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Absorption and Excretion of ^{14}C-Perfluorooctanoic Acid (PFOA) in Angus Cattle (*Bos taurus*)

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ABSTRACT: Perfluoroalkyl substances (PFASs), such as perfluorooctanoic acid (PFOA), are environmentally persistent industrial chemicals often found in biosolids. Application of these biosolids to pastures raises concern about the accumulation of PFOA in the edible tissues of food animals. Because data on the absorption, distribution, metabolism, and excretion (ADME) of PFOA in cattle were unavailable, a study was conducted to determine pharmacokinetic parameters following a single oral exposure (1 mg/kg body weight of ^{14}C-PFOA) in four Lowline Angus steers. Radiocarbon was quantified in blood, urine, and feces for 28 days and in tissues at the time of slaughter (28 days) by liquid scintillation counting (LSC) or by combustion analysis with LSC with confirmation by liquid chromatography−tandem mass spectrometry (LC-MS/MS). ^{14}C-PFOA was completely absorbed and excreted (100.7 ± 3.3% recovery) in the urine within 9 days of dosing. The plasma elimination half-life was 19.2 ± 3.3 h. No ^{14}C-PFOA-derived radioactivity was detected in edible tissues. Although PFOA was rapidly absorbed, it was also rapidly excreted by steers and did not persist in edible tissues, suggesting meat from cattle exposed to an acute dose of PFOA is unlikely to be a major source of exposure to humans.

KEYWORDS: *perfluorooctanoic acid, beef cattle, absorption, elimination, pharmacokinetics, residues*

INTRODUCTION

Perfluoroalkyl substances (PFASs) are a widely used class of industrial compounds. Because of their amphiphilic properties, these chemicals are used extensively in surfactants and coatings for a variety of materials such as carpet, cookware, and paper.[1,2] Perfluorooctanoic acid (PFOA) is a common precursor used in the manufacture of other PFASs that are incorporated into consumer and industrial products. PFOA can also be the breakdown product from other PFASs and as a result is widely found in humans and the environment.[3,4]

On the basis of PFOA's bioaccumulation factor/bioconcentration factor (2−570 L/kg), it has a high probability of bioaccumulating in humans and the environment.[1] In humans, PFOA has an estimated half-life of 3.8 years, and toxic effects associated with prolonged exposures are a concern.[5] For example, toxicity studies in rats, mice, monkeys, and rabbits indicated that PFOA exposures may reduce body weight gains and overall body weight at higher doses.[3,4] Increases in liver weights with hepatocellular hypertrophy and developmental anomalies in neonates were also observed.[3,4] PFOA is also a peroxisome proliferator, which can cause abnormal cell and tissue growth, leading to the possible formation of tumors.[3,4] Considering the potential for bioaccumulation and possible health effects, limiting human exposure to PFOA is important. On the basis of its physicochemical properties, an important potential route of PFOA exposure is consumption of contaminated food.[2,6]

Due to the facts that PFOA is observed in humans, numerous consumer goods, and industrial waste, it is often found in biosolids (80−100% detection frequency) from wastewater treatment plants (WWTPs) at concentrations of 1−244 ng/g dry weight (dw).[7−9] To lessen the amount of sludge that is put in landfills, biosolids are often used as fertilizers and applied to pastures where animals graze or where food crops (i.e., hay and grain) for animals such as beef cattle are raised. The transfer of PFOA from biosolids to the soil in amended fields has been observed.[10] Concentrations of PFOA in the soil of biosolid-amended fields ranged from 0.17 to 317 ng/g dw, indicating transfer and possible accumulation are occurring in the soil.[10] Stahl et al. studied the uptake of PFOA from soil into maize, rye grass, and wheat and showed transfer of PFOA into the stalks, stems, and produce of the plant.[11] The uptake of PFOA into the plants was directly proportional to the PFOA concentrations in the irrigated soil.[11] A recent study looking at the PFOA concentrations in barley, tall fescue, bermuda grass, and Kentucky bluegrass from a contaminated area in the Decatur, AL, region found that PFOA was the major PFAS, with concentrations ranging from 10 to 200 ng/g dw.[12] Cattle were also being raised on this contaminated area.[13]

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Because a portion of a beef animal's life cycle is spent on a pasture, cattle are more likely to be exposed to environmental contaminants, such as PFOA, than other food animals, through consumption of contaminated soils, forages, or grains. Cattle can also be exposed to PFOA through contaminated water, which may be caused by the application of biosolids to fields.

