (10b)$$

We thus fit the normalized model eqs 10 to self-consistent, mass-balanced data. Because of this normalization, the amount excreted in feces A_E (estimated from sheep data) is only relevant for the mass balance of Figure 2, but the terms A_E and f_E disappear from eqs 10 and do not have an effect on the fitted model constants k_{12}, k_{21}, and k_U for the pig. Physiologically this is due to the fact that A_E is an unabsorbed substance which bypasses distribution into the animal's tissues (Figure 1).

Numerical Method To Fit the Model to Experiment. To provide estimates for the model parameters k_{12},k_{21}, and k_u for each substance (j) and pig (k), we perform numerical integration of eqs 10 to best fit the experimentally observed A_1^{exptl} (7 data points, eq 5) and A_2^{exptl} (2 data points, eq 6). In some but not all cases, measurements for the PFAA concentrations in urine were available. In such cases, the best fit is performed as described before and simultaneously integrating eq 8 for the model A_U^{total} and fitting to $A_U^{\text{exptl, total}}$ (1 data point, eq 7). We are thus using 3 model fit parameters and a total of 9 (and in some cases 10) experimental data points. We have chosen to perform fixed effects modeling, meaning that we treat each pig and each substance separately. With this approach, we guarantee a best fit for each case and explore the intersubject variability a posteriori. An alternative approach not followed would have been to fit all pigs at the same time with one random effects model per substance, in a population pharmacokinetics fashion.[44] The MATLAB-compatible program code was written and run in the freely available GNU Octave 3.2.4 language. The dynamic behavior of the amount of substance in the compartments, eqs 10, is integrated using the explicit Runge–Kutta method of order (4,5) in Octave's ode45 algorithm. For the relevant cases, the cumulative amounts excreted in urine in experiment, eq 7, and in model, eq 8, are integrated using the trapezoidal rule. The feed intake $A_I(t)$ is calculated using eq 4. The best fit is performed by nonlinear least-squares minimization of the difference between model and experimental results using the Levenberg–Marquardt algorithm.[34]

Elimination Half-Life. From the fitted model constants, we may calculate the elimination half-life

$$\tau_{1/2} = \frac{\ln(2)}{k_U}(1 + k_{12}/k_{21}) \quad (11)$$

This equation is valid for a two-compartment model with fast equilibrium between compartments. The elimination half-life is thus valid for both compartments, blood plasma and edible tissue.

RESULTS AND DISCUSSION

Twelve PFAAs were analyzed[30] in every sample. Among those 12, 7 substances were detected well above the LOD of 0.2 μg/kg in feed and found in samples of most tissues, blood plasma, and urine: PFHxA, PFHpA, PFOA, PFBS, PFHxS, PFHpS, and PFOS. We present full results for these seven substances, as they were consistently found in amounts that allow for numerical analysis. A special case is PFNA, found in very small amounts in most plasma, liver, and kidney samples, but below the LOD of 0.2 μg/kg in feed and other tissues. Four further substances were below the LOD in all matrices except for the liver: PFDA, PFUnA, PFDoA (LOD = 0.2 μg/kg), and PFDS (LOD = 0.5 μg/kg).[30] The above five substances will not be further discussed, as the data preclude numerical analysis. See Table S2 in the Supporting Information for a complete list of relevant substances.

PFAA Concentration in Contaminated Feed. The mean concentration of each substance $\overline{C_I^{\text{exptl}}}$ in the PFAA-contaminated feed is listed in Table 2. The measured concentrations of PFAA varied slightly because of separate batch preparation for each of the three pig genders and for each of the 3 weeks (reflected as standard deviation in Table 2). These details are given in Table S4 of the Supporting

Table 2. Mean Concentration of Each PFAA $\overline{C_I^{\text{exptl}}}$ in Contaminated Intake (I) Feed on a Dry Basis (Arithmetic Mean ± Standard Deviation)[a]

substance	$\overline{C_I^{\text{exptl}}}$ (μg/kg)
PFOS	137 ± 13
PFBS	132 ± 11
PFHxS	91.3 ± 8.0
PFHxA	47.8 ± 4.4
PFOA	22.4 ± 2.6
PFHpA	10.2 ± 1.7
PFHpS	3.99 ± 0.50

[a]Averaging was performed for days 1–21 of the experiment and all 24 pigs. The LOD was 0.2 μg/kg. For the slightly variable individual measured values for each feed preparation by gender and week, see Table S4 in the Supporting Information.

