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REVISED REPORT

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, Newark, Delaware 19711

HASKELL LABORATORY REPORT NO. 589-80 [redacted]

Material Tested	Haskell No.	Other Codes
Octanoic Acid, Pentadecafluoro-, Ammonium Salt*	12,037	[redacted]

Study Initiated/Completed
10/1//79 - 12/14/79

Material Submitted by
[redacted]
Polymer Products
Washington Works

RAT SKIN ABSORPTION SUBACUTE STUDY WITH [redacted]

I. Introduction: One object of this subacute study was to determine any toxic effects related to applying [redacted] on the shaved intact skin of rats at various concentrations. Another objective was to define the correlation between the effects seen and determined blood organofluorine concentrations.

II. Protocol and Procedures:

A. General Protocol: Male ChR-CD® rats were exposed dermally to [redacted] applied as an aqueous paste, at concentrations of either 20, 200 or 2,000 mg [redacted] kg body weight. A control group of rats was exposed simultaneously to distilled water only. Four groups of 15, 8 wk old rats (initially weighing from 210 to 245 gms) were exposed for 6 hrs/day (compound wiped off after 6 hrs), 5 days/wk for 2 wks. Doses were based on daily, individual body weights. Exposure period was followed by a 42-day recovery-observation period. Five rats per group were randomly selected for sacrifice on exposure day 10, and recovery days 14 and 42.

Rats were collared by securing 1-1/2" ID x 2-1/2" OD Teflon® rings around their necks. Collars prevented ingestion of compound by rats when preening and grooming for 2 wk exposure period. After collaring, rats were housed individually and held for a 1 wk pretest period to ensure suitability.

Compound was applied to the backs of each rat on a 3" x 1-1/2" area shaved free of hair. Less than 0.5 ml of compound was applied in aqueous form to the shaved area. Daily 6 hr exposures ended when compound was wiped from the rats backs with a gauze pad. Collars were removed after exposure day 10. Throughout the test period, Purina Rodent Chow® and water were available ad libitum. All rats were weighed and observed daily (except weekends) throughout the exposure period and approximately every 3 days thereafter.

B. Clinical Pathology Procedure: After exposure day 10, recovery day 14 and 42, blood was taken from the tails of 5 rats from each group for hematology measurements including: erythrocyte count, hemoglobin, hematocrit, leukocyte and relative number of neutrophils, lymphocytes, eosinophils, monocytes, basophils, mean cell volume, mean cell hemoglobin, mean corpuscular hemoglobin concentration. Clinical chemistry measurements included alkaline phosphatase, glutamic-pyruvic transaminase, glutamic-oxaloacetic transaminase and total protein.

C. Pathology Procedure: Gross autopsy and histopathological examinations were done on 5 rats/group after exposure 10, and on recovery days 14 and 42. Histopathology was done on the bone marrow, brain, cecum, colon, epididymides, esophagus, eyes, heart, kidneys, liver, lungs, skin, small intestines, spleen, stomach, testes, thymus, thyroid, trachea and lymph nodes.

Mean absolute and relative organ to body weight analyses were done on the following: liver, spleen, testes, kidneys and thymus.

D. Organofluorine Analysis: After exposure 10 and on recovery days 14 and 42, blood was collected from 5 rats/group by cardiac puncture for organofluorine determinations.

E. Ocular Examinations: Eye examinations were done on each rat after exposure 9, and on recovery days 13 and 41. Procedure included gross observation of the eyes using a bright light and semimicroscopic observation using a hand magnifying lamp and a slit-lamp biomicroscope.

IV. Results: There was no difference in body weight gain between the controls and the 20 mg/kg group during the test. The 2,000 mg/kg and 200 mg/kg groups lost weight from exposure days 1 to 10. Both groups recovered the weight loss and exhibited normal weight gain after exposure day 10. A graph of the body weights is presented in Appendix IV. No rats died during exposure or recovery periods.