Several studies have observed PFOA in retail beef samples ranging from sub parts per billion to 4.26 ppb in edible tissues, indicating that the meat had been contaminated with PFOA either through accumulation in the animal or through the food packaging.[2,6,14−17] Although food packaging may contribute to PFOA levels in retail food, a study by Guruge et al. measured levels of PFOA in the blood and liver of beef cattle from Japanese farms (0.01−0.24 ppb), indicating that some exposure of cattle is occurring in the field.[18] Due to the occurrence of PFOA in beef and the possible exposure of humans to PFOA by consumption of contaminated beef, this study was completed to determine the uptake, absorption, distribution, metabolism, and excretion (ADME) of ^{14}C-PFOA in Lowline Angus cattle. Results from this study will help to assess the persistence of PFOA in beef cattle and whether monitoring of this chemical in beef is needed.

■ MATERIALS AND METHODS

Chemicals and Materials. Radiolabeled [1-^{14}C]perfluorooctanoic acid (^{14}C-PFOA) was synthesized (purity > 98%, 3760 dpm/μg PFOA specific activity) and purchased from Perkin-Elmer (Boston, MA). Perfluorooctanoic acid (purity > 95%), tetrabutylammonium hydrogen sulfate (TBAHS), and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO). Other reagents included sodium carbonate (Fisher, Pittsburgh, PA), methyl *tert*-butyl ether (MTBE, BDH, Radnor, PA), HPLC grade methanol (MeOH, Honeywell Burdick and Jackson, Morristown, NJ), and 1,1,1,3,3,3-hexafluoroisopropanol (HFP, TCI, Portland, OR).

Experiment Setup and Sample Collection. Four Lowline steers (no. 179−182, *Bos taurus*) were purchased from Effertz EZ Ranch (Bismarck, ND) and raised in open pens in-house for 8 months prior to the study. The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) and the USDA Radiation Safety Committee. The steers (329 ± 36 kg) were acclimated to stainless steel metabolism crates 24 h prior to dosing, during which period jugular catheters were surgically inserted for blood collection. Before and during the course of the study the steers were provided feed and water ad libitum. Blood, urine, and feces were collected before dosing to determine background levels of PFOA in the steers and serve as control samples. After the 24 h acclimation period, the four steers were administered single oral bolus doses of [1-^{14}C]PFOA (1 mg/kg body weight (bw), 0.6 mCi per steer) contained in a gelatin capsule and maintained in the metabolism crates for 28 days. Three of the steers (no. 179, 180, and 182) were also given a simultaneous oral dose of perfluorooctane sulfonate (PFOS, 10 mg/kg bw) to assess the pharmacokinetics of PFOS and possible effects from simultaneous exposure (data to be reported at a later date).

Blood was collected prior to and after dosing at 0, 1, 2, 4, 8, 12, 24, 36, 48, 72, and 96 h and at 6, 8, 10, 13, 15, 17, 21, 24, and 28 days. Whole blood was collected from jugular catheters via a syringe, which contained heparin to prevent clotting. When the animals accidently pulled out the jugular catheters, whole blood was collected into Vacutainer (BD, Franklin Lakes, NJ) tubes also containing heparin. Whole blood was centrifuged (1160*g* for 15 min at 5 °C) to separate the plasma and the corresponding red blood cell fraction, which were frozen at −20 °C until analysis.

Urine and feces were collected quantitatively in 24 h increments for the duration of the study. Urine was collected through modified incontinence bags attached to the steers with Tygon (Saint-Gobain Performance Plastics, Akron, OH) tubing running into polypropylene buckets as described by Paulson and Cottrell.[19] Daily urine was weighed, thoroughly mixed, and subsampled. Feces were collected in stainless steel trays in the bottom of the metabolism crates, combined in a large stainless steel mixing bowl, thoroughly mixed, weighed, and subsampled. All urine and feces samples were kept frozen at −20 °C until analysis.

On day 28 the steers were euthanized, eviscerated, and deboned. Tissues including liver, kidneys, spleen, lung, brain, pancreas, thyroid, diaphragm, adrenals, eyes, muscle, gastrointestinal (GI) tract (esophagus through large intestine), bone, skin, bile, and carcass remainder (all trimmings and other parts) were quantitatively collected. Representative samples of a rib bone, skin from the side, back fat, and intraperitoneal fat were subsampled for analysis. All other whole organs/tissues were ground and homogenized before a subsample was removed. The GI tract was completely flushed of all contents before grinding. All samples were frozen at −20 °C until analysis.