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Inormation. The control feed was verified to have a PFAA concentration below the LOD.[30]

PFAA Partitioning to Blood and Edible Tissues. PFAAs distributed into all studied tissues of the pigs, blood plasma, muscle tissues, fat tissue, liver, and kidney (Figure 2), in line with the results in other species.[1] The mass balance of the PFAAs found in blood plasma and edible tissues accounts on average for 97% of the PFAA mass in the feed. The remaining 3% may be attributed to measurement errors and/or partitioning into tissues that were not measured, such as the brain and gastrointestinal tract. For most investigated substances (PFHxS, PFHpS, PFOA, PFHpA, PFBS, and PFHxA), blood plasma became the largest reservoir of unexcreted PFAAs by amount in the body of pigs. PFAAs are known to concentrate in the blood circulation of mammalian organisms, where they bind to proteins such as serum albumin.[45,46] The results show that PFAAs have an even higher affinity for blood plasma relative to other tissues in pigs than in cows and sheep.[21,22] PFAAs are not alone among persistent pollutants in their ability to bind to blood proteins. A study in pigs found that hydroxylated PCBs also accumulate in their blood.[47] This ability to strongly bind to blood proteins and the low clearance and slow excretion in the urine were recently proposed as the best predictors for a chemical's bioaccumulation potential and long half-life.[48] All three factors are present simultaneously in PFAAs, especially in long-chain PFSAs for pigs. After blood plasma, the next most important reservoirs for PFAAs were dorsal muscle, ventral muscle, and fat tissues. Considered together as meat, these three tissues account for between 40% (PFOS) and 49% (PFOA) of unexcreted, accumulated PFAAs. Note that the percentages in Figure 2 refer to both PFAA unexcreted and excreted during the time of the experiment. In contrast to exclusively lipophilic contaminants such as PCDD/Fs,[35] all seven PFAAs were found in similar amounts and concentrations in both fat as well as muscle tissues, in line with findings of PFAA in fat of mice[1] and rats.[49] This supports pooling of the results for muscle tissue and fat as "meat". Likewise, it contradicts the notion that PFAAs are always lipophobic and thus should be absent from fat tissue. This notion may stem from confusion between the (strongly lipophobic) perfluoroalkyl group, a protonated PFAA and its deprotonated version. A study using ion transfer cyclic voltammetry found deprotonated PFSAs and PFCAs to be around 100 times more lipophilic that their nonfluorinated alkyl counterparts,[50] for example, regular fatty acids. At physiological pH, PFSAs and PFCAs are predominantly deprotonated, lending credibility to our findings in fat tissue. Accumulated PFAA in the kidney accounted for under 2% of unexcreted substance. The liver accounted for under 7% of unexcreted PFAAs for all substances except PFOS. The substance PFOS showed a somewhat lower affinity for pig blood plasma and a markedly higher affinity for the liver (35% of unexcreted PFOS). This is likely related to specific protein binding. A study on the binding of several PFAS to human liver fatty acid binding protein (L-FABP)[51] showed that all studied PFAS bound to L-FABP but that PFOS had the strongest binding affinity. In this work, we have used a partitioning-type model, where the interaction with proteins is not an input of the model, as it was in ref 52. Our results may thus be compared a posteriori to in vitro protein binding affinities, because that information was not explicitly used in the model. Finally, because we were able to quantify all 12 PFAAs analyzed in liver samples, even the 5 PFAAs that we could not detect in other matrices, we support the use of liver samples in monitoring programs of farm animals and wild boars.

Baseline Levels of PFAAs in Control Pigs and Background Contamination. Even newborn animals may have body burdens, as maternal–fetal PFOA and PFOS transfer has been demonstrated in rats and humans.[17] Here, PFAA concentrations were found to be below LOD in the six control animals for all tissues and substances, except for PFOS in some liver samples. PFOS in the liver of control animals was always below 1 μg/kg BW, or >1000 times lower than in the experimental animals. PFAAs were also below LOD in drinking water and animal litter.[23]

