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Office of Toxic Substances (WH-557)
Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Subject: EPA Document Control No.: 8EHQ-1180-0373S
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Dear Sir:

This is in response to your letter dated May 15, 1981, received May 22, 1981, including a request for additional information on 3M's 8(e) submission on "Perfluoroalkyl Mixtures".

Mr. Larry Magill, to whom your letter was addressed, has assumed responsibility for Regulatory Affairs in another sector of 3M; I have been assigned his previous function within the Commercial Chemical and Chemical Resources Divisions.

The requested bibliographical citation is to the preceding article in the August, 1980 "American Industrial Hygiene Journal", starting on page 576. For your convenience, I am including a reprint of the article. You should keep in mind that the article refers to the ammonium salt of perfluorocarboxylic acids; the results are not necessarily applicable to the effect of sulfonic acid salts.

A continuing monitoring study of 3M employees is in progress. From time to time, as definitive results are obtained, reports will be prepared and your agency kept informed.

Yours very truly,

W.H. Pearlson, Ph.D
Corporate Scientist, Regulatory Affairs
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/tjl

These studies were conducted to evaluate the potential toxicity of ammonium perfluorooctanoate, a commercial surfactant. They include acute and subchronic feeding studies with rabbits, mice, rats and monkeys as well as *in vitro* mutagenicity assays with *Salmonella typhimurium* and *Saccharomyces cerevisiae*. The compound was non-irritating to the skin and moderately irritating to the eyes of rabbits. The rat oral LD₅₀ was 540 mg/kg; no deaths resulted from a one hour rat inhalation exposure at a nominal concentration of 18.6 mg/L. All *in vitro* assays were negative. The liver was the target organ in rodents in both the 28 day and 90 day feeding studies with males showing a greater response than females. Serum and liver concentrations of organic fluorine were greater in male than in female rats. In a 90 day oral study in rhesus monkeys the gastrointestinal tract and the reticuloendothelial system were the sites of toxic effects. The gastrointestinal effects were attributed to the potent surface activity of the compound. Histopathological effects were noted in the spleen, lymph nodes and bone marrow. Unlike the rats, sex related differences were not evident in the monkeys. Toxicological evaluations of ammonium perfluorooctanoate are continuing.

Animal toxicity studies with ammonium perfluorooctanoate

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These studies were conducted in two phases. In the initial product development phase, acute oral toxicity, primary skin irritation, eye irritation and one-hour inhalation studies were conducted. When worker exposure to and absorption of the compound became evident, other studies were initiated. These included an *in vitro* mutagenicity assay, 28-day oral studies in rats and mice, and 90-day oral studies in rats and monkeys.

The studies are summarized below. They represent 3M's effort to date in the elucidation of the toxic effects of ammonium perfluorooctanoate. Additional studies on the dermal toxicity of the compound are in progress and other studies are being considered.

materials and procedures

Ammonium perfluorooctanoate is a white, free-flowing powder with a bulk density of 0.6 to 0.7 grams per cubic centimeter. It has a vapor pressure of approximately 7×10^{-5} mm Hg at 20°C and it sublimates at 130°C. The compound is highly soluble in water and exhibits powerful surfactant properties only in aqueous solution. It is commonly used commercially in the aqueous polymerization of fluorinated monomers.

Six healthy, young, adult albino rabbits were used to evaluate the primary skin irritation potential of the compound. The procedure was similar to that described in the Federal Hazardous Substances Act (FHSA), Section 1500.41. A 0.5 g test sample was applied to two intact and two abraded sites (dry and moistened), covered with gauze and wrapped with an impervious material. The wrapping was removed after 24 hours and the skin test sites were

scored according to the Draize⁽¹⁾ procedure immediately and again after 48 hours.

Six healthy, young adult albino rabbits were used to evaluate the eye irritation potential of ammonium perfluorooctanoate according to the FHSA, Section 1500.42. A 0.1 g sample was instilled in the conjunctival sac of the right eye of each test animal. The left eyes remained untreated and served as control. The eyes were examined and scored at 1, 24, 48 and 72 hours and 5 and 7 days post-instillation.