Radiochemical Analysis. Radioactivity derived from ^{14}C-PFOA in the plasma and urine was analyzed in triplicate by liquid scintillation counting (LSC, Beckman Coulter, Inc., Brea, CA). Aliquots (100 μL) of plasma or urine were added to 16 mL of Ecolite (+) (MP Biomedicals', Solon, OH) LSC fluid and counted for 10 min each. Equal volumes of control matrices were counted for 10 min and subtracted from test matrices. The limits of quantification (LOQs) in plasma and urine for LSC were 0.017 and 0.017 μg/mL, respectively, as calculated by Smith et al.[20]

Aliquots (0.1−0.3 g) of feces or red blood cellular fractions (in triplicate) were weighed into combustion thimbles, dried, and combusted in a tissue oxidizer (Packard, Meridan, CT) to $^{14}CO_2$, which was trapped in Carbo-Sorb E (Perkin-Elmer, Waltham, MA) and quantified by LSC in Permafluor E (+) scintillation fluid (Packard). In the same manner, the homogenized tissues were combusted without drying. The LOQ for combustion analysis was 0.0053 μg/g.

Extraction Procedure and LC-MS/MS Analysis. For LC-MS/MS analyses of PFOA in plasma and urine the ion-pairing extraction of Hansen et al. was modified.[21] Briefly, 0.5 mL of plasma or urine was partitioned with 1 mL of 0.5 M TBAHS solution (pH 10), 2 mL of 0.25 M sodium carbonate, and 5 mL MTBE followed by shaking (Magni Whirl, Blue M, Blue Island, IL) for 15 min and centrifugation (1160*g* for 15 min) for the extraction of PFOA, which was repeated two more times with subsequent additions of MTBE. Combined MTBE extracts were then evaporated to dryness under nitrogen, reconstituted in 0.5 mL of MeOH, and filtered through a 0.2 μm nylon syringe filter.

Tissue (1−2 g) and feces (1−2 g) were extracted using the same ion-pairing liquid−liquid extraction method, but with two additional steps.[22] Samples were homogenized in the TBAHS (1 mL) and sodium carbonate (8 mL) using an Ultra-Turrax Tissumizer (Tekmar Co., Cincinnati, OH), and then MTBE was added for extraction as above. After MTBE extraction and evaporation under nitrogen to 0.5 mL of extract, 0.5 mL of HFP was added to each extract and vortexed for 30 s to precipitate proteins. Extracts with HFP were centrifuged at 3210*g* for 25 min, and the resulting supernatants were filtered through a 0.2 μm nylon syringe filter. Filtered extracts were evaporated to dryness under nitrogen, reconstituted with 0.5 mL of MeOH, and filtered again through a 0.2 μm nylon syringe filter before LC-MS/MS analysis.

Adipose tissue and carcass remainder samples were extracted using a MeOH extraction method previously described by D'eon et al.[23] Briefly, 1−2 g of tissue was homogenized for 30−60 s in MeOH (8 mL) with an Ultra-Turrax Tissumizer (Tekmar Co.). The sample was shaken for 20 min on a Magni Whirl shaker (Blue M) and centrifuged at 2060*g* for 15 min, and the MeOH was removed. Addition of MeOH (8 mL), shaking, and centrifugation were repeated two more times. Extracts were combined and evaporated to approximately 0.5 mL under nitrogen. Each sample was filtered through a 0.2 μm nylon syringe filter before LC-MS/MS analysis.

For quality assurance and control purposes, each sample set consisted of eight samples (plasma, urine, feces, or tissue), one control matrix, and one recovery matrix (a control matrix spiked at 0.3 μg/mL

for plasma or urine and 0.1 μg/g for tissue or feces) for a total of 10 samples processed at one time. The control matrix allowed for detection of any laboratory or residual contamination, and the recovery matrix assured the extraction was working properly. The control and recovery matrices for plasma, urine, and feces were from the steers used in this study and were collected prior to dosing. The control and recovery matrices for tissues were from beef liver, swine muscle, and swine fat from previous studies conducted in our laboratory. Extraction recoveries for PFOA in plasma, urine, feces, liver, muscle, and fat were 82.7 ± 13% (n = 10), 74.6 ± 7% (n = 12), 81.4 ± 13% (n = 7), 113.4 ± 35% (n = 3), 105.6 ± 7% (n = 3), and 82.2 ± 6% (n = 3), respectively. Extraction recoveries and reproducibility in different matrices were within acceptable standards for LC-MS/MS analysis.

