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Via Federal Express

Document Processing Center (Mail Code 7407M)
Room 6428
Attention: 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, DC 20460

CONTAIN NO CBI

Dear 8(e) Coordinator:

8EHQ-0381-0394
Ammonium Perfluorooctanoate (APFO Linear)

This letter is to inform you of the results of a recently conducted 28-day immunotoxicity study in male rats and mice with the above referenced substance.

The immunotoxicity study in male rats and mice was conducted to evaluate the potential of Ammonium Perfluorooctanoate (APFO-linear) to suppress the primary humoral immune response to sheep red blood cells (SRBC) when administered by oral gavage for at least 28 days. The study was conducted according to EPA OPPTS 870.7800 *Immunotoxicity, Health Effects Test Guidelines (1998)*. Additional hematological, clinical chemistry, organ cell counts, and histopathological endpoints were included as well. The oral route of administration was selected because it is a potential route of human exposure.

Six groups of 10 male Crl:CD® (SD)IGS rats or 20 male Crl:CD1(ICR) mice were dosed by intragastric intubation at a dose volume of 10 mL/kg body weight for 28 consecutive days with 0 (control), 0.3, 1, 10, 30, or 30 (satellite recovery group) mg/kg APFO. NANOpure® water was used as the diluting vehicle. The recovery group was dosed with 30 mL/kg APFO for 22 (rat) or 23 (mice) consecutive days. Following injection of SRBC on test day 23 (rat) or 24 (mice), these groups were dosed with NANOpure® water at a dose volume of 10 mL/kg body weight until sacrifice.

The overall conclusions are 1) the rat study was negative for immunosuppression and 2) in the mouse, a number of immune-related findings, that only occurred at 10 and 30 mg/kg, likely represent secondary responses to the systemic toxicity and stress observed at these two doses.

Rat

- No suppression of the ability to make anti-sheep RBC antibody was observed at any dose
- 10 and 30 mg/kg resulted in systemic toxicity as evidenced by the following:
  - Decreases in body weight gain of 74 and 37%, respectively.
  - Serum corticosterone levels increased to 135 and 196% of control in the 10 and 30 mg/kg treatment groups, respectively. [This effect was reversed in the 30 mg/kg (recovery) group.]

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Mouse

- The top two doses administered in this study, 10 and 30 mg/kg, resulted in marked general toxicity and stress, as evidenced by the following:
  - a loss in body weight of 3.8 and 6.6g, respectively.
  - ~230% increase in serum corticosterone and
  - increases in absolute numbers of blood neutrophils and monocytes with an accompanying decrease in absolute lymphocyte numbers.

- A number of immune-related findings occurred at 10 and 30 mg/kg that likely represent secondary responses to the systemic toxicity and stress observed at these doses:
  - Statistically significant suppression of the ability to make anti-sheep RBC antibody was observed at 10 mg/kg (20% suppression) and 30 mg/kg (28% suppression).
  - Serum corticosterone levels increased to about 230% of control in the 10 and 30 mg/kg groups.
  - Spleen and thymus weights (relative to body weight) declined to 55-65% of control values after exposure to 10 and 30 mg/kg.
  - Spleen cell numbers declined to 53 and 37% of control and thymocytes declined to 44 and 18% of control in the 10 and 30 mg/kg groups, respectively.
  - Microscopic depletion/atrophy of lymphoid tissue was considered to be treatment related starting at 10 mg/kg in the thymus and 30 mg/kg in the spleen.
- In addition, liver weight relative to body weight in mice increased to 350 and 373% of control, respectively.

Summary tables for rats and mice detailing these and other findings are attached.

Under these experimental conditions, the findings described above are being reported in accordance with the guidance given in the EPA TSCA Section 8(e) Reporting Guide (June 1991).

A copy of the final report(s) will be sent to the Agency when available.

