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Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey

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ABSTRACT

Ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate has been developed as a processing aid used in the manufacture of fluoropolymers. The absorption, distribution, elimination, and distribution (ADME) and kinetic behavior of this substance has been evaluated in rats, mice, and cynomolgus monkeys by oral and intravenous routes of exposure and studied in both plasma and urine. The test substance is rapidly and completely absorbed in both rats and mice and both *in vivo* and *in vitro* experiments indicate that it is not metabolized. The test substance is rapidly eliminated exclusively in the urine in both rats and mice, with rats eliminating it more quickly than mice (approximately 5 h elimination half-life in rats, 20 h half-life in mice). Pharmacokinetic analysis in monkeys, rats, and mice indicate rapid, biphasic elimination characterized by a very fast alpha phase and a slower beta phase. The beta phase does not contribute to potential accumulation after multiple dosing in rats or monkeys. Comparative pharmacokinetics in rats, mice, and monkeys indicates that the rat is more similar to the monkey and is therefore a more appropriate rodent model for pharmacokinetics in primates.
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1. Introduction

The widespread presence of long-chain perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) has spurred a move to alternative fluorinated substances which have more favorable environmental and biological properties, most notably rapid elimination from living systems (Ritter, 2010; Buck et al., 2011; US EPA, 2006). Per- and poly-fluorinated ether carboxylates have been developed as alternative polymer processing aids for the aqueous emulsion polymerization of tetrafluoroethylene (TFE) and other fluorinated monomers in the synthesis of fluoropolymers (Buck, 2015; Buck et al., 2011; Gordon, 2011). They have replaced ammonium perfluorooctanoate which was historically used for this purpose (Feiring, 1994).

Ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate has been developed as a processing aid used in the manufacture of fluoropolymers. The acid form is a liquid and the ammonium salt is a solid at ambient temperature (20 °C). Both are infinitely soluble in water above the pK_a (2.84) of the acid. An aqueous solution is made and used in the fluoropolymer manufacturing process. The processing aid is either captured for re-use or thermally destroyed during fluoropolymer processing (Brothers et al., 2009). Some fluoropolymer aqueous dispersions that contain the processing aid are used to coat surfaces such as metal for making non-stick cookwear. The fluoropolymer is sintered onto the substrate surface at temperatures >265 °C. As shown in Table 1, the processing aid decomposes at 150–160 °C.

The objective of the present study was to determine the absorption, distribution, metabolism and elimination (ADME) profile following oral and/or intravenous dosing of ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, [Fig. 1, $CF_3CF_2CF_2OCF(CF_3)COOH{\cdot}NH_3$, CAS# 62037-80-3] in the rat, mouse and cynomolgus monkey. The chronic toxicology and carcinogenicity of this test substance was recently described (Rae et al., 2015), as was the aquatic toxicology (Hoke et al., 2015).

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Table 1
Physical-chemical properties of the test substance.

	Test substance		
	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate salt Aqueous solution ~85 wt% in water	Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate salt Ammonium salt dried powder 99.4% pure	2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)-propanoic acid Protonated acid 98% pure
Molecular formula	$C_6F_{11}O_3^- \cdot H_4N^+$	$C_6HF_{11}O_3 \cdot H_3N$	$C_6HF_{11}O_3$
Molecular weight	347.08	347.08	330.05
CAS number	62037-80-3	62037-80-3	13252-13-6
Appearance	Clear colorless liquid	Solid powder	Clear, colorless liquid
Melting point °C			<−40
Sublimation point °C		~130–140 °C	
Boiling point °C			129
Decomposition point °C		~150–160	
Dissociation constant (pK_a)			2.84
Density (g/mL, 20 °C)	1.569	~1.7	1.690
Aqueous surface tension mN/m (1 g/L)	66.3		59.4 mN/m
Vapor pressure (Pa, 20 °C)		~0.01	100–300
Solubility (g/L)	Aqueous infinite	Aqueous infinite	Aqueous infinite
K_{ow}			2.0 (ChemSilico) 1.34 (SPARC)
Hydrolysis	Stable (pH 4, 7, 9 at 50 °C)		

2. Materials and Methods

2.1. Test substance information

The test substance, ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, [$CF_3CF_2CF_2OCF(CF_3)COOH \cdot NH_3$, CAS# 62037-80-3],was prepared, characterized and provided by DuPont Chemicals and Fluoroproducts. The properties of the test substance, an aqueous solution of the test substance, and the acid form of the test substance are shown in Table 1. Solvents and reagents were purchased from VWR International (Radnor, PA, USA). Stable isotope labeled perfluorooctanoic acid, perfluoro-*n*-[1,2-$^{13}C_2$]-octanoic acid, [$CF_3(CF_2)_5{}^{13}CF_2{}^{13}COOH$], was purchased from Wellington Laboratories (Ontario, Canada).