Sample extracts were analyzed by LC-MS/MS on an Alliance HPLC (Waters, Milford, MA) coupled to a quadrupole time of flight mass analyzer (QTOF, Waters) to confirm the identity of the radioactive residues. Chromatography was achieved with a Waters C-18 symmetry column (2.1 mm × 100 mm, 3.5 μm particle size) using 95:5 water/MeOH with 2 mM ammonium acetate (mobile phase A) and 100% MeOH with 2 mM ammonium acetate (mobile phase B). An injection volume of 10 μL was used, and the flow rate was 0.3 mL/min. The mobile phase gradient started at a composition of 70:30 A:B, was held for 0.2 min, then increased linearly to 15:85 A:B at 21 min, and held for 5 min, after which the mobile phase composition was returned to starting conditions and allowed to equilibrate for 7 min. PFOA eluted from the column at 15.3 min. Analytes were ionized by electrospray ionization in negative ion mode. A desolvation temperature of 350 °C and a source temperature of 120 °C were used, along with a desolvation gas flow of 500 L/h and a cone gas flow of 20 L/h. The capillary, cone, and collision voltages were 2.5 kV, 10 V, and 10 V, respectively. The PFOA precursor and fragment ions of m/z 412.9, 368.9, 218.9, and 168.9 were monitored in MS/MS scan mode.

An external standard curve was freshly made in MeOH the day of analysis from a stock solution (10 μg/mL) in acetonitrile to limit any possible degradation of PFOA in MeOH. The standard curve consisted of eight points ranging from 0.007 to 1 μg/mL. All extracts were quantified using the linear portion of the standard curve; if concentrations were above the linear portion, appropriate dilutions were made and extracts were analyzed again. Regression analysis of LSC urine PFOA concentrations and LC-MS/MS urine PFOA concentrations demonstrated that ion suppression was not occurring due to matrix effects. A blank injection was completed after sets of four sample injections, and a standard check injection was completed after sets of 20 injections to ensure no carry-over was occurring and that the instrument was working properly, respectively. Quantification of check standards had to be within ±20% of actual concentration over the course of a sample list run to ensure accuracy of the calculated PFOA concentrations in the extracts. A method limit of detection (LOD) and LOQ were determined in plasma to be 0.020 and 0.067 μg/mL, respectively.

Kinetic Parameter Estimates. Parameters (A, E, α, and β) describing PFOA absorption and elimination were estimated on each individual animal from plasma versus time data using noncompartmental methods (PK-Solutions software; Summit Research Services, Montrose, CO); absorption and elimination half-lives are reported as the mean ± SD of four animals. For predictive purposes, plasma concentrations at each time point were averaged across animals, and least-squares parameter estimates were calculated after curve-stripping. Predicted plasma PFOA concentrations (C) were calculated using

$$C = A\,e^{-\alpha t} + E\,e^{-\beta t}$$

where t is time (h) and A and E are Y intercepts of lines having slopes of α and β, respectively. Addition of an exponential term during curve stripping to describe two-phase depletion resulted in poor fits.

RESULTS

^{14}C-PFOA Concentrations and Elimination Half-Life in Plasma. Average and model-predicted concentrations of ^{14}C-PFOA in plasma as a function of time following a single oral dose of 1 mg/kg bw ^{14}C-PFOA are shown in Figure 1, whereas

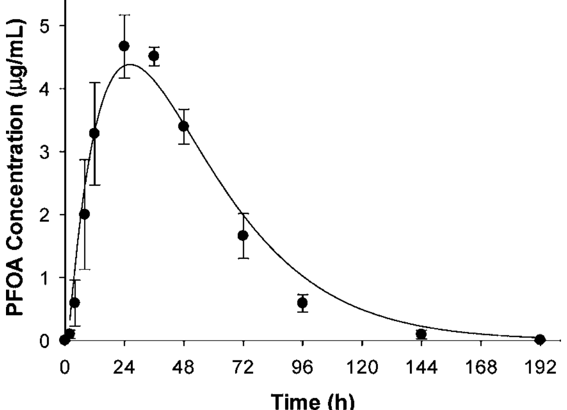

Figure 1. Plasma ^{14}C-PFOA concentrations (μg/mL) and time (h) data through 8 days after a single oral dose given to beef steers. Points represent mean concentrations of four animals from LSC analysis ± one standard deviation. The curve was predicted using a two-phase noncompartmental method.