Sincerely,

A. Michael Kaplan

A. Michael Kaplan, Ph.D.
Director – Regulatory Affairs and Occupational Health

AMK/DMH: clp
(302) 366-5260

Ammonium Perfluorooctanoate: 28-Day Immunotoxicity Study in Male Rats

Male rats	**III**	**V**	**VII**	**IX**	**XI**
Dose (mg/kg):	**0.3**	**1**	**10**	**30**	**30 (Recovery)**
Results:					
Mortality:					
Comment:	All rats survived to scheduled sacrifice.				
Clinical Signs:	M: 0/10	M: 0/10	M: 0/10	M: 0/10	M: 1/10
Comment:	One rat (1109) in 30 mg/kg recovery group was not dosed on d6-8 due to decreased body weight observed on day 6. (64% decrease from day 0). Clinical Signs for rat 1109 on days 6-8 included: lethargic, high carriage, feces absent, stained fur/skin (abdomen, forepaw, inguen, perineum, ventral body, perinasal and perioral), wet fur ventral and not eating.				
Final Body Weights (day 28):			***90%***	***75%***	***79%***
Body Weight Gain:			***74%***	***37%***	***50%***
Daily Food Consumption:				***83%***	***84%***
Hematology					
RBC			95%	98%	***88%***
HGB			***91%***	***91%***	***86%***
HCT			***92%***	***92%***	***87%***
MCV			97%	***95%***	99%
MCH			***95%***	***94%***	97%
MCHC					
RDW			***111%***	***115%***	***123%***
ARET			109%#	112%#	***197%#***
PLT					
WBC			130%	***137%***	111%
ANEU			114%	123%	100%
ALYM			***133%***	***140%***	114%
AMON			132%	140%	116%
AEOS					
ABAS					
ALUC			133%	147%	127%
Comments:	#- Microscopically, some of the rats in these 3 groups had increased anisocytosis (variation in red cell size, also observed in rats dosed with 1 mg/kg/day), macrocytosis (increased #s of large cells), polychromasia (increased bluish staining of red blood cells), and hypochromasia (pale staining of red blood cells). These changes were consistent with minimally increased reticulocytes in some animals.				
Serum Chemistry					
CHOL	***64%***	***69%***	81%	84%	
TRIG	***69%***	75%	***68%***	***66%***	***69%***
TP					107%
ALB		106%	***112%***	***115%***	***112%***
GLOB			***89%***	***89%***	96%
HDL	***75%***	***79%***	***75%***	79%	
LDL	***58%***	***63%***	85%	88%	
SCORT			135%	196%	
Comment:	Hemolyzed serum at 10, 30, and 30R				

Ammonium Perfluorooctanoate: 28-Day Immunotoxicity Study in Male Rats

Male rats	**III**	**V**	**VII**	**IX**	**XI**
Dose (mg/kg):	**0.3**	**1**	**10**	**30**	**30 (Recovery)**
Gross Pathology:					
Liver, Discoloration, tan			1	2	1
Comment:	Underlined values were interpreted to be test-substance related increases, as compared to control values.				
Absolute Organ Weight:					
Liver		***131%***	***163%***	***142%***	***123%****
Spleen					
Thymus				86%	113%*
Brain					
Relative Organ Weight (% of body weight)					
Liver			***182%***	***191%***	***156%****
Spleen					
Thymus				115%	***144%****
Brain			***112%***	***134%***	***121%****
Histopathology					
Liver, Hypertrophy, hepatocellular	5 (1.0)	10 (1.7)	10 (3.0)	10 (3.0)	10 (3.0)
Liver, Necrosis, focal				4 (1.0)	
Spleen, EMH, increased					7 (1.3)

Comment:	( ) = Number in parentheses is the average grade (grades 1 – 4) when lesion is present (i.e., sum of grades ÷ # animals with lesion). Grading scale: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
	EMH = Extramedullary hematopoiesis.
	Underlined values were interpreted to be test-substance related increases, as compared to control values.				

Spleen Cell Count:					
Thymus Cell Count:				95%	***142%****
IgM:	103%	98%	99%	98%	95%

* Group XI mean is significantly different from group IX.