2.2. Animal welfare

The experiments described below complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9CFR) and the 1996 National Research Council Guidelines from the Guide for the Care and Use of Laboratory Animals. All studies conducted by or for DuPont Haskell Global Centers adhere to the principles that (1) all experiments do not unnecessarily duplicate previous experiments, (2) whenever possible, procedures used have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals, (3) animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, (4) methods of euthanasia used conform with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia. DuPont Haskell Global Centers and the contract research organization used for work involving animals are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

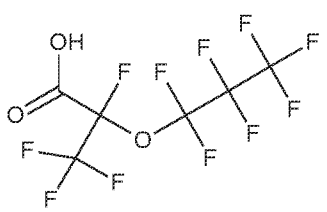

Fig. 1. 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid.

2.3. Rat hepatocyte metabolism

Hepatocytes were prepared from male and female Sprague–Dawley rats by two-stage collagenase perfusion (Seglen, 1976). Hepatocyte incubations were carried out in 20 mL glass vials containing 3 mL of hepatocyte suspension. Cell concentration was 1×10^6 cells/mL with 5 μM test substance for the clearance reactions and 5×10^6 cells/mL with 50 μM test substance for the metabolite identification experiment. Hepatocyte suspensions were preincubated for 5 min at 37 °C with gentle orbital shaking and initiated by addition of the test substance. All dose solutions were prepared in acetonitrile. The amount of organic solvent introduced into the incubation solution did not exceed 1% of the total reaction volume. Incubation vials were sealed with PTFE-lined septum caps and incubated for a total of 120 min. Reactions were terminated by transferring 250 μL of sample to microcentrifuge tubes pre-filled with 500 μL of acetonitrile at 0, 30, 45, 60, 90, and 120 min. The tubes were vortexed for 10 min, centrifuged at 15,000 × *g* for 5 min and supernatant transferred to HPLC vials for analysis by HPLC-MS. Heat inactivated hepatocytes were used as negative controls.

2.4. Single dose pharmacokinetic studies in rats and mice

Three Sprague–Dawley rats of each sex per dose level were dosed orally with 10 or 30 mg/kg of the test substance in water with a dose volume of 4 mL/kg. Rats were fasted overnight prior to dosing and through the first 2 h of blood collection. Blood (approximately 0.1 mL per sample) was collected from the tail vein prior to dosing and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h after dosing. Additional blood samples were collected once daily on test days 2–7. Samples were centrifuged to generate plasma and then frozen at −20 °C until analyzed by HPLC-MS.

Forty-five male and forty-five female Crl:CD1 (ICR) mice per dose level were dosed orally with 10 or 30 mg/kg of the test substance in water with a dose volume of 4 mL/kg. Blood was collected *via* serial sacrifice ($n = 3$ mice per time point) prior to dosing and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h after dosing. Additional blood samples were collected once daily on test days 2–7. Samples were centrifuged to generate plasma and then frozen at −20 °C until analyzed by HPLC-MS.

2.5. Rat and cynomolgus monkey intravenous plasma pharmacokinetics

A total of 6Crl:CD SD rats (3 males and 3 females) per dose level were assigned to the study. The animals were fasted overnight prior to dosing and through the first 2 h of blood collection. The low dose group received a single 10 mg/kg intravenous (*via* tail vein) bolus of the test substance formulated in sterile phosphate buffered saline, pH 7.6 at a dose volume of 1 mL/kg. The high dose group received a single 50 mg/kg dose of the test substance formulated in the same manner as the low dose. Blood (approximately 0.1 mL per sample) was collected from the tail vein prior to dosing and at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h after dosing. Additional blood samples were collected once daily on test days 2–7. Samples were centrifuged to generate plasma and then and then frozen at −20 °C until analyzed by HPLC-MS.

A total of 6 non-naïve cynomolgus monkeys (3 male and 3 female) were assigned to the study. The animals were fasted overnight prior to dosing and through the first 4 h of blood sample collection. All monkeys received a single 10 mg/kg intravenous bolus of the test substance delivered *via* a peripheral vein. The dose was formulated in sterile phosphate buffered saline, pH 7.6 at a dose volume of 2 mL/kg. Blood (approximately 0.5 mL per sample) was collected from the femoral vessel prior to dosing and at 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h post dose. Additional blood samples were collected once daily on test days 3–21. Samples were centrifuged to generate plasma and then frozen at −20 °C and until analyzed by HPLC-MS.

2.6. Absorption, distribution, and elimination in the rat and mouse

The conduct of this study was designed to comply with the Tier 1 requirements of U.S. EPA, OPPTS 870.7485—Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998). Because the test substance was not metabolized in rat hepatocytes nor were any metabolites observed in other studies, a radiolabeled form of the test substance was not required. Rodents were housed individually in glass metabolism units during the study. Rats were fasted overnight and through the first 2 h of blood collection. Mice were not fasted. Five rodents of each sex and species received the test substance orally at 3 (mice) or 30 (rats) mg/kg. Rodents were returned to individual metabolism units following dosing. Urine and feces were collected on dry ice for 24 h prior to dosing, and then at 0–6 h, 6–12 h, 12–24 h, and every 24 h until 168 h after dosing. At the end of the experiment (168 h post dose), rodents were sacrificed by CO_2 asphyxiation followed by exsanguination. The following tissues were collected: liver, fat, gastrointestinal tract, gastrointestinal tract contents, kidney, spleen, and whole blood. The carcass was retained. Tissues and carcass were saved for analysis in the event that the dose was not recovered in the excreta. Individual cages were rinsed with deionized water.