In a subsequent study another six healthy young adult albino rabbits were used to evaluate the effect of washing the treated eyes with water after limited contact with ammonium perfluorooctanoate. After instillation of 0.1 g of sample the eyes of three albino rabbits were washed with 200 mL of water after five seconds of contact and the remaining three were similarly washed after 30 seconds. Again, untreated eyes served as controls. The eyes were examined and scored as above.

Twenty-five male and 25 female ChR-CD albino rats weighing from 180-221 grams were used to determine the acute oral LD₅₀ of ammonium perfluorooctanoate. The rats were housed by sex in groups of five per cage in hanging wire cages in a temperature and humidity controlled room. Water and Purina Laboratory Chow[®] were available *ad libitum* except food was not available for an overnight period immediately prior to treatment. The compound was administered by gavage as an emulsion in 40% acetone - 60% corn oil to groups of five male and five female rats. Doses were 100, 215, 464, 1000 and 2150 mg/kg in a total volume of 10 mL/kg of body weight. The animals were observed for signs of toxic effects during the first four hours after dosing, at 24 hours and then daily for a total of 14 days. The results were evaluated as described by Weil.⁽²⁾ Body weights were recorded immediately prior to dosing, at 7 days and at 14

days. All rats were subjected to gross necropsy shortly after death during study or after sacrifice at 14 days post treatment.

Five male and five female ChR-CD albino rats weighing 211-264 grams were subjected to a one-hour inhalation exposure to ammonium perfluorooctanoate dust. Material was passed through a 60 mesh sieve and hand packed in a Wright dust feed apparatus. Dry air (16 L/min) was passed through the dust feed apparatus which was operated at a gear ratio of 1:6. The resulting atmosphere was passed through a 32.3 L glass exposure chamber. A total of 17.9 g of material was delivered resulting in an average nominal concentration of 18.6 mg/L of atmosphere. The animals were weighed immediately before exposure and on days 1, 2, 4, 7 and 14 days post-exposure. They were then subjected to gross pathologic examination on day 14 post-exposure, serum samples were collected, pooled by sex and analyzed for both organic and inorganic fluoride content.⁽³⁾

Eighty 28-day old ChR-CD albino rats, 40 of each sex, were randomly divided into eight groups of five males and five females. Average body weights at the start of the study for the males and females were 88 and 76 grams, respectively. The animals were placed in individual cages for seven days during which water and Purina Rat Chow[®] were available *ad libitum*. They then received similar feed containing 0, 30, 100, 300, 1000, 3000, 10 000 or 30 000 ppm of ammonium perfluorooctanoate for 28 days. The animals were observed daily; body weights and food consumption were recorded each week. Animals that died during study and survivors, sacrificed at 28 days, were examined for gross pathologic effects. A complete set of organs and tissues were preserved in formalin solution. Livers were weighed for organ-to-body weight ratio calculation then stained with hematoxylin and eosin for histopathologic examination.

A 28-day feeding study similar to the rat study was conducted with ChR-CD albino mice. The average weight of each group of males and females was 33 and 23 g, respectively, at study initiation; all other study parameters were the same as in the rat study.

In a 90-day feeding study, sixty ChR-CD albino rats, 30 of each sex, were randomly divided into six groups of five males and five females. The animals were placed in individual cages for a pretest conditioning period during which Purina Laboratory Chow and water were available *ad libitum*. They then received similar feed containing 0, 10, 30, 100, 300, or 1000 ppm ammonium perfluorooctanoate. Observations for signs of toxicity were made daily; body weights and food consumption were recorded each week.

Once during pretest and at one and three months, orbital sinus blood and urine were collected following overnight fasting. Hematology included hemoglobin, hematocrit, total erythrocytes, reticulocytes, and total and differential leucocyte count. Biochemical evaluations were for fasting blood glucose, blood urea nitrogen, plasma glutamic pyruvic transaminase, plasma glutamic oxalacetic transaminase, plasma alkaline phosphatase, gamma-glutamyl peptidase, creatinine phosphokinase and calcium. Urinalysis included color and appearance, volume, pH, specific gravity, qualitative tests for protein, glucose,

ketone, bilirubin, occult blood and microscopic examination. Serum samples were collected before sacrifice and pooled by group and sex for ammonium perfluorooctanoate analysis.