Dynamic Behavior of PFAAs in Pigs. A very good fit was found for most pigs and substances between the dynamic model of eqs 10 and the experimental data. To exemplify the results, we chose gilt 8 for the plots in Figure 3, because this pig most closely resembles the average pig. The complete plots for all 24 pigs and the average pig are presented in the Supporting Information (Figures S2–S8) for all seven substances. The substances are ordered from slowest (PFHxS) to fastest (PFHxA) urinary excretion (Figure 3). On day 22, all curves display a slight to marked downward trend for both compartments. The curves do not mean a steady state was established, but rather reflect a short depuration period as the animals were fasted before slaughter. The numerical method to fit eqs 10 to experiment is not easily convergent when k_{12} and k_{21} (the rate of mass flow from compartment 1 to 2 and the rate between 2 and 1) are independently fitted. The physiological correlate for this lack of convergence is a fast equilibrium between the blood plasma and edible tissue compartments compared to the urinary excretion rate (so fast that it cannot be convergently calculated precisely). In mathematical language, this means that $k_{12} \gg k_U$ and $k_{21} \gg k_U$ simultaneously. Because we are not interested in the precise independent values of k_{12} and k_{21}, we opt for fitting only the partitioning ratio k_{21}/k_{12} with fast equilibrium between compartments 1 and 2. We thus reduce the number of fitted model constants to only two (k_{21}/k_{12} and k_U). In this way, the numerical method becomes convergent. Another validation of the model prediction of a fast equilibrium between blood plasma and edible tissue is the linear increase of the amount of PFAA in blood plasma for most substances. The red circles in Figure 3a–f display an increase that is almost linear with intercept zero (see also Figures S2–S8 in the Supporting Information). This experimental observation is only mediated by the pig growth functions and no further modeling. A linear increase in PFAA amounts can happen only if the excretion is very slow (compared to the 21 days of the experiment) and if k_{12} and k_{21} are large in comparison. PFHxA displayed nonlinear behavior approaching the steady state. However, the experimental red circles in Figure 3g also suggest a steady state curve shape consistent with fast equilibrium between compartments 1 and 2. We have de facto created a one-compartment model, not a priori, but as a result of the calculations. The Supporting Information section "Concise description of the toxicokinetic model and its constants" provides a compact implementation-ready overview.

Gender Differences in Elimination. This study with pigs found no statistically significant gender difference in elimination half-life between gilts, barrows, and young boars. The statistical insignificance result was obtained with the nonparametric Kruskal–Wallis test (p = 0.01) using the program SPSS 12.0.2G. This is in line with the lack of sex differences in renal elimination in many species, such as rabbits and mice,[53] and

differs from the marked testosterone-regulated difference in the renal elimination of PFCAs found in rats[49] and some fish.[54] The elimination half-life, eq 11, includes information from all model parameters. As no gender differences were found in pigs, averaging was performed using the results for all pigs.

Plasma Elimination Half-Life. From the fitted model constants, we may calculate the elimination half-life (Figure 4)

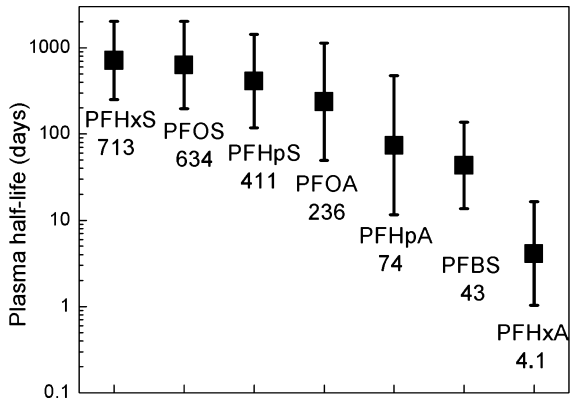

Figure 4. Elimination half-life $\tau_{1/2}$ of PFAAs in pigs. The squares denote the geometric average over up to 24 pigs, and the bars show 95% variability (Student's t statistics). These values reflect both the blood plasma and the edible tissue elimination half-lives, because plasma and edible tissue were found to be in fast equilibrium with each other.