To determine if metabolism had occurred, mass spectra data from the urine samples were screened for suspected metabolites manually and automatically for unexpected metabolites using the IntelliExtract™ (ACD/Labs, Toronto, Ontario, Canada) control-comparison data processing tool.

2.7. Sample processing and analysis

2.7.1. HPLC-MS analysis of urine and plasma

The urine samples were prepared for analysis by pipetting 950 μL of 15% acetonitrile in HPLC grade water solvent and 50 μL of sample. The samples were then mixed with a vortex mixer for 1 min prior to analysis. As necessary, additional sample dilutions were performed using the 15% acetonitrile in HPLC grade water solvent to ensure that the sample responses were within the calibration curve.

The plasma samples were prepared for analysis by pipetting 150 μL acetonitrile into a 1.7 mL microcentrifuge tube, and pipetting 50 μL of sample. The sample tubes were then vortexed for 1 min and centrifuged at 14,000 RCF for 30 min. After centrifugation, 100 μL of sample supernatant was placed into a HPLC vial and 400 μL of HPLC grade water was added and mixed. As necessary, additional sample dilutions were performed using the 15% acetonitrile in HPLC grade water solvent to ensure that the sample responses were within the calibration curve. An internal standard (^{13}C-PFOA) was added to all prepared samples and standards at a concentration of 5 ng/mL. The LOQ was established from the lowest calibration standard at 1 ng/mL multiplied by the sample dilution factor. The sample dilution factor for all plasma samples was 20× which corresponds to an LOQ of 20 ng/mL. The lowest calibration standard had a response of at least 5 times the blank.

Samples were chromatographically separated on an Agilent Model 1200HPLC using a Zorbax® RX-C18 2.1 × 150 mm ID, 5 micron particle size and analyzed using an Applied Biosystems API 4000 mass spectrometer equipped with a Turbo Spray ion source set to negative mode. Mobile phase A was 0.15% acetic acid in water and mobile phase B was 0.15% acetic acid in acetonitrile. The separation was isocratic at 70:30 A:B. The separation was followed by a 2.5 min wash at 100% B and equilibration at initial conditions for 2.5 min. The flow rate was 600 μL. The mass spectrometer mode was MRM monitoring the test substance (329.1 → 284.9 m/z) and the internal standard (415 → 370 m/z) with a dwell time of 150 ms.

The urine samples were analyzed using the method described above but with a Phenomenex® Luna C8 150 × 2 mm column, 5 micron particle size and the addition of 0.15% triethylamine to the mobile phase. An internal standard was not used for the urine analysis or the rat single oral dose experiment. The plasma samples from the rat single oral dose experiment were analyzed using essentially the same method but utilizing a Micromass Quattro Micro mass spectrometer using an Agilent Model 1100HPLC.

2.7.2. Pharmacokinetic calculations

Pharmacokinetic parameters were calculated using PK Plus, a module of GastroPlus (Simulations Plus, Lancaster, California, USA). The data was entered and the goodness-of-fit was calculated for both one and two compartment models. In all cases the two-compartment model provided a better fit to the observed data. The absorption rate constant, the alpha elimination rate constant, the beta elimination rate constant, and the volumes of distribution of both the central and peripheral compartment were calculated for the two-compartment model. These pharmacokinetic constants were also used to model the effects of repeated exposure using GastroPlus.

3. Results

Analyzed concentrations of the test substance are reported as nanograms of the solubilized anion 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate per milliliter of plasma or urine. All data are presented as mean and standard deviation.

3.1. Rat hepatocyte metabolism

There was no difference in clearance rate between hepatocytes and heat inactivated hepatocytes, and no metabolites were detected. The test substance is not metabolized by rat hepatocytes.

3.2. Pharmacokinetic studies in rats, mice, and monkeys

3.2.1. Oral pharmacokinetics (rat and mouse)

Absorption of the test substance was rapid in both the rat and mouse following a single 10 or 30 mg/kg oral dose with absorption times of between 0.2 to 0.5 h (Table 2). The test substance was rapidly eliminated from plasma and exhibited biphasic kinetics. The rapid elimination phase of the male rat pharmacokinetic curve has a half-life of approximately 3 h. The female alpha phase half-life was faster with a half-life of 0.2 h. These half-lives are so rapid that the alpha phase elimination is completed by 24 h (Table 2; Fig. 2, 10 mg/kg; Supplementary material, 30 mg/kg). The beta phase half-life was approximately the same in male and female rats (approximately 70 h) following an oral dose (Table 2). Alpha phase plasma elimination half-life of the test substance from the mouse was slower than rats (male mice: 6 h, female mice: 5 h). The beta-phase elimination half-life was approximately 37 h in male mice and 24 h in female mice (Table 2,Fig. 2, 10 mg/kg; Supplementary material, 30 mg/kg).