The animals were sacrificed by carbon dioxide inhalation and subjected to gross pathologic examination. Absolute and relative organ weights were determined for spleen, liver, kidney, adrenals and pituitary. The thyroid/parathyroid was weighed after fixation. Tissues were preserved in 10% neutral buffered formalin (eyes in Russel's fixative). One sample of each sex from the 300 ppm group was analyzed for organic fluorine content.

Histopathologic examinations of the following tissues were performed for all rats in control, 100, 300, and 1000 ppm groups:

lumbar spinal cord	salivary gland
brain with cervical cord	small intestines (duodenum, jejunum, ileum)
peripheral nerve	colon
eyes	pancreas
pituitary	liver
thyroid with parathyroid	kidneys
adrenals	urinary bladder
lung	testes
heart with coronary vessels	ovaries
aorta	prostate
spleen	uterus
mesenteric lymph node	skin (mammary gland)
thymus	any tissue(s) with gross lesions
bone with marrow (sternum)	

The livers from rats from the 10 and 30 ppm dosage level were also microscopically examined.

Statistical analysis compared the treatment groups with the control group by sex. The results were compared by analysis of variance (one-way classification) using Bartlett's test for homogeneity and the appropriate t-test for equal or unequal variances using multiple tables to judge significance of differences.^(4,5)

Ten male and ten female rhesus monkeys were assigned to five groups, two per sex per group for a 90 day study. The test material was suspended in 0.5% Methocel[®] and administered by stomach tube at dose levels of 0, 3, 10, 30 and 100 mg/kg/day. Animals were housed individually in hanging wire mesh, squeeze-type cages and fed Purina Monkey Chow[®] twice daily. They received fresh apples three times each week and water *ad libitum*. Tuberculin tests were conducted during the pretest period and bimonthly during the treatment period.

The animals were observed daily for general appearance and signs of toxicity; physical examinations were conducted monthly. Blood and urine samples were collected once in the pretest period and at one and three months of treatment following overnight fasting. Hematology included all parameters evaluated in the 90-day rat study plus prothrombin time, activated partial thromboplastin time, and platelet count. Biochemical measurements included fasting blood glucose, blood urea nitrogen, serum alkaline phosphatase, serum glutamic oxalacetic and pyruvic

TABLE I
28-Day Oral Toxicity Study - Albino Rats
Mean Body Weights (grams)

Group and Dietary Level (ppm)	Sex	Week				
		0	1	2	3	4
Control (0)	M	88	142	200	244	299
	F	76	120	163	196	215
T-I (30)	M	89	140	200	247	295
	F	76	118	159	183	210
T-II (100)	M	80	141	196	234	281
	F	76	117	154	175	203
T-III (300)	M	88	134	183	225	264
	F	76	112	163	186	211
T-IV (1000)	M	88	112**	147**	174**	201**
	F	76	115	156	173	196
T-V (3000)	M	88	90**	105**	119**	138**
	F	76	93**	126**	153**	171**

**Statistically different from control group ($p < 0.01$)

transaminases, cholesterol, total protein, albumin, sodium, potassium, chloride, inorganic phosphate, gamma-glutamyl transpeptidase and creatinine phosphokinase. Urinalysis was similar to that in the 90-day rat study.

All animals were examined for gross pathologic changes at necropsy. The heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries, brain and thyroid, parathyroid were weighed and preserved as described for the 90-day rat study. All tissues were subjected to histopathologic examination.

Statistical analyses were as described earlier.^(4,5)

Ammonium perfluorooctanoate was examined for mutagenic activity in microbial assays employing Ames *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538 and *Saccharomyces cerevisiae* strain D4. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor® pretreated rats.

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effect at the highest dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.1 µg to 500 µg per plate. The test with TA-100 was repeated at 100, 500, and 1000 µg because of increased revertants observed at 500 µg dose level in the initial test.

results

Ammonium perfluorooctanoate was non-irritating to the skin of albino rabbits under the test conditions. The primary skin irritation score was 0.

Ammonium perfluorooctanoate produced moderate eye irritation characterized by iridal and conjunctival effects. The effects were most pronounced at the one hour reading (mean score 14.0, highest possible score 110.0). The irritation was persistent but by day 7 the mean score was 2.0.

In the wash-out study the ocular effects were limited to conjunctival irritation. Those eyes washed after 5 seconds had a maximum score of 5.3 noted at 72 hours and 5 and 7 days. The mild conjunctival effects were immediate and persistent.

