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RESEARCH ARTICLE

Toxicokinetics of ammonium perfluorohexanoate

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Abstract

Excretion patterns and rates of ammonium perfluorohexanoate (APFHx) after administration of a single and multiple (14 days) oral dose(s) at 50 mg/kg to male and female mice and rats were examined. The test substance was [^{14}C]-labeled APFHx. After a single oral administration, total excretion was rapid, with mean recoveries of over 90% of the dose at 24 hours after administration, irrespective of gender or species. The major route of elimination was via the urine (means of percentage recovery between 73.0 and 90.2% of the dose), followed by the feces (means of percentage recovery between 7.0 and 15.5% of the dose). Elimination via expired air was negligible. For the multiple dose tests, multiple (13 daily doses) oral administration of APFHx was followed by a single oral administration of [^{14}C]-APFHx. Excretion was rapid, with mean recoveries of over 90% of the administered dose (mean values >95% of the ultimately recovered material) at 24 hours after dosing, irrespective of gender or species. The major route of elimination was via the urine (means of percentage recovery between 77.8 and 83.4% of the dose), followed by the feces (means of percentage recovery between 9.6 and 12.9% of the dose).

Keywords: Toxicokinetics, ammonium perfluorohexanoate, APFHx, C6

Introduction

Ammonium perfluorooctanoate (APFO) has long been used as a polymerization aid to manufacture fluorocarbon resin and fluorocarbon rubber. During its use, it is rapidly transformed to perfluorooctanoate (PFO). PFO can also be produced as a by-product of the production of certain fluorotelomers (particularly those now being phased out of commercial production in the United States and Europe, with a carbon chain of eight carbon atoms), which have been used as water and oil repellents.

PFO has been reported to persist in the environment (Kennedy et al., 2004; Lau et al., 2007; Prevedouros et al., 2006). In response to this property of the compound, industrial companies have been working to replace PFO and 8-carbon (C8) telomers with alternatives. In particular, industrial companies have investigated the manufacturing and use properties of perfluorocarboxylate (PFC) with a carbon chain length of six carbon atoms and related C6 telomers. Because of the acceptable performance characteristics of these substances, industrial companies are also studying the health and environmental profile of these promising alternatives.

Kudo et al. (2006) compared PFCs of different carbon chain lengths, reporting that those with seven carbon atoms or fewer were pharmacologically cleared from mice more quickly than carboxylates of C8 and greater lengths. Chengelis et al. (2009) and Loveless et al. (2009) examined the toxicity of perfluorohexanoate (PFHx, C6) and concluded that the toxicity was low, compared to C8.

The aim of this study was to examine the bioaccumulation potential of PFHx in greater depth.

Methods

Test guidelines

Environmental Protection Agency (EPA), Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Health Effects Test Guidelines, 850.7485 Metabolism and Pharmacokinetics were the test guidelines used for this study.

Study design

This study was designed as a single-dose study, followed by a multiple-dose study. The multiple-dose study was

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conducted in two phases: phase 1, in which the elimination of the test compound was observed, and phase 2, in which the organ distribution was observed. This study design complies with the guidelines of the United States EPA.

Test material

Test materials were labeled and unlabeled ammonium perfluorohexanoate (APFHx). Unlabeled APFHx was a 50% aqueous solution received from Daikin Industries, Ltd. (Osaka, Japan). Carbon 14 (^{14}C)-labeled APFHx was supplied by GE Healthcare, Ltd., (Waukesha, WI) and stored at–20°C in the dark. The radiolabeled material was supplied as a nominal 100% APFHx powder (purity, 99.6%; high-performance liquid chromatography analysis) with a stated specific activity of 6.59 MBq/mg. The terminal carbon, which binds two oxygen atoms at the end of the carbon chain, was labeled.

Animals

Male and female Sprague-Dawley (Crl:CD[SD]) rats and CD-1 mice were supplied by Charles River Ltd. (Margate, UK) Ltd. All animals were 7–10 weeks of age at the time of dosing.

Single oral administration

Compound and dose administration

The formulations were administered by gastric gavage at a target volume of 10 mL/kg body weight to achieve a target concentration of 50 mg/kg body weight (target radioactive dose: 3–5 MBq/kg body weight).