3.2.2. Intravenous pharmacokinetics (rat and monkey)

The test substance was rapidly eliminated in monkeys following a single 10 mg/kg intravenous dose with an alpha phase half-life of approximately 2 h in both male and female monkeys and a beta-phase half-life of 64 h (males) and 80 h (females). The rapid elimination phase was complete by 24 h. (Table 2, Fig. 2). Based on the presence of a beta phase in the rodent data, blood was collected from monkeys for a total of 21 days to ensure that there would be enough data points to adequately determine any beta phase half-life. The test substance was not detected in any sample collected after the 384 h (16 day) collection in monkey.

The pharmacokinetics in rats following a 10 mg/kg intravenous dose are similar to the pharmacokinetics following the 10 mg/kg oral dose in monkeys, with a biphasic elimination curve where the rapid elimination half-life was 4 h in males and 0.4 h in females (Table 2). The beta phase elimination half-lives appear to be considerably different (males 90 h; females, 23 h) between the sexes. However, this may be due to the uncertainty in the data in the beta phase since this apparent difference was not observed after oral dosing (Table 2; Fig. 2, 10 mg/kg; Supplementary material, 50 mg/kg).

3.3. Absorption, distribution, and elimination in the rat and mouse

Following single oral dose administration of the test substance, 98 ± 1.4% (male) and 95 ± 8.6% (female) of the administered dose was accounted for in urine from the 0–12 h time interval. At the conclusion of the study at 168 h, the total accumulated amount of the test substance detected in urine was 103% ± 2.7% and 100% ± 6.4% of the dose for male and female rats, respectively (Table 3). Elimination of the test substance *via* urine was rapid and accounted for essentially all of the administered dose for both male and female rats (Fig. 3). Negligible levels of the test substance were detected in feces from male (1% ± 1.0%) and female rats (1% ± 0.6%), likely due to contamination with urine. Cage wash accounted for 1% ± 0.5% and 5% ± 5.1% of the administered dose for male and female rats, respectively (Table 3).

The total amount of test substance recovered in all compartments at 168 h after dosing was 105 ± 2.2% and 106 ± 1.4% male and female rats, respectively (Table 3). The elimination half-life for the test substance in male and female rats following a single oral dose at 30 mg/kg was estimated to be 3 and 8 h, respectively. Tissues were not analyzed because the entire dose was recovered in the urine, feces, and cage wash.

Following single oral dose administration of the test substance to mice, 31% ± 5.4% and 39% ± 5.6% of the administered dose was accounted for in urine from the 0–12 h time interval. At the conclusion of the study at 168 h, the total accumulated amount of the test substance detected in urine was 90% ± 6.9% and 92% ± 6.0% of the administered dose for male and female mice, respectively (Table 3). Elimination of the test substance *via* urine accounted for a majority of the administered dose for both male and female mice. The low levels of test substance detected in feces from male (2% ± 1.0%) and female mice (2% ± 0.6%) were likely due to contamination with urine. Cage wash accounted for 10% ± 4.0% and 6% ± 3.2% of the administered dose for male and female mice, respectively (Fig. 4). The total amount of test substance recovered in all compartments at 168 h after dosing was 101% ± 3.2% (male) and 99% ± 3.2% (female) (Table 3). The elimination half-life for the test substance in male and female mice, following a single oral dose at 3 mg/kg, was estimated to be 21 and 18 h, respectively. Tissues were not analyzed because the entire dose was recovered in the urine, feces, and cage wash.

Samples of urine from the rat and mouse studies were evaluated for metabolites using LC-MS Mass spectra data were

Table 2
Pharmacokinetic parameters.

		Rat, intravenous		Rat, oral		Mouse, oral		Cynomolgus monkey, intravenous	
Constant	Units	Male	Female	Male	Female	Male	Female	Male	Female
Absorption									
Rate constant (k_a)	1/h	NA	NA	3.30	1.52	3.83	3.11	NA	NA
Time	h	NA	NA	0.21	0.46	0.18	0.22	NA	NA
Alpha phase									
Elimination rate constant	1/h	0.20	1.72	0.25	2.78	0.12	0.15	0.30	0.37
Half-life	h	3.6	0.4	2.8	0.2	5.8	4.6	2.3	1.9
Beta phase									
Rate	1/h	7.8E-03	3.1E-02	9.6E-03	1.0E-02	1.9E-02	2.9E-02	1.1E-02	8.7E-03
Half-life	h	89.1	22.6	72.2	67.4	36.9	24.2	64.1	79.6
Volume of distribution									
Central	L/kg	0.168	0.178	0.142	0.057	0.117	0.148	0.068	0.056
Peripheral	L/kg	0.155	1.508	0.161	2.462	0.130	0.078	0.029	0.021