A. Clinical Observations: There were no differences in clinical signs displayed by the control and the 20 mg/kg groups during the exposure or recovery periods. Rats exposed to concentrations of 200 mg/kg or the 2,000 mg/kg showed slight redness in the treatment areas throughout the test. Rats exposed to 2,000 mg/kg were salivating during the 2 wk exposure period and had wet stained perineal areas during the recovery period.

Treated and control groups showed hair loss and cutaneous lesions in areas where collars rubbed the rats' necks. Treated and control rats also displayed red discharge from the nose and eyes while wearing collars. When collars were removed, discharge subsided. Neck irritation, red nose and eye discharge were probably not compound-related.

B. Clinical Chemistry: After the last (10th) exposure the treated groups showed dose-related increases in serum enzyme activities such as alkaline phosphatase, GPT and GOT. In the 2,000 mg/kg group the serum total protein was lower than controls after the last (10th) exposure. In all treated groups the erythrocyte counts, hemoglobin and hematocrits were lower than controls on recovery day 14. These measurements from the treated groups were similar to controls after exposure 10. Erythrocyte counts, hemoglobin and hematocrit measurements in the 200 mg/kg group remained lower than the control at recovery day 42. The 20 mg/kg, and 2,000 mg/kg showed measurements similar to controls at recovery day 42. Clinical chemistry results are reported in detail in Appendix 1.

C. Pathology: Liver changes, basically characterized by one or more foci of coagulative necrosis containing large vesicular nuclei with markedly increased numbers, were seen in all treated groups of rats sacrificed following exposure 10. The incidence and severity was dose-related although in each group there were rats which did not show this lesion. In the 2,000 mg/kg group, 3 rats sacrificed after 14 and 1 rat after 42 recovery days showed this lesion. None of the recovery rats in the 200 mg/kg group and 1 rat from each recovery interval in the 20 mg group displayed the change. The liver lesion, both in terms of incidence and severity, appears to be reversible.

Coagulative necrosis of the epidermis at the dose site of 2 rats exposed to 2,000 mg/kg and sacrificed immediately after the last dose was observed. No other skin lesions were observed microscopically and all other tissues and organs examined appeared normal. Details of pathologic results are presented in Appendix II.

D. Organ and Body Weight Analysis: There was a statistically significant increase in the mean absolute and relative liver weights in all treated groups on exposure day 10. By recovery day 14, this increase was still significant in the 2,000 and 200 mg/kg groups. At recovery day 42, the mean absolute liver weights were significantly increased in the 2,000 mg/kg group only. On exposure day 10, the mean absolute spleen and kidney weights from the 2,000 mg/kg group were significantly less than controls and in the 20 mg/kg group the kidney weights were significantly greater than controls.

The mean relative testicular weights from the 2,000 mg/kg group showed a statistically significant increase on exposure day 10. On recovery day 14, there was a statistically significant increase in the mean relative kidneys and testes weights of the 2,000 mg/kg and 200 mg/kg groups. Details of mean absolute and relative organ to body weight ratios are in Appendices V and VI.

E. Organofluorine Analysis: Organofluorine measurements in each treated group were greater than controls after exposure 10, and recovery days 14 and 42. These increases were dose-related and appeared to be reversible with time. The controls had the lowest measurements of 10.2 ppm, 2.6 ppm and 0.6 ppm (after exposure 10, recovery days 14 and 42). The 2,000 mg/kg group averaged the highest values of 117.8 ppm, 44.8 ppm and 8.2 ppm. The 200 mg/kg group averaged 80.8 ppm, 26.2 ppm and 3.7 ppm. The 20 mg/kg group had average values of 52.4 ppm, 9.5 ppm and 1.2 ppm. There were rats in the 20 mg/kg group which had net values significantly greater than the controls. [At recovery day 14 one rat had organofluorine concentration of 3.1 ppm and recovery day 42, two rats had values of 0.9 ppm.] Control values appear somewhat elevated at the exposure 10 and recovery day 14; however, all control rats received only distilled water, applied with uncontaminated, sterile syringes and were housed in separate rooms from treated groups from study onset to sacrifice. Results of the nonvolatile fluorine measurements are reported in Appendix III.