corresponding individual plasma concentrations along with mean ± SD plasma concentrations are provided in the Supporting Information (Table S1). ^{14}C-PFOA absorption was rapid, with a calculated absorption half-life of 9.6 ± 1.1 h. The calculated maximal plasma ^{14}C-PFOA time occurred at 19.1 ± 1.7 h with a concentration of 4.9 ± 0.4 μg/mL. Plasma ^{14}C-PFOA decreased with a calculated first-order elimination half-life of 19.2 ± 3.3 h and had reached background by 8 days (192 h).

^{14}C-PFOA Concentrations in Urine, Feces, and Tissues and Mass Balance. Figure 2 shows the relationship between

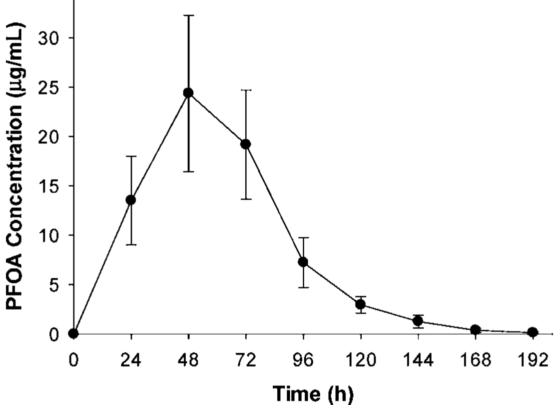

Figure 2. Urine ^{14}C-PFOA concentrations (μg/mL, mean ± SD) and time (h) data through 9 days after a single oral dose given to beef steers. Points represent mean concentrations for four animals from LSC analysis ± one standard deviation.

^{14}C-PFOA concentrations in urine and time, whereas Table 1 shows total mass and percentage of the total dose excreted at each time period analyzed by LSC. ^{14}C-PFOA concentrations in urine peaked at 24.4 ± 7.9 μg/mL on day 2, and >50% of the dose was excreted in the urine by day 2 (Table 1). Total mass (mg) of ^{14}C-PFOA excreted in urine also peaked on day 2 (Table 1). The cumulative percentage of ^{14}C-PFOA derived

Table 1. Total Mass (Mean ± SD) of ^{14}C-PFOA Excreted in Steer Urine and Feces As Calculated from LSC or Combustion Analyses[a]

	urine			feces[b]		
time (days)	total mass (mg)	% excreted	cumulative total %	total mass (mg)	% excreted	cumulative total %
1	77.4 ± 9.9	21.8 ± 2.8	21.8	1.3 ± 0.4	0.37 ± 0.1	0.37
2	132.9 ± 14.1	37.5 ± 4.0	59.4	4.7 ± 2.9	1.3 ± 0.8	1.7
3	86.1 ± 5.7	24.3 ± 1.6	83.7	6.4 ± 8.6	1.8 ± 2.4	3.5
4	35.9 ± 7.3	10.1 ± 2.0	93.8	2.1 ± 1.7	0.60 ± 0.5	4.1
5	16.9 ± 2.0	4.8 ± 0.6	98.6	0.70 ± 0.6	0.20 ± 0.2	4.3
6	4.5 ± 1.5	1.3 ± 0.4	99.9	0.84 ± 0.5	0.24 ± 0.1	4.5
7	2.0 ± 0.6	0.55 ± 0.2	100.6	0.15 ± 0.1	0.042 ± 0.03	4.6
8	0.60 ± 0.2	0.17 ± 0.06	100.7	0.08 ± 0.04	0.023 ± 0.01	4.6
9	0.34 ± 0.1	0.097 ± 0.04	100.7	<LOQ		
10−28	<LOQ			<LOQ		

[a]Corresponding excretion percentages for each time period (24 h) and cumulative percentages for mass balance purposes. Calculations are for all four steers. [b]Percent of PFOA excreted in feces could be overestimated due to contamination from the urine.

radioactivity that was recovered in the urine was 100.7 ± 3.3%, the entire dose, and radiocarbon levels were at background within 9 days of dosing.