The acute oral LD₅₀ with 95% confidence levels in rats was:

Males: 680 (399-1157) mg/kg
 Females: 430 (295-626) mg/kg
 Combined: 540 (389-749) mg/kg

One male in the 100 mg/kg group died on the seventh day. There were no other deaths in this group or in the 215 mg/kg group. Two males died on day 2 and three females died on days 2 or 4 in the 464 mg/kg group. Three males died on days 2 or 4 and all females died on days 1, 2, or 3 in the 1000 mg/kg group. All animals died by the end of the first day in the 2150 mg/kg group. Non-lethal signs included ptosis, piloerection, hypoactivity, decreased limb tone, ataxia and corneal opacity. All signs were intermittent and there was no apparent dose-severity relationship. The average body weight gain (pretreatment to 14 days post-treatment) was 98 and 99 g for the males and 36 and 48 g for the females in the 100 and 215 mg/kg groups, respectively. Average weight gain was not calculated for the remaining groups because of the high number of deaths.

The lungs of most animals that died on study were congested, pitted or had red foci. There was also a high incidence of stomach effect including distension, excess fluid, hyperemic mucosa and thickened mucosa. Red, fluid filled intestines were reported for 3/10 animals at the 2150 mg/kg level. The only liver effect reported was pale coloration for 1/10 also at the 2150 mg/kg level.

One female in the 215 mg/kg group had lung damage foci at the 14 day sacrifice but other lung effects were not evident. Most of the animals had stomach effects similar to those

TABLE II
28-Day Oral Toxicity Study - Albino Rats
Food Consumption (g/rat/7 days)

Group and Dietary Level (ppm)	Sex	Week			
		1 ^a	2 ^b	3	4
Control (0)	M	132	133	166	178
	F	120	121	142	143
T-I (30)	M	135	140	171	176
	F	114	119	136	135
T-II (100)	M	129	138	163	169
	F	108	113	133	130
T-III (300)	M	117	129	173	185
	F	108	123	145	137
T-IV (1000)	M	85	105 ^c	128	142
	F	103	114	122	124
T-V (3000)	M	67	74	92	102
	F	75	91	117	115

^afood consumption represents an 8-day interval.

^bfood consumption represents a 6-day interval.

^cvalue represents 4 animals.

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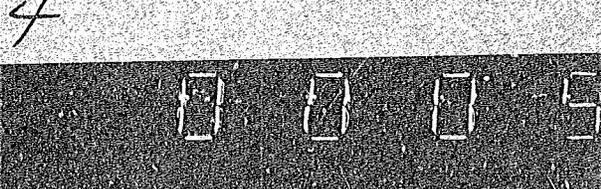


TABLE III
28-Day Oral Toxicity Study - Albino Rats
Final Sacrifice
Mean Liver Weights and Ratio Data

Group	Dietary Level (ppm)	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)	
		Males	Females	Males	Females
Control	0.0	12.98	9.29	4.35	4.30
T-I	30.0	17.86*	8.89	6.04	4.24
T-II	100.0	16.44	8.40	6.40	4.13
T-III	300.0	20.82**	10.45	7.95	4.98
T-IV	1000.0	15.79	11.64*	7.87	5.95
T-V	3000.0	12.24	12.49**	8.88**	7.31

*Statistically different from control group (p < 0.05).

**Statistically different from control group (p < 0.01).

TABLE IV
28-Day Oral Toxicity Study - Albino Mice
Mean Body Weights (grams)

Group and Dietary Level (ppm)	Sex	Week				
		0	1	2	3	4
Control (0)	M	33	34	37	35	40
	F	23	24	25	24	28
T-I (30)	M	33	33	34	32	32
	F	23	24	25	22	21**
T-II (100)	M	33	28*	25**	23**	25**
	F	23	21	18**	16**	17**
T-III (300)	M	33	23**	23**	21**	20**
	F	23	17**	17**	17*	(1)

*Statistically different from control group (p < 0.05).

**Statistically different from control group (p < 0.01).

(1) All animals died.

noted above for animals that died on study. There were mottled kidneys at all levels that had survivors at 14 days. Uterine hydrometra was evident in 1 and 2 animals in the 100 and 215 mg/kg group, respectively. There were no gross liver effects in these groups.