from eq 11. The fast equilibrium between central (plasma) and peripheral (edible tissue) compartments is very advantageous for consumer risk management, because the values in Figure 4 are directly relevant to the elimination of PFAAs from edible tissues. The elimination half-life of PFAAs in pigs is mostly determined by the chemical end-group (sulfonic vs carboxylic acid); half-lives of PFSAs are much longer than for PFCAs. The chain length[10] plays a secondary role. The elimination half-lives are longer than those in trout,[55] rat,[1] Angus cattle,[56] mouse, and monkey[1] but shorter than those for humans.[1,14,17,25] PFHxS has a longer average half-life than PFOS, despite having a shorter chain, in agreement with results in humans.[1,14,17,25] Likewise, a study[14] in humans found a relatively large half-life variability using data from up to 66 individuals, similar to the large variability (vertical bars in Figure 4) in the current study with 24 pigs. It is impossible to separate the systematic from the stochastic variability, but these results may reflect an inherent variability in pig metabolism. Each PFAS may exist in several chain isomers (one linear and several branched). Other studies have found the half-life of each chain isomer to be different.[12,14] In this study, we were able to ensure that only linear PFOS was analytically quantified, without interference from branched isomers. By methodological analogy, although not with full certainty, the quantification of the rest of the substances was also only for the respective linear isomer. See Quantification and Isomer Separation for more details.

Biomagnification Factors. The biomagnification factor (BMF) (also called the bioconcentration factor) measures the ability of a substance to concentrate in an organism's living tissues in relation to the concentration in feed. The BMF is meaningful only in the steady state (ss) and is defined as

$$\mathrm{BMF}_X = \frac{C_X^{ss}}{\overline{C_I^{exptl}}} \tag{12}$$

for each substance, where X = plasma (P), liver (L), kidney (K), fat (F), dorsal muscle (D), ventral muscle (V), or whole pig. The concentration in feed $\overline{C_I^{exptl}}$ is in Table 2. The steady state plasma concentration is obtained as $C_P^{ss} = A_1^{ss}/m_P$. To obtain the amount in blood plasma at steady state A_1^{ss}, one sets the differential eqs 3 to zero and algebraically solves for A_1. Likewise, we solve for the steady state total amount in edible tissue A_2^{ss}. If we assume fast equilibrium between edible tissues, we may use the experimental relative amounts in each tissue (cf. eq 8) to obtain

$$C_Y^{ss} = \frac{A_Y^{exptl}(t_{end})}{A_2^{exptl}(t_{end})}\frac{A_2^{ss}}{m_Y(t_{end})} \tag{13}$$

where Y = liver (L), kidney (K), fat (F), dorsal muscle (D), ventral muscle (V), or whole pig, where $m_{whole} = M(t)$, eq 1. Here we have presupposed that the tissues have reached their final weight at t_{end} = 22 days of the experiment. In this 3 week study, the steady state was only observed for PFHxA in some pigs (Figure 3g). The model predicts that the steady state is approached to 95% completion in $t_{95\%ss}$ = 22 ± 14 days for PFHxA. We may use the model to numerically extrapolate and predict $t_{95\%ss}$ for other substances. For PFBS, $t_{95\%ss}$ = 217 ± 120 days and between 1 and 10 years for the rest of the compounds (Supporting Information Table S5). Both BMF and $t_{95\%ss}$ depend only on the compound through the model constants and do not depend on $\overline{C_I^{exptl}}$. The extrapolated steady state (eq 13) assumes a linear half-life behavior, which may be a flawed assumption if renal resorption saturation[57] is relevant for the concentrations considered (see section on Limitations). The computed BMF, eq 12, is listed in Table 3 by tissue groups, where meat is an average of D, V, and F.

Table 3. Biomagnification Factor, Dimensionless (Arithmetic Mean) from Equation 12[a]

substance	mean BMF whole pig	mean BMF meat	mean BMF liver
PFHxS	20.1	13.1	48
PFHpS	12.7	8.3	81
PFOS	17.9	9.7	503
PFOA	7.9	5.3	32.8
PFHpA	2.7	1.8	7.0
PFBS	1.2	0.8	6.
PFHxA	0.13	0.08	0.42

[a]Mean for all 24 pigs. For more detailed information on tissue-specific BMF, see Supporting Information Table S5.

Limitations. A drawback worth mentioning is the inability of this study to detect a possible interaction among substances regarding absorption, distribution, and excretion. Because we have chosen to study a mixture of PFAAs arising from a contaminated cultivation site, we cannot rule out that the specific pattern of substances (Table 2) results in a specific toxicokinetic profile. Likewise, we have not analyzed any PFAA precursors possibly present in the feed. The presence of precursors cannot be ruled out in the industrial waste that contaminated the soil.[27] The mass balance closes to 97% (Figure 2). Possible explanations for this good agreement in terms of PFAA precursors are (a) the feed did not contain precursors, (b) precursors were present, but the pigs' metabolic bioconversion is too slow to show up in 3 weeks, or (c) part of the PFAAs go into tissues not tested, such as the brain, whereas