IV. Summary: Three groups of 15 male ChR-CD® rats were exposed dermally for 6 hrs/day, 5 days/wk for 2 wks. to concentrations of either 2,000 mg [redacted] kg body weight. A 4th group, exposed simultaneoulsly to distilled water, served as a control. Groups of rats were sacrificed and examined immediately following exposure 10, after 14 recovery days (no additional exposures), and after 42 recovery days.

During the 10-day exposure period, rats treated with either 200 or 2,000 mg/kg lost weight followed by a normal growth after exposure period. Slight redness of the skin was seen in these 2 groups along with salivation in the 2,000 mg/kg group only. Rats treated with 20 mg/kg showed normal body weights and no unusual clinical signs during the experiment.

Clinical enzyme determinations monitoring liver function (alkaline phosphatase, GPT, GOT) showed dose-related increases in all treated groups after exposure 10. These values returned to normal at recovery days 14 and 42.

Liver damage characterized by coagulative necrosis was seen in all treated groups following the 10th dose. The incidence and severity of liver damage was, in general, dose-related. Recovery was complete in the 20 mg/kg group 14 days following the 10th dose and was essentially complete in the 200 mg/kg group at the same time. On recovery day 42, reversal of liver damage was essentially complete in the 2,000 mg/kg group.

Two rats exposed to 2,000 mg/kg had coagulative necrosis of the epidermis at the dose site following 10 exposures.

Liver weights, both on an absolute and relative to body weight basis, showed a dose-related increase on exposure day 10 with a return to normal weight seen in the 20 mg/kg group at 14 days and in the 200 mg/kg group at 42 days recovery. The increased liver weight persisted for 42 days in the 2,000 mg/kg group although a trend toward normal was seen.

Blood organofluoride levels showed dose-related elevation on exposure day 10 followed by a decrease in the levels at recovery days 14 and again at 42. These values after the tenth exposure ranged from 52 ppm (20 mg/kg), 81 ppm (200 mg/kg), and 110 ppm (2,000 ppm) to 1, 4, and 8 ppm (20, 200, and 2,000 ppm, respectively), at recovery day 42. At a blood organofluoride concentration of approximately 10 ppm there were normal liver weight to body weight ratios, serum enzyme activity, no clinical signs or body weight differences from controls. One rat showed liver changes at this level. At a blood organofluoride level of approximately 50 ppm there were marked liver changes and significant increases in the mean absolute and relative liver weights. No other toxic effects were evident at this blood organofluoride concentration.

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* Synonym: [redacted]

Purity: [redacted]

Contaminants: [redacted]

Report by: Lynn S. Silber

Lynn S. Silber
Technician

Approved by: Gerald L. Kennedy

Gerald L. Kennedy
Chief, Acute Investigations Section

LSS:jrg
Study Director: O. L. Dashiell
Date Issued: October 10, 1980
[redacted]
Report No. 589-80

Date Reissued: January 15, 1982

APPENDIX I

DERMAL SUBACUTE TOXICITY STUDY IN RATS WITH OCTANOIC ACID, PENTADECAFLUORO, AMMONIUM SALT; [redacted]

[redacted]

HASKELL LABORATORY NO. 12037

CLINICAL PATHOLOGY REPORT

PROCEDURE:

Sixty male rats were divided into four groups of fifteen each. These four groups were exposed dermally to 0 mg/kg, 20 mg/kg, 200 mg/kg and 2000 mg/kg [redacted] Blood was taken from the tails of five rats from each group after the tenth exposure, then these rats were sacrificed for pathology. Fourteen days later (REC 1), blood samples were again collected from five rats from each group and these were also sacrificed for pathology. Forty-two days after the tenth exposure (REC 2) blood was collected from the remaining five rats in each group.