Fecal concentrations of ^{14}C-PFOA were measurable through day 8 but decreased to background levels at 9 days. Figure 3 shows the relationship of ^{14}C-PFOA concentrations in feces with time, whereas Table 1 shows the total mass (mg) of ^{14}C-PFOA excreted along with the fractional and cumulative excretion of radiocarbon in feces as a percentage of the dose. The maximum ^{14}C-PFOA concentration in feces was 0.379 ± 0.25 μg/g wet wt on day 2. Total ^{14}C-PFOA elimination in feces peaked on day 2 but was minimal compared to the elimination via urine. The amount of ^{14}C-PFOA-derived radioactivity recovered in the feces was only 4.6 ± 2.8% of the dose. The larger relative standard deviations observed between animals in the feces data are most likely due to urine contamination, which occurred occasionally when collecting tubes were disconnected from incontinence bags by cattle stepping on or otherwise placing pressure on the tubes. No ^{14}C-PFOA was observed in the kidney, liver, or plasma at slaughter, which were considered to be the major compartments for PFOA distribution (data not shown).[24] No ^{14}C-PFOA was observed in the muscle of the steers.

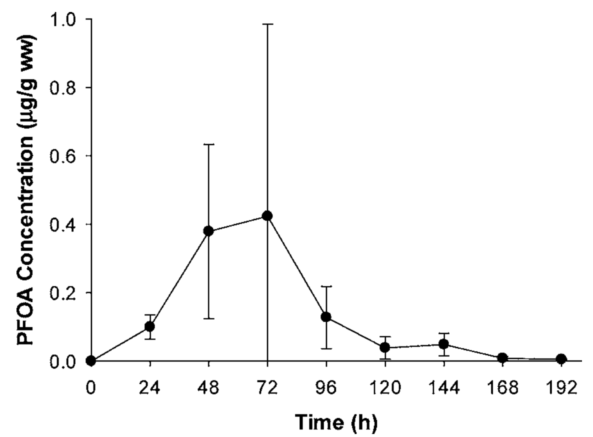

Figure 3. Fecal ^{14}C-PFOA concentrations (μg/g wet wt) and time (h) data through 8 days after a single oral dose given to beef steers. Points represent mean concentrations for four animals from LSC analysis ± one standard deviation.

^{14}C-PFOA Metabolism. There were no detectable ^{14}C-PFOA metabolites in plasma, urine, feces, or tissue that were analyzed by LC-MS/MS. To confirm that there was no metabolism, a regression analysis was completed comparing ^{14}C-PFOA concentrations derived from LSC data in the urine to PFOA concentrations calculated from LC-MS/MS data in the urine (Figure 4). The analysis gave a line with a slope of 1.152 (r^2 = 0.9956, $p < 0.01$, Figure 4) and an intercept of 0.075, indicating that the concentrations between analytical techniques were highly correlated and the radioactivity observed by LSC was only from ^{14}C-PFOA.

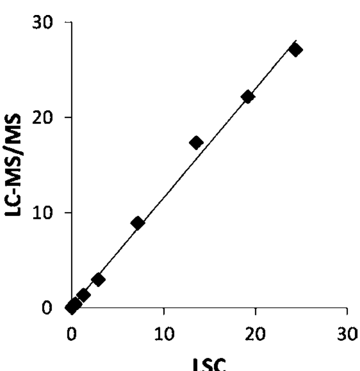

Figure 4. Regression analysis of concentrations (μg/mL) of PFOA in steer urine analyzed by LSC and LC-MS/MS (slope = 1.152, intercept = 0.075, r^2 = 0.9956, and $p < 0.01$).

DISCUSSION

This study was completed to determine the absorption, distribution, metabolism, and excretion of a single oral dose of ^{14}C-PFOA (1 mg/kg bw) in Lowline cattle. Beef cattle are an important ruminant animal system, and to this date there is no literature published on the fate of PFOA in ruminants. Other animal models have been studied with PFOA, such as monkeys, chickens, and rats.[25−33] Beef cattle are considered to be an important food animal in which to study the fate of environmentally persistent chemicals because of their high rate of production, they graze over large tracts of land, and they are fed grain and hay that could be contaminated.[6,34,35] Beef also makes up a large percentage of the American diet, which possibly contributes to our exposure to PFOA and other toxic chemicals.[6,34,35] The Angus breed (full and crossbred) is the

major breed in U.S. beef production with a majority of commercial cattle producers reporting their herds as Angus;[36−38] therefore, Lowline Angus, a small-stature Angus breed, was used in this study to simplify dosing and handling.[39]