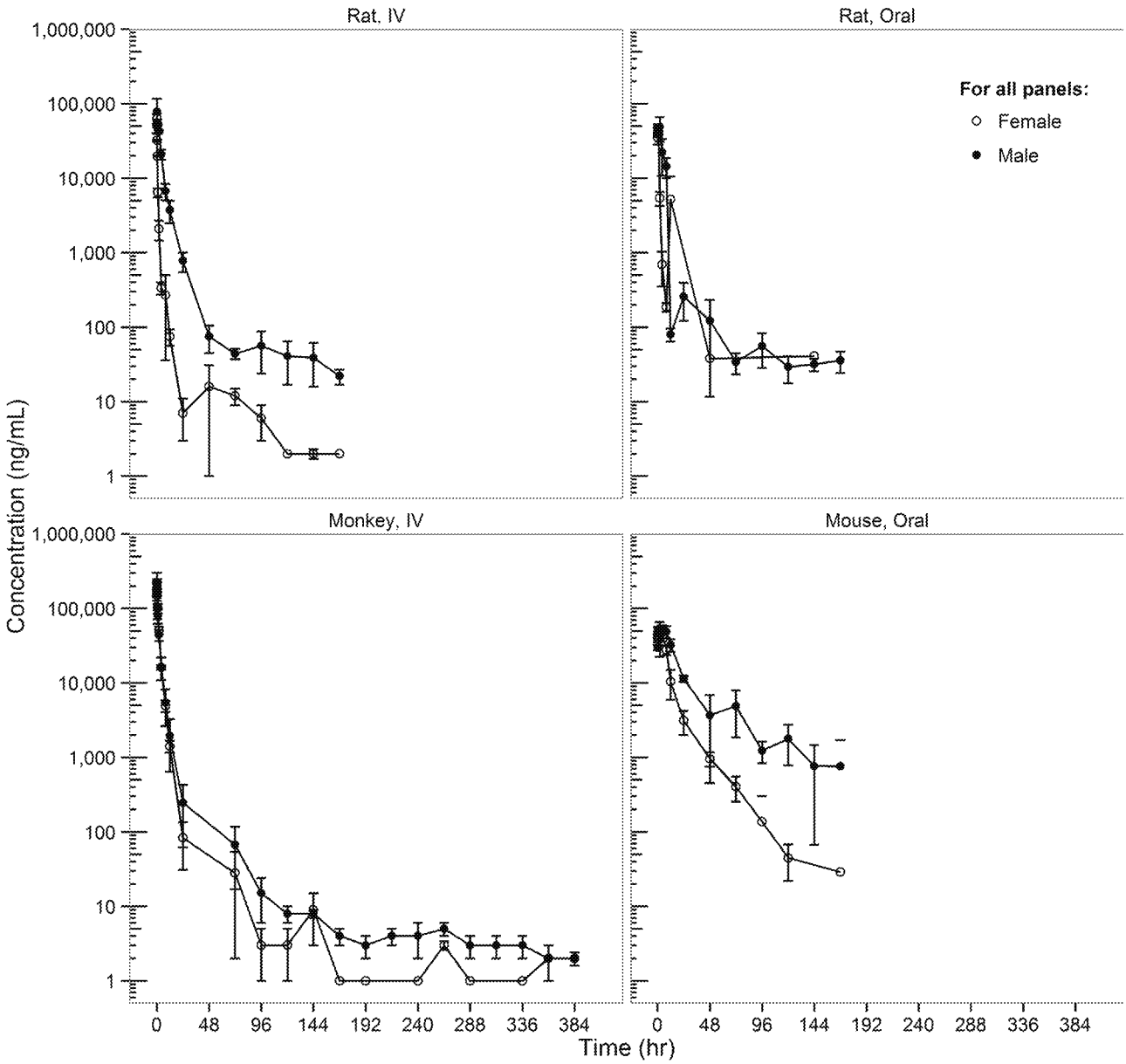

Fig. 2. Concentration of the test substance (ng/mL) in plasma from rats following a 10 mg/kg intravenous dose; rats following a 10 mg/kg oral dose; cynomolgus monkeys following a 10 mg/kg intravenous dose; and mice following a 10 mg/kg oral dose. The last blood collection time was 168 h in rodents and 504 h (21 days) in monkey. The analyte was not detected after the 384 h (16 day) collection in monkey. Data points without error bars indicate that the analyte was detected in less than three of the individual samples at that time point. In the mouse dataset, the standard deviation was approximately equal to the average for the 96 h female and the 168 h male point. These points are labeled with a horizontal bar (¯) above the symbol. The limit of detection was 20 ng/mL for oral data sets and 1 ng/mL for the intravenous data sets.

Table 3
Material balance of the test substance following oral dosing.

	Rat (30 mg/kg)				Mouse (3 mg/kg)			
	Male		Female		Male		Female	
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)
Urine	103	2.7	100	6.4	90	6.9	92	6.0
Feces	1	1.0	1	0.6	2	1.0	2	0.6
Cage wash	1	0.5	5	5.1	10	4.0	6	3.2
Total	105	2.2	106	1.4	101	3.2	99	3.2

screened for suspected metabolites manually and automatically for unexpected metabolites using the IntelliExtract™ (ACD/Labs, Toronto, Ontario, Canada) control-comparison data processing tool. In all cases, there was no evidence of metabolism observed in any of the samples by either method. Only the anionic form of the test substance was detected. This finding, taken with near complete recovery of the dose in urine confirms that the test substance was rapidly absorbed and eliminated without metabolism in urine following oral dosing in rodents.