Hematology measurements on the blood included the following: erythrocyte count (RBC), hemoglobin (Hb), hematocrit (Ht), leucocyte (WBC), and relative number of neutrophils (Neut), lymphocytes (Lymph), eosinophils (Eosin), monocytes (Mono) and basophils (Baso). Mean cell colume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the appropriate data.

Clinical chemistry measurements included alkaline phosphatase (AP), glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT) and total protein (TPROT).

STATISTICS:

The data were analyzed statistically by a one-way analysis of variance and least significant difference tests, comparing treated groups with the controls when the ratio of variance (F) indicated an effect due to treatment. Significance was judged at the 0.05 probability level.

RESULTS:

Results of the clinical pathology measurements are summarized in Table 1; statistical analysis in Table 2.

A dose-related increase in the activity of the serum enzymes, GPT and GOT, in all groups occurred after the tenth treatment. The alkaline phosphatase activity in the serum of these groups was also higher than the controls at this time. The statistical analysis, however, indicated only the GOT in the group treated with 2000 mg/kg was significantly elevated. The serum total protein was also lower than the controls in this group.

Increased activity of the serum enzymes and a decrease in serum protein are associated with liver involvement. Similar effects have been observed in the serum enzymes of rats inhaling [redacted] and in dogs ingesting [redacted]. Fourteen and 42 days after the last treatment the serum enzymes and total protein of the treated rats were not different than the controls.

Erythrocyte counts, hemoglobins and hematocrits were lower than the controls in all treated groups 14 days after the last treatment. For the group treated with 2000 mg/kg these measurements were also lower 42 days after the last treatment.

Report by: Bobby L. Moore

Bobby L. Moore
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Approved by: John R Barnes

John R. Barnes
Chief, Clinical Pathology

BLM:JRB:ljm

Date: February 29, 1980

TABLE I

AVERAGE CLINICAL LABORATORY VALUES ON MALE RATS EXPOSED (DERMAL) TO [redacted]

	TEN DAYS				FOURTEEN DAYS				FORTY-TWO DAYS			
	Exposure Concentration			mg/kg	Exposure Concentration			mg/kg	Exposure Concentration			mg/kg
	0	20	200	2000	0	20	200	2000	0	20	200	2000
RBC X 10^6/mm^3	6.05	5.34	5.23	6.30	6.38	6.01	6.02	5.74	5.87	5.94	6.02	5.56
Hb g%	14.8	14.1	14.0	14.6	15.2	14.1	14.2	13.1	15.1	15.4	15.5	14.5
Ht %	45	45	47	44	45	44	45	39	45	48	47	42
MCV μ^3	75	84	89	70	71	73	74	69	76	81	77	76
MCH pg	25	27	27	23	24	24	24	23	26	26	26	26
MCHC %	33	32	30	33	34	32	32	33	34	32	33	34
WBC X 10^3/mm^3	16.3	16.8	23.4	15.1	18.2	16.0	16.5	14.9	14.6	18.5	17.7	14.8
Neut %	23	24	28	23	35	28	28	29	25	17	17	23
Lymph %	72	72	68	72	59	69	68	67	71	80	79	72
Eosin %	1.6	1.0	1.4	1.0	0.2	0.4	1.8	0	0.6	0.4	1.2	1.6
Mono %	3.6	2.6	2.8	4.8	5.4	3.0	3.0	4.0	3.6	3.2	2.6	3.4
Baso %	0	0	0	0	0	0	0	0	0	0	0	0
AP IU	250	332	370	330	253	199	256	307	160	176	178	187
GPT IU	27	44	52	67	33	28	27	36	24	24	23	23
GOT IU	62	97	95	155	72	53	48	65	53	50	[redacted]	45
TPROT g%	5.86	6.20	6.42	5.16	6.44	6.50	7.12	6.38	6.84	7.08	7.22	7.[redacted]