At least one incident of cattle being raised on PFOA-contaminated pastures in the United States has also caused concern over human exposure to PFOA from edible meat tissues.[13] Using the highest PFOA concentrations from this contaminated location (Decatur, AL)[10,12,40] and an estimated cattle consumption of dry matter, soil, and water,[41] a high daily PFOA intake for a 300 kg steer can be calculated to be 0.014 mg PFOA/kg bw. This estimated high daily intake of PFOA is roughly 70 times lower than the PFOA dose administered to the steers in this dosing study. Nonetheless, the single oral doses of ^{14}C-PFOA were absorbed quickly and efficiently into the steers with an absorption half-life in plasma of 9.6 h and ∼100% of the dose being eliminated via the urine. The coadministered dose of PFOS (10 mg/kg bw) to three of the steers did not seem to hinder the uptake or excretion of the ^{14}C-PFOA dose (1 mg/kg bw) as there were no differences in ^{14}C-PFOA excretion or plasma half-life between the steer that was administered only ^{14}C-PFOA (no. 181) and the three steers administered both PFOS and ^{14}C-PFOA (no. 179, 180, and 182) (Table S1). Accordingly, simultaneous exposure to PFOS and PFOA in the field might not have an effect on an animal's ability to absorb or eliminate PFOA. The entire dose was systemically available as evidenced by complete elimination through the kidneys. No metabolites were observed in any of the samples analyzed in this study, confirming the recalcitrant nature of PFOA, and data are consistent with other studies that did not observe any metabolite formation in rats through defluorination or conjugation of PFOA.[3,27]

A first-order elimination half-life in plasma of 19.2 h indicates beef cattle can clear an acute dose of PFOA relatively quickly. When comparing the beef steers' half-life of PFOA to other animal systems, it can be seen that beef steers have the ability to eliminate PFOA more rapidly than other animal species. The half-lives of PFOA in female and male monkeys following a single intravenous dose (10 mg/kg bw) and in male monkeys following chronic oral exposure (10 mg/kg bw/day for 6 months) were 32.6, 20.9, and 19.5 days, respectively, and reflected first-order elimination.[30] With the comparable half-lives of 20.9 and 19.5 days for a single dose and a chronic dose in male monkeys, respectively, there does not seem to be any difference between elimination half-lives from acute and chronic exposure of PFOA in monkeys. Both half-lives are much longer than the 19.2 h observed in the plasma of beef steers. Elimination rates are also much shorter in beef steers than in 1-day-old and 6-week-old male chicks that received a subchronic dose of PFOA for 3 and 4 weeks, respectively. The male chickens had first-order elimination half-lives of 3.9−4.6 days.[31,32] The half-lives of PFOA in the blood of male rats have been reported to range from 4.4 to 13.4 days after single doses.[25−29] These half-lives are longer than the half-life for the steers used in this study. The subchronic half-life in male rats is 16 days after a 12 week dosing.[30] However, the PFOA elimination rates in female rats following a single intraperitoneal dose of 50 mg/kg bw or a single intravenous dose of 20 mg/kg bw are similar to beef steers, that is, 24 and 2 h, respectively.[25,27] Humans appear to have the longest PFOA half-life of all studied species at 3.8 years.[5] This long half-life is thought to be due to human's high renal absorption, low renal clearance, and specific complement of transporter proteins.[42]

Kudo et al. observed that differences in excretion rates of PFOA in female and male rats could be linked to circulating sex hormone levels.[27] They observed that castrated male rats cleared PFOA almost as rapidly as female rats. When the castrated male rats were administered testosterone, their clearance of PFOA decreased to that of the control.[27] Whereas when intact male rats were administered estradiol, their clearance of PFOA increased significantly above the control.[27] Kudo et al. also observed that estradiol and testosterone controlled expression of specific organic anion transporting polypeptides (OATPs) and organic anion transporters (OATs) in the kidney, which are thought to be related to reabsorption of PFOA from the kidneys to the liver or elimination of PFOA through the kidneys via urine, respectively.[27,42,43] The expression of OATPs in the kidney is known to be controlled by the levels of testosterone present.[42,43] Higher levels of testosterone allow for more expression of OATPs, which in turn is likely to result in increased reabsorption of PFOA into the liver from the kidneys.[42,43] Given that steers are castrated young bulls, low levels of testosterone following castration may be related to the rapid elimination rates of PFOA measured in the present study because reabsorption from the kidneys to the liver is unlikely to occur due to decreased expressions of OATPs. All of these factors could contribute to the rapid elimination of PFOA from beef steers, although more studies would be needed to determine the exact mechanisms underlying the short half-life calculated in beef steers.