Two male and 2 female rats, and 2 male and 2 female mice, received a single oral administration of [^{14}C]-APFHx.

Urine and feces samples from each animal were collected into containers cooled with solid carbon dioxide for the following periods: 0–6 hours after dosing (urine only), 6–24 hours after dosing, and then at 24-hour intervals until 72 hours after dosing. Cages were washed with water at the time of each feces collection, and the water was saved as sample. Expired air was collected over 0–24 and 24–48 hours after dosing. The expired air was collected into an absorbing solution of ethanolamine:ethanediol (3:7; v/v).

At the end of the 72-hour collection period, each animal was humanely killed by CO_2 asphyxiation. The gastrointestinal tract and residual carcass from each rat were weighed and retained as samples.

Levels of total radioactivity were determined in each sample collected.

Multiple oral administrations

Compound and dose administration

The formulations were administered by gastric gavage at a target volume of 10 mL/kg body weight to achieve a target concentration of 50 mg/kg body weight (target radioactive dose: 3–5 MBq/kg body weight).

APFHx was orally administered at a target daily dose of 50 mg/kg body weight for 13 consecutive days, followed by a single oral dose of [^{14}C]-APFHx (day 14). The dose was administered to 8 male and 8 female rats (4 animals each for the phase I and II studies) and 16 male and 17 female mice (4 animals each for the phase I study, and 12 animals each for the phase II study; 1 female mouse died as a result of gavage error).

Phase I

After administration of the last dose (day 14), urine samples were collected into containers cooled with solid carbon dioxide from each animal for the following periods: 0–6 hours after dosing, 6–24 hours after dosing, and then at 24-hour intervals until 168 hours after dosing. Feces samples were collected into containers cooled with solid carbon dioxide at 24-hour intervals until 168 hours after dosing. Cages were washed with water at the time of each feces collection, and the water was saved as sample. At the end of the 168-hour collection period, each animal was humanely killed by CO_2 asphyxiation and body weight was measured. A terminal whole blood sample was collected into heparinized tubes (approximately 5–10 mL for rats and 0.5–1 mL for mice from the vena cava and heart, respectively).

Phase II

After administration of the last dose (day 14), serial blood samples were taken from all rats (approximately 0.4 mL). Samples were collected from the tail vein at 12 hours after dosing. A terminal whole blood sample was taken (approximately 5–10 mL) from the vena cava at 24 hours after dosing. All blood samples were collected into heparinized tubes. Plasma was separated from the whole blood samples. After taking the blood sample, each animal was humanely killed by CO_2 asphyxiation and body weight was measured.

Blood samples were taken from the hearts of mice (approximately 0.5–1 mL). Samples were taken from 4 male and 4 female mice at predosing and 12 and 24 hours after dosing. All blood samples were collected into heparinized tubes. Plasma was separated from all blood samples by centrifugation. Before sampling, each animal was humanely killed by CO_2 asphyxiation and body weight was measured.

The gastrointestinal tract, white fat, kidney, liver, spleen, and residual carcass were separately weighed and retained separately as samples.

Levels of total radioactivity were determined in each sample collected.

Preparation of samples for total radioactivity analysis

Liquid samples Duplicate aliquots of liquid samples were made up to 1 mL with water (if necessary) and mixed with scintillation fluid. Duplicate aliquots of each blood sample were combusted using a PerkinElmer 307 Sample Oxidizer (PerkinElmer Life Science and Analytical Instruments Inc, Sears Green, UK).

Solid samples Feces samples were weighed, an appropriate amount of water was added, and total weight was recorded before homogenization. Duplicate aliquots of fecal material from each sample (approximately 0.2–0.3 g) were combusted using a PerkinElmer 307 Sample Oxidizer. Carcass samples were minced and then analyzed as previously described for feces. All gastrointestinal-tract and tissue samples were finely chopped using scissors and then analyzed as previously described for feces.

All aliquots were combusted using a PerkinElmer 307 Sample Oxidizer. The [^{14}C]-carbon dioxide generated was absorbed and mixed with scintillant before analysis by liquid scintillation counting. The efficiency of oxidation of the test samples was determined relative to [^{14}C]-standard oxidation efficiencies at regular intervals during each series of oxidations. Combustion of standards showed recovery efficiencies of >97%.