TABLE 2

SUMMARY OF STATISTICAL ANALYSIS FOR RATS EXPOSED (DERMAL) TO [redacted]

	TEN DAYS				FOURTEEN DAYS				FORTY-TWO DAYS			
		Exposure Conc. mg/kg				Exposure Conc. mg/kg				Exposure Conc. mg/kg		
	F	2000	200	20	F	2000	200	20	F	2000	200	20
RBC	8.9	0	-	-	3.4	-	-	-	2.5	0	0	0
Hb	1.0	0	0	0	14.7	-	-	-	3.4	0	0	0
Ht	0.8	0	0	0	20.8	-	0	-	9.9	-	+	+
MCV	28.4	-	+	+	1.9	0	0	0	2.4	0	0	0
MCH	25.9	-	+	+	1.0	0	0	0	0.2	0	0	0
MCHC	7.1	0	-	0	1.3	0	0	0	5.0	0	0	-
WBC	2.5	0	0	0	0.9	0	0	0	0.2	0	0	0
Neut	0.6	0	0	0	0.5	0	0	0	1.4	0	0	0
Lymph	0.6	0	0	0	0.7	0	0	0	1.5	0	0	0
Eosin	0.6	0	0	0	2.3	0	0	0	1.5	0	0	0
Mono	1.1	0	0	0	1.2	0	0	0	0.2	0	0	0
Baso	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0
AP	1.7	0	0	0	4.9	+	0	0	0.4	0	0	0
GPT	2.3	0	0	0	1.1	0	0	0	0.2	0	0	0
GOT	4.0	+	0	0	2.9	0	0	0	1.0	0	0	0
TPROT	15.2	-	+	0	5.8	0	+	0	4.7	+	+	0

0 = not significantly different from control - = significantly lower when compared to controls

+ = significantly higher when compared to controls

APPENDIX II

[redacted]

Octanoic acid, pentadecafluoro-, ammonium salt [redacted]

H-12,037 - [redacted] - Petrochemicals Department

Subacute Dermal Toxicity Study - ChR®-CD Rats

May 27, 1980

Introduction

Male ChR®-CD rats were arranged into four treatment groups as follows:

Group I - Distilled water control (0.05 ml)

Group II - 2,000 mg/kg (as an aqueous paste)

Group III - 200 mg/kg (as an aqueous paste)

Group IV - 20 mg/kg (as an aqueous paste)

Results

The morphologic changes observed at necropsy are reported in Table I. Representative sections were prepared for histomorphologic evaluation from bone marrow (sternum), brain, cecum, colon, epididymides*, esophagus, eyes, heart, kidneys*, liver*, lungs*, skin (control area), skin (treated area), small intestine (duodenum), spleen*, stomach, testes*, thymus*, thyroid, trachea, and lymph node (mediastinal). The results of histomorphologic evaluation of tissues

from the control and high-dosed rats are presented in Table II. Upon finding a compound-related effect in the cutaneous and hepatic tissues of animals in the high dose group, similar tissues were examined microscopically from all animals in the intermediate (200 mg/kg) and low dose (20 mg/kg) groups. The results of these examinations are reported in Table III. Compound-related histomorphologic changes are summarized for between group comparisons in Table IV.

Discussion and Conclusions

A compound-related hepatic effect was observed in rats from all three compound-treated groups (Table IV). Affected livers contained one or more foci of coagulative necrosis. The Kupffer cells, within the foci of hepatocellular necrosis, contain large vesicular nuclei and were markedly increased in number. Inflammatory cells were occasionally present within and at the periphery of the necrotizing lesions.

Dermal lesions at the site of compound application were observed in two animals in the high dose (2,000 mg/kg) group (Tables II and IV). Affected areas of skin were characterized by coagulative necrosis of the epidermis and papillary and reticular dermis. A minimal lympho-histocytic inflammatory infiltrate was present in the subadjacent dermis bordering the necrotic zones.