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the PFAAs from precursors balance this mechanism out. The two-compartment model includes no provision for saturation of binding,[52,57] but we know from experiments that blood proteins can bind much larger amounts than those observed here.[45,46] However, a mechanism known to be saturable in other organisms is renal PFAA resorption through organic anion transporters.[17] In our model, renal resorption is implicitly part of the renal excretion constant k_U and includes no saturable equations.[52,57] Saturable renal resorption may lead to nonlinear half-life, with faster elimination at higher plasma concentrations. The present experimental data are not designed for fitting renal resorption, so this caveat should be considered for half-life (Figure 4) and BMF (Table 3).

Consequences for Food Safety. The elimination half-lives are very long compared to the lifetime of a farm pig (~180 days) before slaughter, except for PFHxA. Even if contaminated animals are fed uncontaminated feed for weeks or months, it is not expected to see significant declines in PFAA levels. This has consequences for risk management. The EFSA has set a TDI limit of 0.15 μg/kg BW/day for PFOS and 1.5 μg/kg BW/day for PFOA.[8] No TDI is yet available for other PFAAs.[9] From the EFSA TDI and total exposure, including total diet studies, it is possible to derive exposure guidelines for pork. It is very important to produce consistent regulations for maximum content of PFAAs in animal feed and animal products, which is possible only when both a toxicokinetic model and a maximum exposure are available. This has been done for dioxins and other lipophilic pollutants in, for example, chicken feed/eggs.[58] Although lipophilic pollutants have different toxicokinetic properties from PFAAs, the general concepts needed to relate the maximum levels in feed and food are the same. The present toxicokinetic model enables such a quantitative connection between porcine feed and food product PFAA concentrations.

■ ASSOCIATED CONTENT

Supporting Information

More details, such as a compact description of the model and its constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

PFAAs	perfluoroalkyl acids
PFSAs	perfluoroalkyl sulfonic acids
PFCAs	perfluoroalkyl carboxylic acids
PFBS	perfluorobutanesulfonic acid
PFHxS	perfluorohexanesulfonic acid
PFHpS	perfluoroheptanesulfonic acid
PFOS	perfluorooctanesulfonic acid
PFDS	perfluorodecanesulfonic acid
PFBA	perfluorobutanoic acid
PFPA	perfluoropentanoic acid
PFHxA	perfluorohexanoic acid and
PFHpA	perfluoroheptanoic acid
PFOA	Perfluorooctanoic acid
PFNA	perfluorononanoic acid
PFDA	perfluorodecanoic acid
PFUnA	perfluoroundecanoic acid
PFDoA	perfluorododecanoic acid
DiPAPs	polyfluoroalkyl-diester phosphate surfactants
L-FABP	liver fatty acid binding protein
PBT	persistent bioaccumulative and toxic
TDI	tolerable daily intake
LOD	limit of detection
LOQ	limit of quantification
HPLC-MS/MS	high-performance liquid chromatography coupled with tandem mass spectrometry
MRM	multiple reaction monitoring
UCL-MS	ultraliquid chromatography−mass spectrometry
ESI	electrospray interface
BW	body weight
BMF	biomagnification factor
ss	steady state

■ REFERENCES

(1) Lau, C. Perfluorinated compounds. In *Molecular, Clinical and Environmental Toxicology Volume 3: Environmental Toxicology*; Luch, A., Ed.; Springer: Basel, Switzerland, 2012.

(2) Lindstrom, A. B.; Strynar, M. J.; Libelo, E. L. Polyfluorinated compounds: past, present, and future. *Environ. Sci. Technol.* **2011**, *45*, 7954−7961.

(3) Kissa, E. *Fluorinated Surfactants and Repellents*, 2nd ed.; Dekker: New York, 2001; Vol. 97.

(4) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Progress toward understanding the bioaccumulation of perfluorinated alkyl acids. *Environ. Toxicol. Chem.* **2013**, *32*, 2421−2423.

(5) Buhrke, T.; Kibellus, A.; Lampen, A. In vitro toxicological characterization of perfluorinated carboxylic acids with different carbon chain lengths. *Toxicol. Lett.* **2013**, *218*, 97−104.

(6) Grandjean, P.; Budtz-Jørgensen, E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. *Environ. Health* **2013**, *12*, 35.