There were no deaths during the acute inhalation exposure or the 14-day observation period. Clinical signs during exposure were red nasal discharge, yellow staining of the ano-genital fur, dry rales, red material around the eyes, excessive salivation and lacrimation in most animals. One animal had body tremors. Similar signs were evident during the 14-day post-exposure observation period except that one animal had moist rales and there were no body tremors. Average body weight gain was 59 and 23 g for the males and females, respectively. Bilateral mottling of the lungs from eight of the ten animals was the only abnormality observed at necropsy. The discoloration included white, pink, orange, red, tan and brown spots. Two animals had red and/or pink foci on all lobes.

The pooled serum samples had 42 ppm and 2 ppm of

organic fluorine for the males and females, respectively. Inorganic fluoride content was 0.02 ppm for the males and 0.01 ppm for the females.

All animals at the 10 000 and 30 000 ppm level died before the end of the first week in the 28-day rat feeding study. There were no deaths or unusual clinical signs in the other groups.

The average body weight gains of the remaining treatment groups (see Table I) show a compound related decrease beginning after the first week and persisting throughout the remainder of the study. This effect was consistent in all groups except the females at the 300 ppm level. Food consumption (Table II) shows a similar compound related effect, again with the exception of the 300 ppm females. This suggests that the latter group may have gained more than those at the lower levels because of increased food consumption.

Average liver weights of males fed 30 ppm or more and females fed 300 ppm or more were greater than the control. The average liver weight in males fed 1000 ppm appear

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TABLE V
28-Day Oral Toxicity Study - Albino Mice
Mean Liver Weights and Ratio Data

Group	Dietary Level (ppm)	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)	
		Males	Females	Males	Females
Control	0.0	2.13	1.27	5.24	4.61
T-I	30.0	5.77**	3.83**	17.95	18.17*
T-II	100.0	6.66**	2.93**	18.47*	17.25

*Statistically different from control group (p < 0.05)

**Statistically different from control group (p < 0.01)

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TABLE VI
90-Day Oral Toxicity Study - Albino Rats
Mean Body Weights (g)

Week of Study	Sex	Dietary Level (ppm)					
		0	10	30	100	300	1000
0	M	272	271	272	274	271	270
13	M	466	478	500	457	431	362*
0	F	174	174	177	180	182	179
13	F	259	260	268	287	263	255

*Significantly different from control group (p < 0.05).

Final body weights taken during last week of study.

TABLE VII
90-Day Oral Toxicity Study - Albino Rats
Mean Organ Weight and Organ/Body Weight Ratio

Group Sex	Body Weight g	Liver		Kidneys	
		g	%	g	%
Control:					
M	445	13.43	3.01	3.54	0.79
F	271	7.66	2.85	2.16	0.81
10 ppm:					
M	482	15.26	3.18	4.13	0.86
F	237	7.25	3.06	2.50	1.05*
30 ppm:					
M	500	20.31*	4.09	4.40*	0.88
F	254	7.73	3.04	2.37	0.94
100 ppm:					
M	438	18.59	4.21	4.19	0.96*
F	271	7.97	2.95	2.47	0.91
300 ppm:					
M	412	20.13**	4.88**	3.91	0.95*
F	246	7.44	3.03	2.40	0.98
1000 ppm:					
M	340	19.16**	5.70**	3.47	1.03*
F	240	8.76*	3.65*	2.15	0.90

*Significantly different from control group (p < 0.05).

**Significantly different from control group (p < 0.01).

Body weights taken immediately before sacrifice.

TABLE VIII
90-Day Oral Toxicity Study - Albino Rats
Organic Fluorine in Pooled Serum (ppm)

Dose (ppm)	No. in Pool		No. in Pool	
	Male	Female	Male	Female
0	5	ND	5	ND
10	5	21	5	NM
30	5	34	5	0.15
100	5	36	4	NM
300	5	38	4	0.25
1000	5	49	5	0.65

ND = None detected

NM = Not measured

similar to controls, but this is probably the result of severely reduced body weight gain. There was a dose related increase in liver to body weight ratio in the males. The dose relationship was not consistent in the females although the ratio was higher than controls in the three highest dose levels (Table III). There were no other remarkable gross pathologic effects.