The remaining histomorphologic changes shown in Table I were changes that were observed with similar frequency in the control and compound-treated groups and/or were lesions which commonly occur in laboratory-reared rats.

Summary

The dermal application of octanoic acid, pentadecafluoro- ammonium salt [redacted] for 10 days resulted in hepatocellular necrosis and necrosis of cutaneous tissue at the site of application.

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* = Tissue weighed

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Approved by: William C. Krauss

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RME:vlm
Date Issued: May 27, 1980

TABLE I

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
Group I - Control Males			
12 Days on Test; 0 Recovery Days			
263204	None	S	N.A.D.†
263205	None	S	Thymus - pink
263206	None	S	N.A.D.
263207	None	S	N.A.D.
263208	None	S	N.A.D.
26 Days on Test; 13 Recovery Days			
263209	None	S	Thymus - right lobe - dark red mottling
263210	None	S	Lungs - all lobes - pin-head sized red foci Thymus - right lobe - dark red mottling
263211	None	S	N.A.D.
263212	None	S	Salivary Lymph Nodes - large, mottled red
263213	None	S	Thymus - pink

* Mode of Death: E = Sacrificed in extremis; FD = Found Dead; S = Sacrificed
† N.A.D. = No abnormalities detected.

TABLE I (Cont'd)

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES
OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY
DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
53 Days on Test; 41 Recovery Days			
263215	None	S	Lungs - few dark red foci 0.5 to 1.0 mm in diameter, few grey streaks Nose - red discharge Skin - neck - irritation
263217	None	S	Lungs - grey mottling 1 to 3 mm in diameter
263218	None	S	Skin - neck - necrotic area 1.5 x 0.2 cm; skull - necrotic area 3 mm in diameter Thymus - left lobe - dark red
263219	None	S	Liver - lobular markings prominent
263277	None	S	N.A.D.

TABLE I (Cont'd)

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES
OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY
DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
Group II - 2000 mg/kg Males			
12 Days on Test; 0 Recovery Days			
263220	None	S	Animal - thin Liver - heavy Nose - red discharge Perineal Area - wet and stained Skin - chin - stained and moist
263221	None	S	Eyes - red discharge Liver - heavy Nose - red discharge Perineal Area - stained, wet Skin - irritation Spleen - small Urine - bright yellow
263222	None	S	Animal - very thin Liver - heavy Nose - red discharge Perineal Area - stained, wet Skin - irritation Thymus - very small Urine - stained red
263223	None	S	Liver - heavy Nose - red discharge Perineal Area - stained, wet Skin - test area - dark red crusty area 0.8 cm in diameter Spleen - small Thymus - small Urine - bright yellow
263224	None	S	Eyes - red discharge Liver - heavy Nose - red discharge Perineal Area - stained, wet Skin - irritation

TABLE I (Cont'd)

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
26 Days on Test; 13 Recovery Days			
263225	None	S	Liver - heavy
263226	None	S	Liver - left lobe - numerous pale brown foci up to 1 mm in diameter, heavy
263227	None	S	Liver - heavy Thymus - right lobe - dark red mottling
263228	None	S	Liver - heavy
263229	None	S	Liver - heavy
53 Days on Test; 41 Recovery Days			
263231	None	S	N.A.D.
263232	None	S	N.A.D.
263234	None	S	N.A.D.
263235	None	S	N.A.D.
263236	None	S	N.A.D.