Quantification of radioactivity

All samples prepared in scintillation fluid were subjected to liquid scintillation counting for 5 minutes, together with representative blank samples, using a Packard TR 2100 Liquid Scintillation Analyzer with automatic quench correction by an external method. Where possible, samples were analyzed in duplicate and allowed to heat- and light-stabilize before analysis. Before calculation of each result, a background count was determined and subtracted from each sample count. For scintillation counting, a limit of reliable determination of 30 disintegrations per minute (dpm) above background has been instituted in these laboratories. At the specific activity used, the limit of reliable measurement was approximately 0.06 μg equiv/g for a tissue and blood weight of approximately 0.1 g. The calculated limit of reliable measurement was 0.1 μg equiv/g for a mean plasma weight of approximately 0.05 g. Where results have been derived from data below the limit of reliable determination, this fact was noted.

Results

Single oral administration

Excretion of total radioactivity after a single oral administration of [^{14}C]-APFHx to male and female rats and mice are shown in Figure 1. Irrespective of sex or species, excretion of radioactive material was rapid, with mean recoveries of >90% of the dose at 24 hours after dosing. The major route of elimination was via the urine (means of recovery: 73.0–90.2% of the administered dose), followed by the feces (means of recovery: 7.0–15.5%). Elimination via expired air was negligible.

At 72 hours after dosing, mean recoveries of total radioactivity were 97.4 and 100.8% in male and female rats, respectively, with approximately 0.2% remaining in the gastrointestinal tract and carcass.

At 72 hours after dosing, mean recoveries of total radioactivity were 95.4 and 97.3% in male and female mice, respectively, with approximately 0.6–0.9% remaining in the gastrointestinal tract and carcass.

Multiple oral administration (14 daily doses)

Irrespective of sex or species, in multiple oral administrations (13 daily doses) of APFHx followed by a single oral administration of [^{14}C]-APFHx, excretion of total radioactivity was rapid, with mean recoveries of >90%

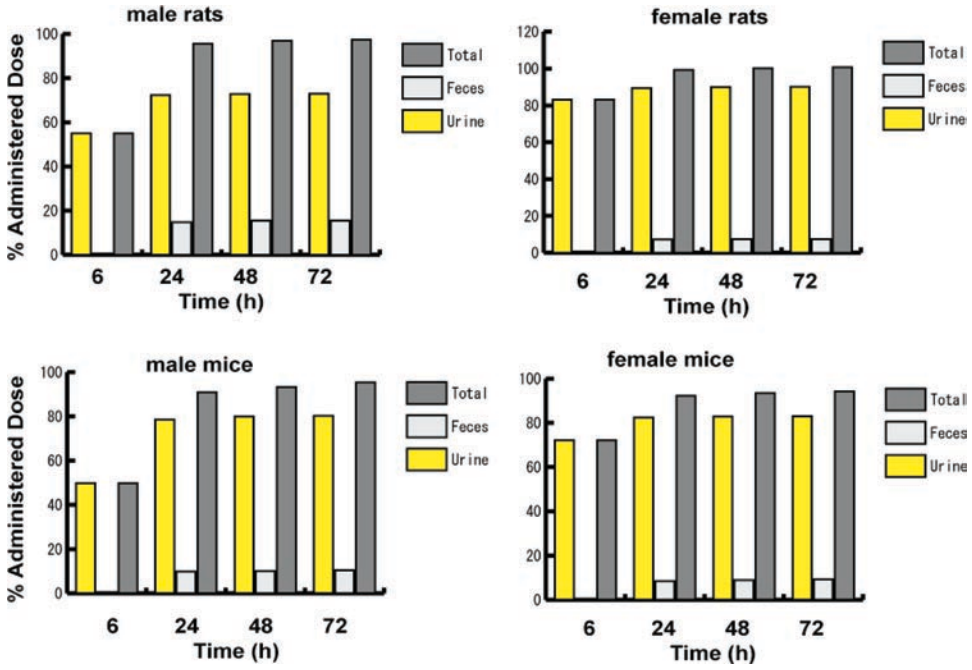

Figure 1. Recovery of total radioactivity after single oral administration.

of the dose administered (mean values >95% of the ultimately recovered material) at 24 hours after dosing. The major route of elimination was via the urine (means of recovery: 77.8–83.4%), followed by the feces (means of recovery: 9.6–12.9%), indicating that the majority of the administered dose had been absorbed (Figure 2).