There were treatment related histopathologic changes in all test groups consisting primarily of focal to multifocal cytoplasmic hypertrophy of the hepatocytes at the 30 to 300 ppm levels. Multifocal to diffuse hypertrophy of hepatocytes was observed at the 1000 to 3000 ppm levels. The hypertrophy was centrilobular to midzonal at the lower levels and panlobular at the higher levels. The lesions were accompanied by acidophilic degeneration and/or necrosis of scattered liver cells. The severity and degree of tissue involvement was slightly more pronounced in the males. There was also degeneration and/or necrosis of hepatocytes and focal bile duct proliferation among animals in all treatment groups.

All test animals in the 1000 ppm level and above died in the first or second week of the 28-day mouse feeding study. Four of the five males and all females in the 300 ppm group died or study before the end of the fourth week. One animal died in each of the 30 and 100 ppm groups. After four days, rough hair coat and muscular weakness were evident in animals fed 3000 ppm or more ammonium perfluorooctanoate. Similar reactions and cyanosis were present in

the 1000 ppm group after six days and in the 300 ppm group after nine days. Some 100 ppm animals had slight cyanosis on days 10 and 11 but appeared normal thereafter. There were no abnormal signs in the 30 ppm group. There was dose related reduced mean weight gain followed by loss in average body weight at the 30, 100 and 300 ppm levels (Table IV). These cannot be related to food consumption because excessive wastage rendered the data unreliable.

There were statistically significant increases in the average liver weight and the organ to body weight ratios in the 30 and 100 ppm groups (Table V). There was also enlargement and/or discoloration of one or more liver lobes in all animals sacrificed at 28 days. No other significant gross pathologic changes were observed. Panlobular diffuse hypertrophy of hepatocytes accompanied by focal to multifocal cytoplasmic lipid vacuoles was found in all livers examined histopathologically.

The mean body weight of the males for the 300 and 1000 ppm levels was lower than the control in the 90-day rat feeding study (Table VI). There were no other differences in mean body weight for any of the other groups. There was a slight decrease in food consumption by the males at the 300 and 1000 ppm levels, but the lower mean body weights are considered compound related. There were no compound related differences in the hematologic, biochemical or urine parameters. These data are not presented.

Absolute and relative liver weights were increased as a result of treatment. There were also differences in absolute and relative kidney weights but as there was no consistent pattern in the kidney weights the effect is not considered compound related (Table VII). The absolute and relative weights of other organs were comparable to controls and the data are not shown. Other gross pathologic observations were limited to enlargement and discoloration of the livers of males at the 1000 ppm level. Histopathologic observations of livers were similar to those in the 28-day study. Changes were predominantly among males and most pronounced at the 1000 ppm level.

The organic fluorine content of the livers from the 300 ppm group was 22 and 0.3 ppm for the males and females, respectively. The serum concentrations (Table VIII) were dose related in both sexes and the 75 to 226 fold difference

TABLE IX
90-Day Oral Toxicity Study - Rhesus Monkeys
Mean Body Weights (kg)

Dose mg/kg/day	Week													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
0	3.43	3.40	3.43	3.44	3.28	3.29	3.38	3.30	3.41	3.49	3.38	3.46	3.56	3.60
3	3.41	3.43	3.45	3.44	3.44	3.40	3.40	3.36	3.48	3.55	3.36	3.40	3.50	3.59
10	3.43	3.48	3.51	3.44	3.39	3.33	3.43	3.36	3.49	3.54	3.50	3.56	3.63	3.73
30	3.44	3.29	3.26	3.13	3.01	2.76	2.64	(1)						
100	3.43	3.09	2.98	(2)										

(1) 2 females and 1 male died in weeks 7-12.

(2) All died in weeks 2-5.

TABLE X
90-Day Oral Toxicity Study -- Rhesus Monkeys
Ammonium Perfluorooctanoate Content

Dose mg/kg/day	Sex	Serum		Liver	
		Number Analyzed	ppm	Number Analyzed	ppm
0	M	0	NM	1	0.05
	F	1	1	1	0.07
3	M	2	48.53	1	3
	F	2	50.65	1	7
10	M	2	45.71	1	9
	F	2	71.79	1	10
30	M	1	145	2	60.125
	F	0	NM	2	80.125
100	M	0	NM	1	100
	F	0	NM	1	325

Samples at sacrifice
 NM = Not measured

between the organic fluorine concentration in males and females is consistent with the difference in the liver concentration noted above.