TABLE I (Cont'd)

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
Group III - 200 mg/kg Males			
12 Days on Test; 0 Recovery Days			
263238	None	S	Liver - heavy
263239	None	S	Liver - cystic lobe - grey area 0.8 cm in diameter, heavy
263240	None	S	Liver - heavy
263241	None	S	Liver - heavy Nose - red discharge Perineal Area - stained, wet Skin - neck - irritation
263242	None	S	Eyes - red discharge Liver - heavy Nose - red discharge Thymus - pink
26 Days on Test; 13 Recovery Days			
263243	None	S	Liver - heavy
263244	None	S	Liver - heavy Thymus - pink
263245	None	S	Liver - slightly heavy Testes - right - yellowish-white subcapsular streaks
263246	None	S	Kidneys - right - hydronephrosis Liver - heavy
263248	None	S	Liver - heavy Lungs - few pin-head size red foci scattered throughout

TABLE I (Cont'd)

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
53 Days on Test; 41 Recovery Days			
263249	None	S	Skin - test area - thick
263250	None	S	Lungs - fine grey mottling scattered throughout
263251	None	S	N.A.D.
263252	None	S	Skin - test area - slightly thick Thymus - right lobe - pink
263253	None	S	Testes - slightly small

TABLE I (Cont'd)

MACROSCOPIC OBSERVATIONS RECORDED DURING NECROPSIES OF ChR®-CD CONTROL AND TEST MALE RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Rat No.	Amount of Autolysis Recorded During Necropsy	Mode of Death*	Observations
Group IV - 20 mg/kg Males			
12 Days on Test; 0 Recovery Days			
263254	None	S	Liver - heavy
263256	None	S	Liver - heavy Skin - neck - scaly, crusty
263257	None	S	Liver - heavy
263258	None	S	Liver - heavy Thymus - left lobe - pink
263259	None	S	Liver - heavy
26 Days on Test; 13 Recovery Days			
263260	None	S	Liver - slightly heavy Lungs - grey mottling scattered throughout
263261	None	S	Skin - neck - necrotic area
263262	None	S	N.A.D.
263265	None	S	N.A.D.
263268	None	S	Skin - neck - necrotic area 8 mm in diameter
53 Days on Test; 41 Recovery Days			
263269	None	S	N.A.D.
263270	None	S	Skin - test area - slightly thick
263271	None	S	N.A.D.
263276	None	S	N.A.D.
263278	None	S	Skin - test area - slightly thick

TABLE 11

HISTOMORPHOLOGIC OBSERVATIONS OF TISSUES
FROM MALE ChR®-CD RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Tissue** and Observation***	Mode of Death* / Days on Test / Recovery Days / Animal Number 263-	Group I - Controls														Group II - 2,000 mg/kg													
		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
		12	12	12	12	12	26	26	26	26	26	53	53	53	53	53	12	12	12	12	12	26	26	26	26	26	53	53	53	53	53
		0	0	0	0	0	13	13	13	13	13	41	41	41	41	41	0	0	0	0	0	13	13	13	13	13	41	41	41	41	41
		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3
		4	5	6	7	8	9	0	1	2	3	5	7	8	9	7	0	1	2	3	4	5	6	7	8	9	1	2	4	5	6
Bone Marrow (sternum):		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Brain:		N	N	N	N	D	N	N	D	D	N	N	D	N	N	N	N	N	N	N	N	N	N	N	N	D	N	N	N	N	N
Cecum:		N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	O	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Colon:		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Epididymides:		L	N	N	L	N	L	N	N	N	N	L	N	L	N	L	N	N	N	L	N	N	L	N	N	L	N	L	N	N	N
Perivascular Lymphocytic Infiltration, Focal/multifocal Bilateral		1																													
Periepididymal Fat, Neutrophilic Inflammation, Unilateral					1																						1				
Perivascular Lymphocytic Infiltration, Focal/multifocal, Unilateral							1					1		1		1				1			1			1					
Periepididymal Fat, Pyogranulomatous Inflammation, Focal/multifocal, Bilateral														2																	
Esophagus:		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Eyes:		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Retinal Rosette, Unilateral																	P														
Heart:		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

* Mode of Death: E = Sacrificed in extremis, F = Found Dead, S = Sacrificed.

** Tissue Accounting Code: A = Autolysis prevented complete evaluation; K = Autolysis present, tissue evaluation adequate; D = Fragmentation or disruption of tissue prevented complete evaluation; L = Lesion observed; N = No lesion observed; O = Tissue not available for histomorphological evaluation.