At 168 hours after dosing in rats and mice, radioactivity in most tissues was generally very low or below the detection limit. Tissue concentrations were below those of blood concentrations, with the exception of the liver, in which the concentration was approximately 4–8 times higher than that of the circulating blood level. Elevated levels of measurable radioactivity in the liver were consistent with its role in metabolism and excretion (Table 1).

At 12 hours after dosing, mean plasma concentrations were 0.8 and 0.4 mg/mL in male and female rats, respectively. At 24 hours after dosing, mean plasma values decreased to 0.5 and 0.3 mg/mL in male and female rats, respectively. At 12 hours after dosing, mean plasma concentrations were 1.3 and 1.0 mg/mL in male and female mice, respectively. At 24 hours after dosing, mean plasma values decreased to 1.0 and 0.5 mg/mL in male and female mice, respectively (Table 2).

Discussion

Our investigation on rats and mice showed that PFHx administered as a single dose of APFHx was rapidly eliminated. Its body distribution in rats and mice was similar. Further, its elimination showed the same pattern in the repeated-dose study. This result is similar to findings reported by Chengelis et al., who examined the toxicokinetics of PFHx in rats and monkeys and found that it was rapidly eliminated in both species (Chengelis et al., 2009a).

Our findings suggest that either the association of PFHx with toxicokinetic mechanisms seen for PFO and higher chain length PFCs is either very weak or that the mechanism is different altogether. Further investigations are warranted to determine which of these hypotheses is true.

In contrast to PFHx, elimination rates of PFO in rats were highly sex dependent: Female rats showed rapid elimination, whereas male rats did not (Ohmori et al., 2003; Kudo et al., 2001; Vanden Heuvel et al., 1991; Lau et al., 2007; Kennedy et al., 2004).

Katakura et al. (2007) reported that in a study of PFO in rats, organic anion-transporting polypeptide Oatp1 and the organic anion transporter, Oat3, of proximal tubule cells of the kidney may play a role as transporters of PFO, suggesting that these transporters reabsorb PFO. They discussed the theory of Oat1p transport, noting that the gender-dependent distribution in rats may be explained by the fact that the distribution of Oatp1 in rat kidney is sex dependent, although that of Oat3 is not (Kato et al., 2002). PFHx does not show the same gender dependency in rats.

A study in rats comparing the elimination of PFCs with carbon chain lengths ranging from C7 to C10 showed that those with a longer carbon chain are eliminated more slowly (Ohmori et al., 2003). Yang et al. (2009) reported that among PFCs with chain lengths between 6 and 10 carbon atoms, those with a longer carbon chain have higher affinities to Oatp1a1. A study of rat kidneys showed that Oatp1a1 levels were higher in male than in female rats. It would fit into this framework that PFHx, which has a C6 chain and, therefore, is shorter than those PFCs studied by Yang et al., may have an even lower affinity to Oatp1a1.

Weaver et al. (2010) considered that Oatp1al plays a key role in the reabsorption of PFCs, particularly for C8,