4. Discussion

The kinetic behavior of the test substance has been evaluated in multiple species by oral and intravenous routes of exposure and repeated measurements in both plasma and urine. The test substance is rapidly and completely absorbed as evidenced by the recovery of 100% of the dose in the urine of both rats and mice over the range of oral doses tested (3 to 30 mg/kg). *In vitro* hepatocyte metabolism indicates that this test material is not metabolized,

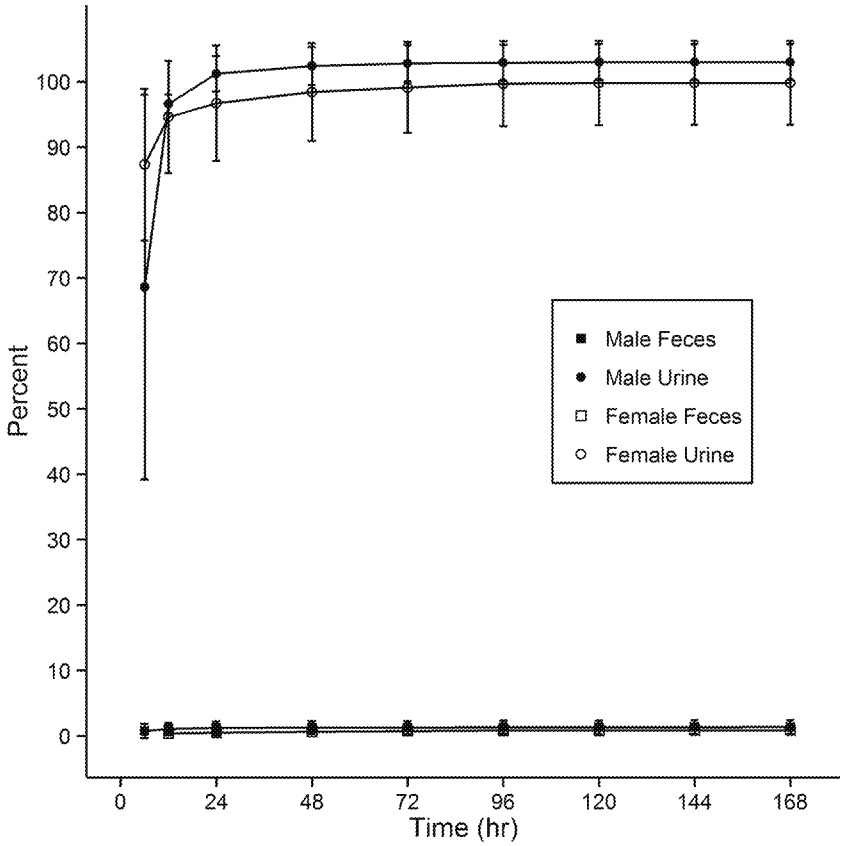

Fig. 3. Cumulative percent recovery of the test substance in the excreta of rats dosed with 30 mg/kg.

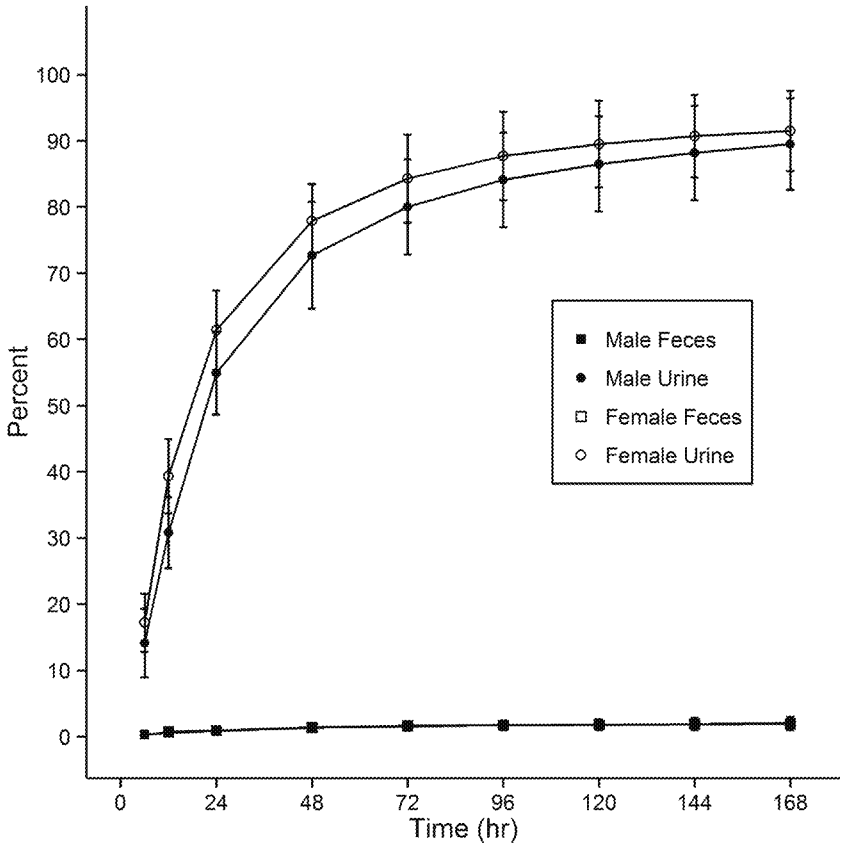

Fig. 4. Cumulative percent recovery of the test substance in the excreta of mice dosed with 3 mg/kg.