In the 90-day monkey study, all of the animals in the 100 mg/kg/day group died during weeks 2-5; one male and both females died during weeks 7-12 in the 30 mg/kg/day group. There was no mortality in the other groups. Weight loss was evident from the first week in the two higher dose groups while average weight gain was comparable to controls in the lower treatment groups (Table IX). All animals in the 100 and 30 mg/kg/day groups had anorexia, emesis, black stool, pale face and gums, swollen face and eyes, hypoactivity and prostration. One male monkey in the 10 mg/kg/day group had anorexia (week 4), pale face and gums (week 7) and black stool (week 12). There were no other abnormalities reported for this group. There were no signs of toxicity in either the 3 mg/kg/day or the control group. Clinical laboratory studies were not conducted for the 100 mg/kg/day group after study initiation. There was a statistically significant increase in activated partial prothrombin time in the 30 mg/kg/day group. All other hematological, biochemical and urine parameters were comparable for control and treated groups.

There were no compound related gross pathologic lesions evident in any of the animals. Histopathologic examination revealed marked diffuse lipid depletion in the adrenals, slight to moderate hypocellularity of bone marrow, moderate atrophy of lymphoid follicles in the spleen and moderate atrophy of the lymphoid follicles of the lymph nodes in the two highest treatment groups. These were considered compound related. There were no histopathologic changes in the 10, 3 and 0 mg/kg/day groups.

Results of serum and liver analyses for organic fluorine are shown in Table X; the compound was found in the serum and liver of all samples analyzed. There was a suggestion of a dose related increase in both the serum and liver values, but the marked sex difference observed in rats was not evident.

The results of the *in vitro* tests conducted on the compound in the absence or presence of a metabolic activation system were all negative. The repeat test was also negative.

discussion and conclusion

The compound was non-irritating to the skin and moderately irritating to the eyes of albino rabbits. Five and 30-second wash-outs greatly reduced the eye irritation scores. Oral LD₅₀ values in rats were in the 500 mg/kg range and no deaths occurred during the one hour acute inhalation study. Microbial assays using five strains of *Salmonella typhimurium* and a single strain of *Saccharomyces cerevisiae* with and without metabolic activation of the compound did not reveal mutagenic activity.

In repeat dose rodent toxicity studies, it was readily apparent that the liver was the target organ. An apparent sex related difference in toxicity was evident in the rats. The males tended to develop hepatotoxic effects at lower treatment levels than the females. At comparable treatment levels the males generally had more pronounced histopathologic effects than the females. Three rat studies indicated that serum and liver concentrations of organic fluorine were appreciably greater in males than in females.

In contrast to the rats and mice the major sites of toxicity in the rhesus monkey appear to be the gastrointestinal tract and the reticuloendothelial system. The gastrointestinal signs could be attributed to the potent surface activity properties of ammonium perfluorooctanoate. Atrophy of the lymphoid follicles in the spleen and lymph nodes and hypocellularity of the bone marrow were the major histopathological observations in the monkeys which died at the two highest treatment levels. At the 30 mg/kg treatment level, the only surviving monkey at three months had slightly reduced hemoglobin level compared to controls. The sex related difference in the concentration of organic fluorine in serum and liver noted in the rat studies was not evident in the monkeys.

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These studies suggest a sex difference in rats and a species difference in the toxicological response to ammonium perfluorooctanoate. These differences add to the difficulty of extrapolation to humans. Another complicating factor is the mode of administration. It is evident in the monkey study that the administration of the compound by gavage caused local irritation in the gastrointestinal tract. The biological significance of the irritation, while not completely defined, no doubt contributed to the toxicity of the compound.

The studies have contributed to the knowledge of the compound. They will serve as a guide to future evaluation of the toxicological effects of ammonium perfluorooctanoate.

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Biosearch, Inc., Philadelphia, PA
Dermal and eye irritation

Industrial Bio-Test Laboratories, Inc., Northbrook, IL
28 day mouse and rat studies
In vitro Salmonella typhimurium studies

International Research and Development Corp.,
Mattawan, MI
Acute oral
90 day rat and monkey studies

Litton Bionetics, Inc., Kensington, MD
In vitro Salmonella typhimurium and
Saccharomyces cerevisiae studies

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