Combined developmental and perinatal/postnatal reproduction oral toxicity study of ammonium perfluorohexanoate in mice

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Introduction

The purpose of this study was to test for toxicity of ammonium perfluorohexanoate (APFHx) to pregnant female mice and embryo and fetus development. Exposure to APFHx was to the dam from time of implantation to closure of the hard palate and continuing through lactation. Observation of toxic effects was continued through sexual maturity of the F1 generation mice because manifestations of effects induced during this period may be delayed in the offspring.

Methods

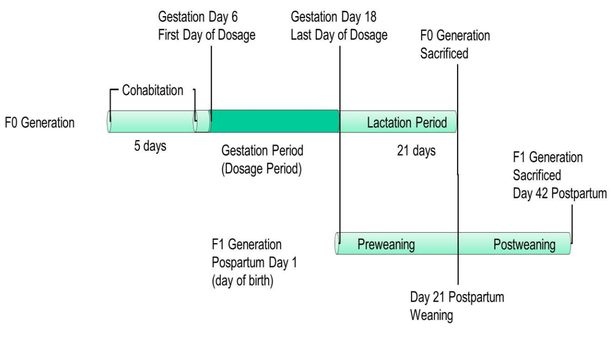
Pharmacokinetic

Sixty three female Crl:CD(1CR) mice were randomly assigned to three dosage groups (Groups I through III), twenty one mice per group. Solutions of the test substance ammonium perfluorohexanoate (APFHx and vehicle (Reverse osmosis deionized water) were administered orally via gavage once to these mice on Day 1 of study (DS 1) at doses of 35, 175 and 350 mg/kg/day, the dose volume was 5 mL/kg. Checks for viability were made at least twice daily. Clinical observations were recorded prior to dosage administration and prior to sacrifice. Body weights were recorded weekly during the acclimation period and once on the day of dosage administration (for main study mice only). All main study mice were sacrificed [by carbon dioxide asphyxiation] according to the blood sample collection timepoints and discarded without further evaluation. On the day of dosage administration, blood samples were collected from three mice per group at each timepoint for main study mice. Samples were collected prior to dosage and at approximately 30 minutes, 2, 4, 6, 8 and 24 hours post dosage. Blood was collected from the vena cava after sacrifice. The samples were transferred into un-coated red top tubes and spun in a centrifuge. The resulting serum was transferred into appropriately labeled polypropylene tubes and immediately frozen on dry ice and maintained frozen until shipment for analysis. Serum concentration data were obtained from 3 animals at each time point, and mean values were used to generate a composite PK profile

Developmental and Perinatal/Postnatal Reproduction Toxicity Study

APFHx was administered via gavage, once daily to pregnant CD-1 mice from day 6 of presumed gestation (DG 6) through DG 18 at dosages of 0, 7, 35, 100, 175, 350 and 500 mg/kg/day. These results come from two sequential studies. The results shown in the tables are the compilation of the two sequential studies (the controls have been combined from the two studies) The dosage volume was 5 mL/kg. After completion of the 20 day postpartum period (PPD 20), F0 generation female mice were euthanized and liver samples were collected from 5 mice per group for pharmacokinetic analysis; mice that did not deliver a litter were sampled on DG 23. Additionally, on PPD 20, all pups not selected for continued evaluation were euthanized. F1 generation mice selected for continued evaluation were sacrificed on PPD 41. Liver samples were collected from five mice per sex per group for pharmacokinetic analysis. The following parameters were evaluated for F0 generation female mice: viability, clinical observations, body weights, body weight changes, maternal behavior, litter observations, natural delivery, pup body weights, dam and pup necropsy observations. The following parameters were evaluated for F1 generation male and female mice: viability, clinical observations, body weights, body weight changes, eye opening, age of sexual maturity and necropsy observations.