Humans have the slowest renal elimination of PFOA compared to other animal species. The longer half-life of PFOA in humans is due to the high percentage (>99%) of reabsorption of PFOA in the kidneys; this, however, was not observed in female rats or the steers from this current study. Male rats, at 94% reabsorption, are similar to humans in their ability to reabsorb PFOA.[42] The differences in elimination of PFOA by female and male rats are not observed in humans.[42] Clearance of PFOA by reabsorption into the blood or secretion into the urine is determined by the specific transporters that are present and their levels. The affinity that these transporters have for PFOA and the rate at which they move into the membrane also affect the reabsorption or elimination of PFOA. Humans and rats have different variations and locations for expression of specific OATs and OATPs, so reabsorption pathways are different.[42] There is minimal information available in publications on the specific OATs and OATPs expressed in cattle, so it is difficult to speculate on the specific reason for the fast elimination of PFOA in these steers.

The dominant pathway of exposure to PFOA for humans depends on their local environment. For example, if humans are exposed to only background levels of PFOA, then the major pathway of exposure is through food, but if humans are exposed to a contaminated water source, then it is more likely that the major pathway will be the water source and not the diet.[44] Vestergren et al. proposed that if humans are exposed to only background levels of PFOA, then approximately 80% of the PFOA exposure is from food, 1−2% is from water, about 10% is through dust inhalation, and roughly 8% is through other sources (i.e., clothing, carpeting, and cookware).[44] A duplicate diet study by Fromme et al. observed that females and males in Germany on average intake 269 ng PFOA/day, with the highest intake being approximately 800 ng PFOA/day from their diet.[45] However, when exposed to a highly contaminated water source >70% of human exposure to PFOA is from the water and only about 20% is from food sources.[44] Trudel et al. reported food

and water exposure (15−20%) and PFOA migrating into food from packaging (∼50%) as separate categories of human exposure.[46] When Trudel et al.'s percentages as a total are compared to Vestergren et al.'s proportions, they are similar, indicating that the majority of PFOA exposure is from food, whether it be directly from the food or indirectly through the food packaging.[44,46] PFOA is found in various food groups including vegetables (i.e., carrots and potatoes) and fruits (i.e., strawberries) as well as wheat flour, fish, and shellfish.[2,15,16] Levels of PFOA in each of these food groups tended to be higher than in beef. However, the daily intake of each food is important when assessing total exposure from food.[2,15,16] Also, preparation of food is important when measuring exposure to PFOA. For example, how and in what medium food is prepared could exacerbate or mitigate exposures.[47]

It was observed in this study that beef steers, which make up a large portion of the commercially available domestic beef supply, have the ability to eliminate PFOA quickly and that PFOA probably would not accumulate to a high extent in the edible tissues of beef steers following acute exposure. Because of the fast clearance of a single dose of PFOA from beef cattle, PFOA-contaminated meat is unlikely to contribute significantly to human exposure to PFOA. PFOA residues in marketed beef products are likely due to low accumulation in the meat from chronic exposure of cattle to PFOA and from food packaging. However, this study takes into account only the absorption and excretion of a single oral dose and does not reflect the effects from chronic exposure of beef cattle to PFOA, which was outside the scope of this study. Residual levels of PFOA have been found in beef on the retail market, indicating that beef currently are either being exposed to PFOA in the field or PFOA is migrating from food packaging to beef. Due to the short half-life of PFOA measured in beef steers, potentially contaminated cattle from chronic exposure could be remediated through relatively short depuration times (1−2 weeks) during which a majority of the PFOA would be cleared from the body, thus reducing any food safety concerns; however, currently there are no regulations on PFOA residues in cattle. Food-packaging contamination can be addressed by altering the type of packaging currently used in the industry. If PFOA contamination in beef is from exposure to PFOA in the field, then chronic PFOA exposure should also be assessed in the future.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table showing the individual steer plasma concentrations for each time point, mean plasma concentrations for each time point, and individual steer plasma elimination half-lives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

SAFETY. The toxicity of PFOA is believed to be minimal at the concentrations utilized for the study; however, all treated animals and corresponding samples produced from the study were considered radioactive and were handled and disposed of by proper laboratory methods.

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