and this is further supported by analysis of urine from rats and mice in which no metabolites were detected. This was expected since structurally similar perfluorinated carboxylic acids like perfluorohexanoic and perfluorooctanoic acid are also not metabolized (Gannon et al., 2011; Kennedy et al., 2004). Since this resistance to metabolism is intrinsic to the strength of the carbon-fluorine bond (Key et al., 1997), it is doubtful that metabolism if this material would occur in any mammal, including humans.

The volume of distribution in the central compartment is low (less than 0.2 L/kg) in rats, mice, and monkeys, indicating that the test substance is most likely confined primarily to the blood volume and does not preferentially partition into the tissues. This is also supported by the observation that total body clearance occurred rapidly in rats and mice. In rats, both plasma kinetics and urinary elimination kinetics indicate that the nearly the entire dose is eliminated within 12–24 h. Because the urinary elimination rate is very rapid, the slight sex difference observed in the plasma kinetics was not readily apparent in the urine kinetics. The elimination rate is slower in mice than in rats and this is observed in both the plasma kinetics and the urinary elimination kinetics. The pharmacokinetics following an intravenous dose in rats or monkeys also shows rapid elimination, consistent with the data presented above. Blood samples from monkeys were collected for 21 days following dosing, but the test substance was not detected after Day 16. This lack of detection after Day 16 (384 h) is consistent with the average calculated beta elimination half-life of ~72 h since at 384 h, between 5 and 6 half-lives have passed and only 1–3% of the initial beta phase plasma concentration is expected to remain.

The pharmacokinetics of this test material differ between rats and mice, the two most commonly used animal models in toxicology. To determine which rodent species might better model the pharmacokinetics in primates, including humans, the pharmacokinetics of the test substance was also examined in cynomolgous monkey. The results described above indicate that the pharmacokinetics in rats are more similar to the monkey. The mouse shows somewhat slower alpha and beta elimination phases than the rat or monkey, and the two compartment nature of the pharmacokinetic curve in rats and monkey is less apparent in mouse. Because of these findings, the rat is a more representative model for pharmacokinetics in primates than the mouse.

Although the data shows a biphasic elimination, the alpha phase elimination is so fast that the beta phase is negligible and does not contribute to accumulation (Toutain and Bousquet-Mélou, 2004) in either the rat or monkey. In both of these species, the concentration is typically 1000 times less than the C_{max} by 24 h (Fig. 2). When the pharmacokinetics of repeated dosing is modeled, the steady state plasma concentration is achieved following the first dose. In contrast, the slower elimination rate in the mouse means that the mouse requires approximately 4 consecutive doses before reaching steady state in plasma (Fig. 5). The physiological reason for the biphasic elimination is not known. Some perfluorinated carboxylic acids were shown to be substrates for organic anion transporters, a class of transporters responsible for renal reabsorption (Yang et al., 2010). A mechanism by which