SCHEMATIC OF STUDY DESIGN AND PROPOSED SCHEDULE



Results

Acute pharmacokinetic study

Table 1 – Pharmacokinetic Exposure Parameters of PFHxA in Female Crl:CD1(ICR) Mice Serum Following Oral Gavage of APFHx

Group No.	Dose Level (mg/kg)	Tmax (h)	T1/2 (h)	Cmax (µg/mL)	Cmax/Dose	AUC(0-inf) (µg •h/mL)	AUC(0-inf)/ Dose
I	35	0.50	0.89	96.6	2.76	1.00	5.10
II	175	0.50	1.24	330	1.88	6.41	6.54
III	350	0.50	0.92	454	1.30	1.65	5.41

Table 2 - Natural delivery observation

Dosage Group Dosage (mg/kg/day)	unit	0	7	35	100	175	350	500
Mice assigned to natural delivery	N	40	20	20	20	20	20	20
Pregnant	N	39	17	20	19	20	19	17
Delivered a litter	N(%)	39(100.0)	17(100.0)	20(100.0)	19(100.0)	20(100.0)	19(100.0)	17(100.0)
Included in analyses	N	39	17	19	19	20	19	17
Duration of gestation	Mean±S.D.	19.7±0.6	19.8±0.8	19.8±0.4	19.9±0.2	19.7±0.5	19.9±0.6	20.2±1.1
Pups delivered (total)	N	470	213	232	250	241	245	177
	Mean±S.D.	12.1±3.5	12.5±3.0	12.2±1.7	13.2±1.6	12.0±2.1	12.9±3.8	11.1±2.4
mice with still born pups	N(%)	2(5.1)	0(0.0)	0(0.0)	0(0.0)	1(5.0)	5(26.3)	7(41.2)**
mice with no liveborn pups	N	0	0	0	0	0	0	1(5.9)
gestation index	%	100	100	100	100	100	100	94.1
	N/N	39/39	17/17	20/20	19/19	20/20	19/19	16/17
Mice with all pups dying Days 0-3 postpartum	N(%)	1(2.6)	1(5.9)	0(0.0)	0(0.0)	0(0.0)	2(10.5)	5(31.3)**
Mice with all pups dying Days 4-20 postpartum	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Delivered a litter with one or more liveborn pups	N	39	17	19	19	20	19	16
Pups Delivered（total）	N	470	213	232	250	241	245	177
Liveborn	N(%)	466(99.1)	211(99.1)	232(100.0)	250(100.0)	238(98.8)	232(94.7)	150(84.7)**
Stillborn	N(%)	4(0.9)	0(0.0)	0(0.0)	0(0.0)	3(1.2)**	5(2.0)	16(9.0)**

Table 3 – Eye Opening and Sexual Maturation

Dosage Group Dosage (mg/kg/day)	unit	0	7	35	100	175	350	500
Eye Opening								
Litters delivered	N	39	17	19	19	20	19	17
litters tested	N	38	16	19	19	20	17	11
Criterion Day	Mean±S.D.	13.8±0.7	13.8±0.6	13.4±0.6	14.2±0.8	14.0±0.8	14.9 ± 1.1**	14.5±1.0
Sextual maturation								
Male Mice	N	40	20	20	20	20	19	20
Preputial separation	Mean±S.D.	29.3±1.5	29.0±1.1	28.0 ± 1.0**	29.8±2.2	28.4±1.0	29.3±2.3	29.4±1.7
Body weight at separation	Mean±S.D.	23.9±2.7	23.9±2.1	22.8±2.3	23.2±2.1	22.6±2.4	22.4±2.3	22.8±2.7
Female Mice	N	40	20	20	20	20	20	20
Vaginal patency	Mean±S.D.	26.6±2.4	25.8±2.9	25.8±1.6	27.5±1.1	25.2±1.9	27.6±2.0	27.5±2.1
Body weight at verginal patency	Mean±S.D.	17.4±2.2	16.2±2.9	17.2±1.7	17.4±2.8	15.7±2.4	16.3±1.7	17.6±2.2

CONCLUSIONS

1 PFHx was rapidly absorbed (Cmax reached within 30 minutes) and in general was not quantifiable at 24 hours after dose administration. The terminal elimination half-life of PFHx ranged from 0.889 to 1.24 hours, and was dose-independent. The increase in Cmax (from 96.6 to 454 µg/mL) was lower than proportional, whereas the increase in AUC(0-inf) (from 178 to 1893 h*µg/mL) was proportional, to the increase in dose from 35 to 350 mg/kg.