(7) Fletcher, T.; Savitz, D.; Steenland, K. C8 Probable Link Reports, http://www.c8sciencepanel.org, 2012.

(8) European Food Safety Authority, Scientific opinion of the panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. *EFSA J.* **2008**, *653*, 1−131.

(9) European Food Safety Authority, Scientific report on perfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA J.* **2012**, *10*, 2743.

(10) Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; de Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr. Environ. Assess. Manage.* **2011**, *7*, 513−541.

(11) D'eon, J. C.; Mabury, S. A. Production of perfluorinated carboxylic acids (PFCAs) from the biotransformation of polyfluoroalkyl phosphate surfactants (PAPS): exploring routes of human contamination. *Environ. Sci. Technol.* **2007**, *41*, 4799−4805.

(12) Ross, M. S.; Wong, C. S.; Martin, J. W. Isomer-specific biotransformation of perfluorooctane sulfonamide in Sprague-Dawley Rats. *Environ. Sci. Technol.* **2012**, *46*, 3196−3203.

(13) Trier, X.; Granby, K.; Christensen, J. H. Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging. *Environ. Sci. Pollut. Res.* **2011**, *18*, 1108−1120.

(14) Zhang, Y.; Beesoon, S.; Zhu, L.; Martin, J. W. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ. Sci. Technol.* **2013**, *47*, 10619−10627.