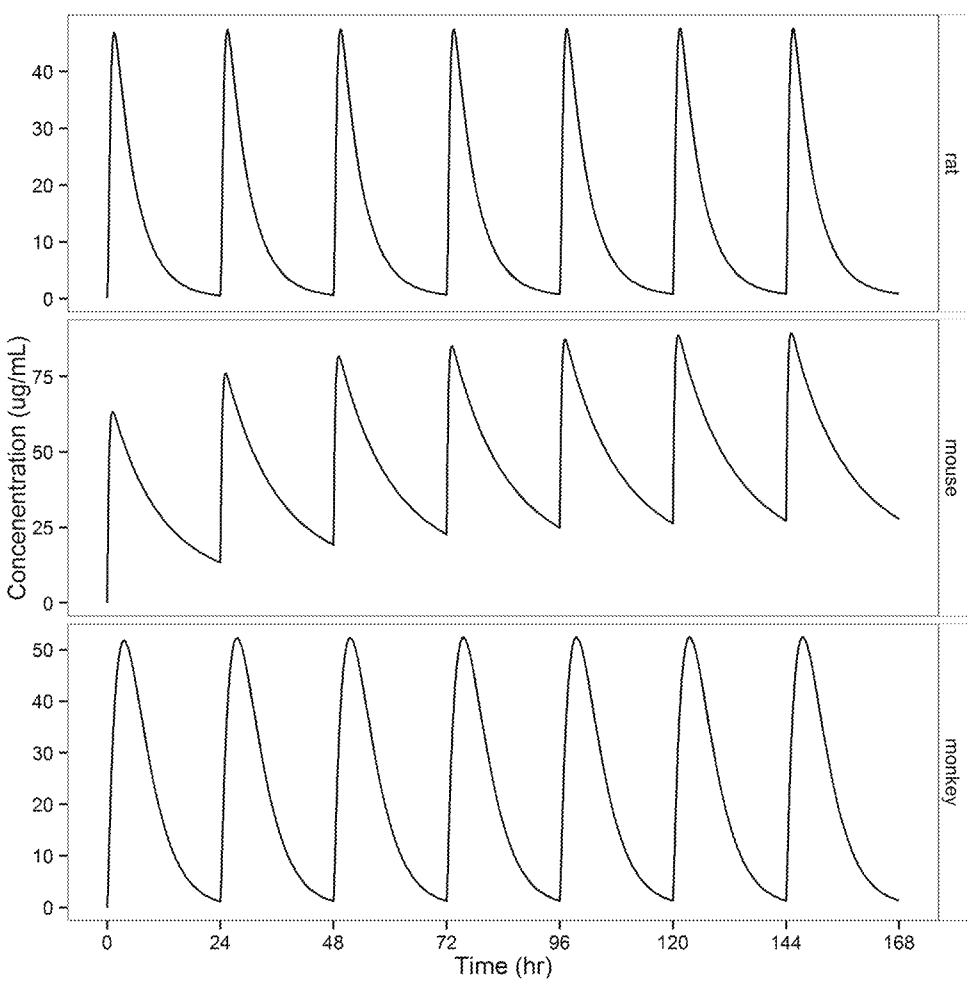

Fig. 5. 2-compartment model of seven consecutive oral doses of 10 mg/kg/day dosing in rats, mice, and monkey.

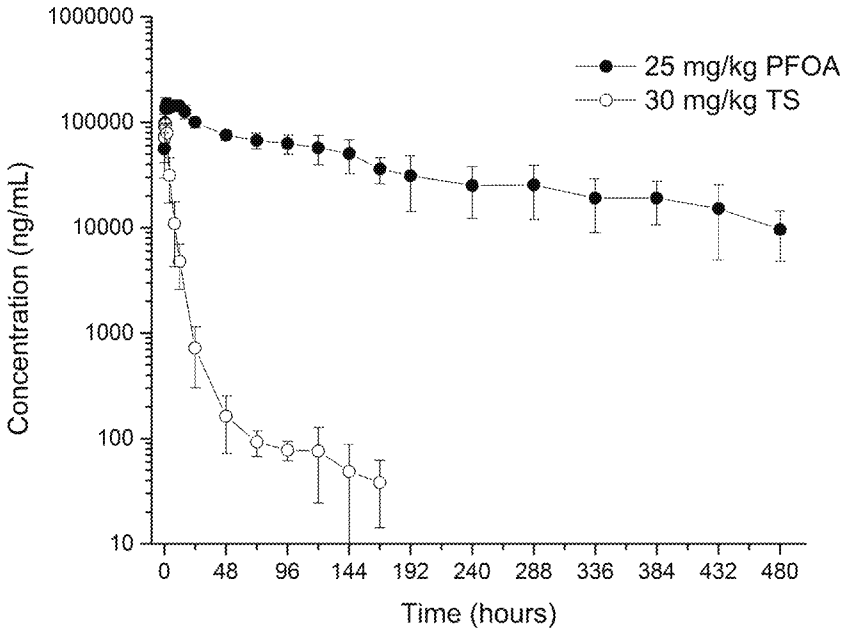

Fig. 6. Comparison of plasma concentration (ng/mL) of test substance (30 mg/kg) and PFOA (25 mg/kg) in male rats following a single oral dose. The PFOA plasma concentration data was reported previously in Kemper and Jepson, 2003.

test material is reabsorbed back into the bloodstream could result in a biphasic elimination profile in plasma, but this mechanism, if applicable here, does not contribute significantly to the overall elimination of this test substance. The rapid elimination observed for this test substance is in contrast to the behavior of perfluorooctanoic acid, PFOA, (Fig. 6) which demonstrates considerably slower elimination in rats (Kudo and Kawashima 2003); (Lau et al., 2007); (Olsen et al., 2007a; Kemper and Jepson 2003), mice (Lau et al., 2006; Lou et al., 2009), hamsters (Hundley et al., 2006), and primates (Butenhoff et al., 2004), along with a remarkable sex difference in rats and hamsters (Hundley et al., 2006).

In summary, the test substance in this study is rapidly and extensively absorbed, not metabolized, and eliminated completely and rapidly in the urine of rats and mice. Oral and intravenous pharmacokinetic experiments also show rapid plasma elimination in rodents and cynomolgus monkeys. Comparative studies in rats, mice, and monkeys indicate that the rat may be more appropriate than the mouse as a rodent model of pharmacokinetics in primates.

Conflict of interest

The experiments presented here were performed by E. I. DuPont de Nemours and Company, Inc. with the exception of the *in vivo* monkey experiments which were paid for by E. I. DuPont de Nemours and Company but performed by MPI Research. The authors of this paper are employees of The Chemours Company or E. I. DuPont de Nemours and Company, Inc. The authors of this paper affiliated with The Chemours Company were employees of E. I. DuPont de Nemours and Company at the time this work was performed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tox.2015.12.006.

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