2. The results in this study and indicate a very minimal effect of PFHx at 100 mg/kg/day with a clear no-observable-effect-level at 35 mg/kg/day

3 On the basis of these data from this study, the maternal no-observable-adverse-effect-level (NOEL) for APFHx is 100 mg/kg/day. The NOAEL in the F1 generation is 100 mg/kg/day. None of the effects observed in the pups preweaning persisted into the postweaning period.

Figure 1 – F0 Generation Maternal Body Weight Change

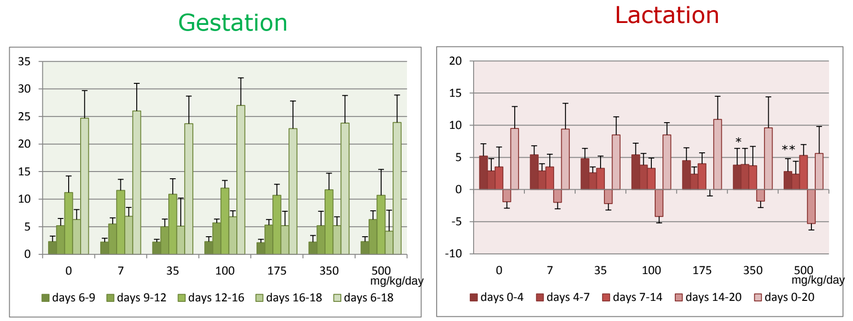
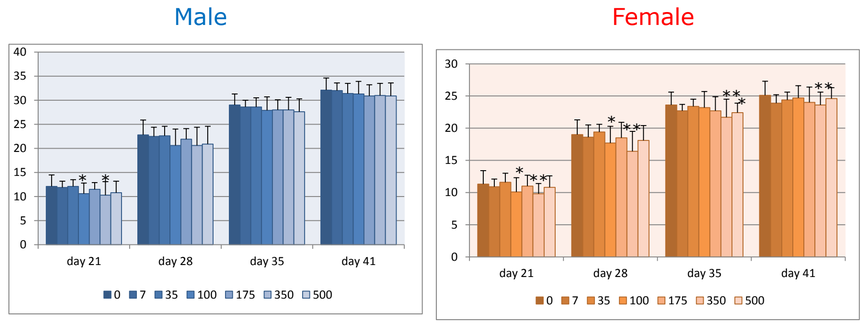


Figure 2 – F1 Generation Body Weights



RESULTS

Pharmacokinetics

1. PFHxA was rapidly absorbed (Cmax reached within 30 minutes) and was not quantifiable after 24 hours post dosing. (Table 1) The terminal elimination half-life of PFHxA ranged from 0.89 to 1.24 hours. (TABLE1). The increase in Cmax (from 96.6 to 454 µg/mL) was lower than proportional, whereas the increase in AUC(0-inf) (from 178 to 1893 h*µg/mL) was proportional, to the increase in dose from 35 to 350 mg/kg. (TABLE 1)

Developmental and Perinatal/Postnatal Reproduction Study

1. Administration of APFHx to pregnant mice resulted in minimal adverse effects at doses below 350 . At higher doses including single mortalities, excess salivation and changes in body weight gains during lactation were seen (FIGURE1)
2. In the F1 generation litters, On postnatal day 21 in males average pup weights per litter were reduced at 100 and 350 mg/kg/day. However in subsequent samplings no change in body weights were noted (FIGURE 2).
3. In the F1 generation litters, in females average pup weights per litter were reduced at 100, 350 and 500 mg/kg/day above on day 21 postnatal. At subsequent samplings changes in body weights were seen 350 and 500 mg/kg/day (FIGURE 2). A number of total pups delivered at 100 and 350 mg/kg/day was larger than other dose groups (TABLE 2).
4. Additional effects, including stillbirths, reductions in viability indices, delays in physical development in F1 generation mice occurred only in the 350 and 500 mg/kg/day dosage groups (TABLES 2 and 3) .
5. Levels APFHx in the livers from dams administered the 100 mg/kg/day dosage were all below the lower limit of quantization.(DATA NOT SHOWN)
6. Adverse effects occurred only in the 175 mg/kg/day dosage group (increased number stillborn pups and pups dying day 1 along with a reduction in pup weights on postnatal day 1, two litters with pups with corneal opacity).(DATA NOT SHOWN)