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Ammonium Perfluorooctanoate: 28-Day Immunotoxicity Study in Male Mice

Male mice	**III**	**V**	**VII**	**IX**	**XI**
Dose (mg/kg):	**0.3**	**1**	**10**	**30**	**30 (Recovery)**
Results:					
Mortality:					
Comment:	• No treatment related deaths occurred.				
Clinical Signs:	M: 0/20	M: 0/20	M: 0/20	M: 2/20	M: 4/20
Comment:	• 2 mice were not dosed on study days 8-10 due to decrease body weight observed on day 8. (66% and 72% decrease from day 0) • 4 mice were not dosed on study days 9-11 due to decrease body weight observed on day 9. (70 - 75% decrease from day 0) • Lethargy: 1 mouse in 0, 30, 30R groups; Abnormal gait: 1 mouse in 0, 1, 10 groups • Prostrate: 1 mouse in 30R				
Final Body Weights (Day 28):			***86%***	***78%***	***88%****
Body Weight Gain: (Day 29)	1.5g	1.5g	***-3.8g***	***-6.6g***	***-3.3g***
Comment:	Control group gained 0.9grams (g)				
Daily Food Consumption:			***108%***	98%	100%
Hematology					
RBC				94%	***86%***
HGB				88%	***82%***
HCT				91%	***85%***
MCH					
MCHC					
RDW					109%
ARET					148%
PLT					
WBC					
ANEU			***236%***	***296%***	***256%***
ALYM				59%	74%
AMON			***285%***	254%	177%
AEOS			57%	64%	64%
ABAS					
ALUC					
Serum Chemistry					
CHOL			***69%***	***51%***	80%
TRIG			***47%***	***32%***	***57%***
TP			***125%***	109%	***134%***
ALB			***145%***	***131%***	***148%***
GLOB					***119%***
HDL		***71%***	***61%***	***44%***	***69%***
LDL			85%	65%	103%
SCORT			***230%***	232%	154%
Comment:	Icteric serum at 10, 30, and 30R				
Gross Pathology:					
Liver, Large			17	16	17
Liver, Discoloration			1	5	1
Spleen, Small			8	8	12
Thymus, Small			3	2	2
Comment:	Control group exhibited Liver, Large incidence of 1				

Ammonium Perfluorooctanoate: 28-Day Immunotoxicity Study in Male Mice

Male mice	**III**	**V**	**VII**	**IX**	**XI**
Dose (mg/kg):	**0.3**	**1**	**10**	**30**	**30 (Recovery)**
Absolute Organ Weight:					
Liver		*162%*	*301%*	*293%*	*317%*
Spleen			*56%*	*44%*	*65%*
Thymus			*50%*	*50%*	*54%*
Brain			*95%*	*93%*	*94%*
Relative Organ Weight (% of body weight)					
Liver		*160%*	*350%*	*373%*	*350%*
Spleen		*86%*	*65%*	*55%*	*70%*
Thymus			*57%*	*61%*	*58%*
Brain			*110%*	*119%*	*103%**
Histopathology					
Liver, Hypertrophy, hepatocellular	20 (2.0)	20 (3.0)	20 (4.0)	19 (4.0)	19 (4.0)
Liver, Necrosis, individual cell		11 (1.1)	20 (1.9)	19 (2.0)	19 (1.7)
Liver, Necrosis, focal		3 (1.0)	4 (1.8)	7 (1.7)	3 (1.7)
Liver, Mitotic figures, increased			10 (1.0)	15 (1.0)	19 (1.4)
Liver, Hyperplasia, bile duct			6 (1.0)	17 (1.2)	12 (1.0)
Liver, Fatty change, nonzonal			9 (1.0)	14 (1.0)	4 (1.0)
Thymus, Depletion/Atrophy, lymphoid			6 (1.2)	7 (2.9)	4 (2.8)
Spleen, Depletion/Atrophy, lymphoid				8 (1.1)	7 (1.1)
Spleen, EMH, increased					15 (2.1)
Bone Marrow, Hyperplasia, granulocytic			3 (1.7)	4 (1.0)	3 (1.7)
Bone Marrow, Hyperplasia, erythrocytic					3 (2.0)

Comment:	( ) = Number in parentheses is the average grade (grades 1 – 4) when lesion is present (i.e., sum of grades ÷ # animals with lesion). Grading scale: 1 = minimal; 2 = mild; 3 = moderate; 4 = severe.
	EMH = Extramedullary hematopoiesis.
	Underlined values were interpreted to be test-substance related increases, as compared to control values.				

Spleen Cell Count:			*53%*	*37%*	*56%*
Thymus Cell Count:			*44%*	*18%*	*49%*
IgM:	98%	92%	*80%*	*72%*	*70%*

* Group XI mean is significantly different from group IX.