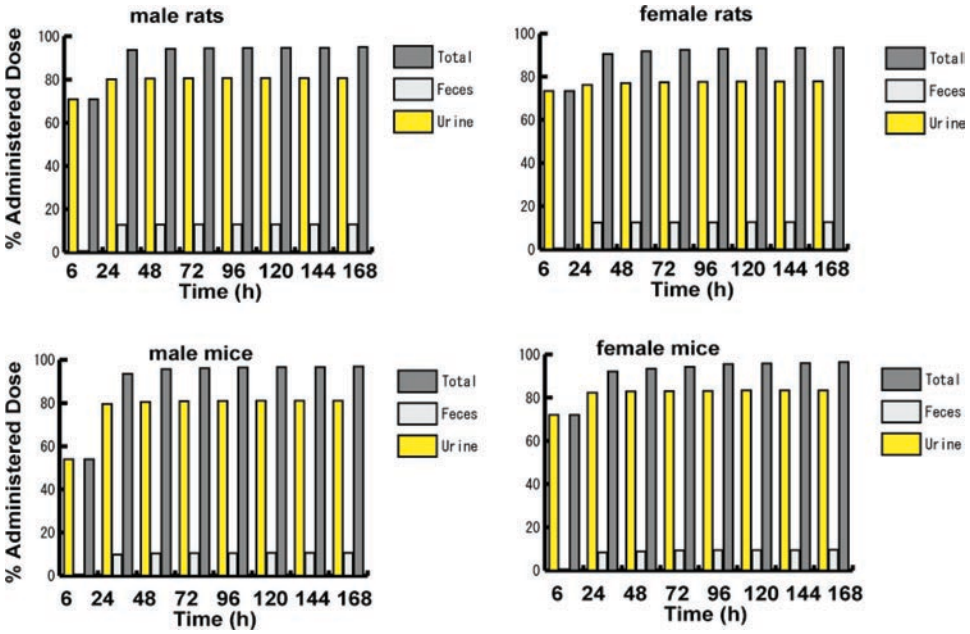

Figure 2. Recovery of total radioactivity after multiple oral administration. Results are expressed as percent administered dose. Total includes cage wash and expired air.

Table 1. Concentration of total radioactivity in tissues at 168 hours after dosing.

Sample	Male rats	Female rats	Male mice	Female mice
White fat	0.03	0.03	0.03	0.04
Kidneys	0.11	0.13	0.05	0.05
Liver	1.16	0.85	0.70	0.61
Spleen	0.03	0.04	0.02	0.02
Gastro-intestinal tract	0.03	0.03	0.04	0.03
Carcass	0.10	0.10	0.06	0.04
Whole blood	0.15	0.16	0.17	0.17

Results are expressed as %percent administered dose.

Table 2. Plasma concentrations of total radioactivity following multiple oral administration.

Time point (hours)	Male rats	Female rats	Male mice	Female mice
12	0.8 (0.1)	0.4 (0.1)	1.3 (0.7)	1.0 (0.5)
24	0.5 (0.0)	0.3 (0.1)	1.0 (0.3)	0.5 (0.1)

Results are expressed as %percent administered dose. All values are presented as mean (standard deviation) of 4 animals.

C9, and C10 PFCs. At the same time, it did not influence the transportation of C6. It is unclear whether this was attributable to the low affinity, as mentioned above, or simply to the low retention time, reducing the opportunity for transport and reabsorption.

In mice, elimination rates of PFO are less likely to be dependent on sex (Lau et al., 2006), but the distribution of Oatp1 in the kidney and liver is sex dependent (Cheng and Klaassen, 2008). Therefore, the distribution of Oatp1 can probably not account for the pharmacokinetics of PFO. It is likely that other transporters are involved.

For perfluorheptanoate (PFHp, C7), Weaver et al. (2010) indicated that there is no uptake by human embryonic kidney cells, which lack the Oat1 transporter. This may be because C7 is hydrophilic and, therefore, less likely to permeate cell membranes without a transporter.

With regard to PFCs in general, 1) the effect of change of the electric potential of short-chain substances is lessened (Kleszczynski and Skladanowski, 2009) and 2) suppression of gap junctional intercellular communication also becomes weaker as the carbon chain becomes shorter (Upham et al., 1998).

In PFHx, which has a relatively short chain, the cell membrane is either not traversed because it is hydrophilic and lacks a transporter, or the ion is not taken up because it has no intracellular usefulness.

Further, the measured bioaccumulation of PFHx is low. The value of the apparent inhibition constant [Ki(app)] is 1,857.8 in Chinese hamster ovary (CHO) cells (Yang et al., 2009). Since Kudo et al. reported that beta oxidation activity in the liver of rats and mice is not in proportion to the carbon chain length of PFCs, but to its concentration in the liver (Kudo and Kawashima, 2003; Kudo et al., 2006), and because the bioaccumulation of PFHx is low, its toxic effects, such as beta-oxidation activity, can be considered low.

Although it seems likely that Oat1 plays no part in the toxicokinetic mechanisms of PFHx, it is probably important to clarify whether the mechanisms of PFHx are replicas of other, secondary mechanisms of PFO and higher chain PFCs or whether the mechanisms are entirely different.

Declaration of interest

The author reports no financial conflicts of interest. The author alone is responsible for the content and writing of this paper.

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