(15) Vestergren, R.; Cousins, I. T. Tracking the pathways of human exposure to perfluorocarboxylates. *Environ. Sci. Technol.* **2009**, *43*, 5565−5575.
(16) Klenow, S.; Heinemeyer, G.; Brambilla, G.; Dellatte, E.; Herzke, D.; de Voogt, P. Dietary exposure to selected perfluoroalkyl acids (PFAAs) in four European regions. *Food Addit. Contam. A* **2013**, *30*, 2141−2151.
(17) Loccisano, A. E.; Longnecker, M. P.; Campbell, J. L., Jr.; Andersen, M. E.; Clewell, H. J., 3rd. Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages. *J. Toxicol. Environ. Health A* **2013**, *76*, 25−57.
(18) Stahl, T.; Heyn, J.; Thiele, H.; Hüther, J.; Failing, K.; Georgii, S.; Brunn, H. Carryover of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from soil to plants. *Arch. Environ. Contam. Toxicol.* **2009**, *57*, 289−298.
(19) Watanabe, M. X.; Kunisue, T.; Tao, L.; Kannan, K.; Subramanian, A.; Tanabe, S.; Iwata, H. Dioxin-like and perfluorinated compounds in pigs in an Indian open waste dumping site: toxicokinetics and effects on hepatic cytochrome P450 and blood plasma hormones. *Environ. Toxicol. Chem.* **2010**, *29*, 1551−1560.
(20) Norden, M.; Berger, U.; Engwall, M. High levels of perfluoroalkyl acids in eggs and embryo livers of great cormorant (*Phalacrocorax carbo sinensis*) and herring gull (*Larus argentatus*) from Lake Vanern, Sweden. *Environ. Sci. Pollut. Res. Int.* **2013**, *20*, 8021−8030.
(21) van Asselt, E. D.; Kowalczyk, J.; van Eijkeren, J. C.; Zeilmaker, M. J.; Ehlers, S.; Fürst, P.; Lahrssen-Wiederholt, M.; van der Fels-Klerx, H. J. Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk. *Food Chem.* **2013**, *141*, 1489−1495.
(22) Kowalczyk, J.; Ehlers, S.; Fürst, P.; Schafft, H.; Lahrssen-Wiederholt, M. Transfer of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) from contaminated feed into milk and meat of sheep: pilot study. *Arch. Environ. Contam. Toxicol.* **2012**, *63*, 288−298.
(23) Kowalczyk, J.; Ehlers, S.; Oberhausen, A.; Tischer, M.; Fürst, P.; Schafft, H.; Lahrssen-Wiederholt, M. Absorption, distribution, and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA by dairy cows fed naturally contaminated feed. *J. Agric. Food Chem.* **2013**, *61*, 2903−2912.
(24) Vestergren, R.; Orata, F.; Berger, U.; Cousins, I. T. Bioaccumulation of perfluoroalkyl acids in dairy cows in a naturally contaminated environment. *Environ. Sci. Pollut. Res.* **2013**, *20*, 7959−7969.
(25) Olsen, G. W.; Burris, J. M.; Ehresman, D. J.; Froehlich, J. W.; Seacat, A. M.; Butenhoff, J. L.; Zobel, L. R. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* **2007**, *115*, 1298−1305.
(26) Groenen, M. A. M.; Archibald, A. L.; Uenishi, H.; Tuggle, C. K.; Takeuchi, Y.; Schook, L. B. Analyses of pig genomes provide insight into porcine demography and evolution. *Nature* **2012**, *491*, 393−398.
(27) Arenholz, U.; Bergmann, S.; Bosshammer, K.; Busch, D.; Dreher, K.; Eichler, W.; Geueke, K.-J.; Grubert, G.; Hähnle, J.; Harff, K.; Kraft, M.; Leisner-Saaber, J.; Oberdörfer, M.; Rauchfuss, K.; Respondek, R.; Reupert, R.; Rose-Luther, J.; Schroers, S.; Tiedt, M.; Just, P.; Poschner, A.; Susset, B. *LANUV − Fachbericht 34 Verbreitung von PFT in der Umwelt: Ursachen - Untersuchungsstrategie - Ergebnisse - Maßnahmen*; Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen: Germany, 2011.
(28) *Empfehlungen zur Energie und Nährstoffversorgung bei Schweinen*, Auflage 1/2006 ed.; DLG-Verlag: Frankfurt, Germany, 2006.
(29) Kärrman, A.; van Bavel, B.; Järnberg, U.; Hardell, L.; Lindström, G. Development of a solid-phase extraction-HPLC/single quadrupole MS method for quantification of perfluorochemicals in whole blood. *Anal. Chem.* **2005**, *77*, 864−870.
(30) Ehlers, S. *Analytik von Perfluoralkylsäuren in verschiedenen Matrices zur Klärung der Toxikokinetik in Tierarten, die der Lebensmittelgewinnung dienen*. Westfälische Wilhelms-Universität Münster, 2012.
(31) Taniyasu, S.; Kannan, K.; So, M. K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *J. Chromatogr., A* **2005**, *1093*, 89−97.
(32) Bernsmann, T.; Fürst, P. Determination of perfluorinated compounds in human milk. *Organohalog. Compd.* **2008**, *70*, 718−721.
(33) Benskin, J. P.; De Silva, A. O.; Martin, L. J.; Arsenault, G.; McCrindle, R.; Riddell, N.; Mabury, S. A.; Martin, J. W. Disposition of perfluorinated acid isomers in Sprague-Dawley rats. Part 2: Subchronic dose. *Environ. Toxicol. Chem.* **2009**, *28*, 555−567.
(34) Marquardt, D. D. An algorithm for least-squares estimation of non-linear parameters. *J. Soc. Ind. Appl. Math.* **1963**, *11*, 431−441.
(35) Adolphs, J.; Kleinjung, F.; Numata, J.; Mielke, H.; Abraham, K.; Schafft, H.; Müller-Graf, C.; Greiner, M. A probabilistic model for the carry-over of PCDD/Fs from feed to growing pigs. *Chemosphere* **2013**, *93*, 474−479.
(36) Pfeiffer, H.; Lengerken, G. v.; Gebhardt, G. *Wachstum und Schlachtkörperqualität*; VEB Deutscher Landwirtschaftsverlag: Berlin, Germany, 1984.
(37) Branscheid, W.; Honikel, K.-O.; Lengerken, G. v. *Qualität von Fleisch und Fleischwaren: Band 1. 2. überarbeitete und erweiterte*, Auflage ed.; Deutscher Fachverlag: Frankfurt, Germany, 2007.
(38) Elsner, F. L. Messung der Nierenperfusion mittels DCE-MRI am narkotisierten Schwein. Freie Universität, Berlin, Germany, 2012.
(39) Aiello, S. E.; Moses, M. A. *The Merck Veterinary Manual Online*, 2011.
(40) *Empfehlung zur Blutentnahme bei Versuchstieren, insbesondere kleinen Versuchstieren*; GV-SOLAS: Marburg, Germany, 2009.
(41) Johnson, J. D.; Gibson, S. J.; Ober, R. E. Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [^{14}C]perfluorooctanoate or potassium [^{14}C]-perfluorooctanesulfonate. *Fundam. Appl. Toxicol.* **1984**, *4*, 972−976.
(42) Fricke, M.; Lahl, U. Risikobewertung von Perfluortensiden als Beitrag zur aktuellen Diskussion zum REACH-Dossier der EU-Kommission. *UWSF* **2005**, *17*, 36−49.
(43) Chiu, W. A.; Barton, H. A.; DeWoskin, R. S.; Schlosser, P.; Thompson, C. M.; Sonawane, B.; Lipscomb, J. C.; Krishnan, K. Evaluation of physiologically based pharmacokinetic models for use in risk assessment. *J. Appl. Toxicol.* **2007**, *27*, 218−237.
(44) Urien, S.; Lokiec, F. Population pharmacokinetics of total and unbound plasma cisplatin in adult patients. *Br. J. Clin. Pharmacol.* **2004**, *57*, 756−763.
(45) Bischel, H. N.; MacManus-Spencer, L. A.; Zhang, C.; Luthy, R. G. Strong associations of short-chain perfluoroalkyl acids with serum albumin and investigation of binding mechanisms. *Environ. Toxicol. Chem.* **2011**, *30*, 2423−2430.
(46) Luo, Z.; Shi, X.; Hu, Q.; Zhao, B.; Huang, M. Structural evidence of perfluorooctane sulfonate transport by human serum albumin. *Chem. Res. Toxicol.* **2012**, *25*, 990−992.
(47) Mizukawa, H.; Nomiyama, K.; Kunisue, T.; Watanabe, M. X.; Subramanian, A.; Takahashi, S.; Tanabe, S. Accumulation features of hydroxylated-PCBs (OH-PCBs) in the blood of pigs collected from a dumping site for municipal wastes in India. In *Interdisciplinary Studies on Environmental Chemistry — Environmental Specimen Bank*; Isobe, T., Nomiyama, K., Subramanian, A., Tanabe, S., Eds.; Global COE Ehime University: Japan, 2010; pp 175−181.
(48) Tonnelier, A.; Coecke, S.; Zaldívar, J.-M. Screening of chemicals for human bioaccumulative potential with a physiologically based toxicokinetic model. *Arch. Toxicol.* **2012**, *86*, 393−403.
(49) Vanden Heuvel, J. P.; Kuslikis, B. I.; Van Rafelghem, M. J.; Peterson, R. E. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J. Biochem. Toxicol.* **1991**, *6*, 83−92.
(50) Jing, P.; Rodgers, P. J.; Amemiya, S. High lipophilicity of perfluoroalkyl carboxylate and sulfonate: implications for their membrane permeability. *J. Am. Chem. Soc.* **2009**, *131*, 2290−2296.
(51) Zhang, L.; Ren, X.-M.; Guo, L.-H. Structure-based investigation on the interaction of perfluorinated compounds with human liver fatty acid binding protein. *Environ. Sci. Technol.* **2013**, *47*, 11293−11301.

(52) Ng, C. A.; Hungerbühler, K. Bioconcentration of perfluorinated alkyl acids: how important is specific binding? *Environ. Sci. Technol.* **2013**, *47*, 7214−7223.
(53) Hundley, S. G.; Sarrif, A. M.; Kennedy, G. L. Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. *Drug Chem. Toxicol.* **2006**, *29*, 137−145.
(54) Lee, J. J.; Schultz, I. R. Sex differences in the uptake and disposition of perfluorooctanoic acid in fathead minnows after oral dosing. *Environ. Sci. Technol.* **2010**, *44*, 491−496.
(55) De Silva, A. O.; Tseng, P. J.; Mabury, S. A. Toxicokinetics of perfluorocarboxylate isomers in rainbow trout. *Environ. Toxicol. Chem.* **2009**, *28*, 330−337.
(56) Lupton, S. J.; Huwe, J. K.; Smith, D. J.; Dearfield, K. L.; Johnston, J. J. Absorption and excretion of ^{14}C-perfluorooctanoic acid (PFOA) in Angus cattle (*Bos taurus*). *J. Agric. Food Chem.* **2012**, *60*, 1128−1134.
(57) Loccisano, A. E.; Campbell, J. L., Jr.; Butenhoff, J. L.; Andersen, M. E.; Clewell, H. J., 3rd. Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. *Reprod. Toxicol.* **2012**, *33*, 452−467.
(58) Van Eijkeren, J. C. H.; Zeilmaker, M. J.; Kan, C. A.; Traag, W. A.; Hoogenboom, L. A. A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. *Food Addit. Contam.* **2006**, *23*, 509−517.