*** Severity of Lesion Code: 1 = Minimal change; 2 = Mild change; 3= Moderate change, Marked change; P = Change present, severity not graded; B = Bilateral change present, severity not graded.

TABLE II (Cont'd)

HISTOMORPHOLOGIC OBSERVATIONS OF TISSUES
FROM MALE ChRo-CD RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

	Group I - Controls															Group II - 2,000 mg/kg													
Mode of Death*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Days on Test	12	12	12	12	12	26	26	26	26	26	53	53	53	53	53	12	12	12	12	12	26	26	26	26	26	53	53	53	53	53
Recovery Days	0	0	0	0	0	13	13	13	13	13	41	41	41	41	41	0	0	0	0	0	13	13	13	13	13	41	41	41	41	41
Animal Number 263-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	4	5	6	7	8	9	0	1	2	3	5	7	8	9	7	0	1	2	3	4	5	6	7	8	9	1	2	4	5	6
Tissue** and Observation***																														
Kidneys:	N	N	N	N	N	N	L	N	L	L	N	N	N	N	N	L	N	L	N	N	N	N	L	N	L	N	L	L	N	L
Proximal Tubular Epithelium, Regeneration, Focal/multifocal							1									1														
Perivascular Lymphocytic Infiltration/s									1	1															1		1			
Interstitial Nephritis, Subacute, Focal																		1										1		2
Medulla, Cyst/s, Tubular																														
Liver:	N	L	N	N	N	N	N	N	N	L	N	L	N	N	N	L	L	N	L	L	N	L	P	N	L	N	N	L	L	L
Extramedullary Hematopoiesis		1																										1		
Perivascular, Lympho-histiocytic Infiltration/s										1																			2	
Focus of Cellular Alteration, Basophilic												2							P											
Centrolobular Vacuolar Cytoplasmic Change																4			1	2		3		1	1			2		
Hepatocellular Coagulative Necrosis With Kupffer Cell Proliferation																	2			1										
Fibrosis, Focal																							1						2	
Periportal Lymphocytic Infiltration/s																														
Periportal Fibrosis and Hemosiderin Deposition, Multifocal																														
Centrolobular Sinusoidal Ectasia																														
Lung:	N	N	N	N	N	N	L	N	N	N	L	L	L	L	L	N	N	N	N	N	N	L	L	L	L	L	L	L	M	M
Perivascular Lymphocytic Infiltration/s							3				3	1	2	2	1							2	2	2	1	1				
Granulomatous Inflammation, Focal/Multifocal							3						1	2	1								1	2	1	1				
Histiocytosis, Focal/Multifocal															1									2	1	1				
Lymph Nodes (Mediastinal):	0	N	N	0	N	0	N	0	N	N	0	N	0	0	0	0	N	N	0	L	0	N	0	N	N	0	C	0	0	0
Cortex, Hemorrhage																				3										

TABLE II (Cont'd)

HISTOMORPHOLGIC OBSERVATIONS OF TISSUES
FROM MALE CnRe-CD RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Tissue** and Observation***	Mode of Death* / Days on Test / Recovery Days / Animal Number 263-	Group I - Controls															Group II - 2,000 mg/kg													
		S 12 0 2 0 4	S 12 0 2 0 5	S 12 0 2 0 6	S 12 0 2 0 7	S 12 0 2 0 8	S 12 0 2 0 9	S 26 13 2 1 0	S 26 13 2 1 1	S 26 13 2 1 2	S 26 13 2 1 3	S 53 41 2 1 5	S 53 41 2 1 7	S 53 41 2 1 8	S 53 41 2 1 9	S 53 41 2 2 7	S 12 0 2 2 0	S 12 0 2 2 1	S 12 0 2 2 2	S 12 0 2 2 3	S 12 0 2 2 4	S 26 13 2 2 5	S 26 13 2 2 6	S 26 13 2 2 7	S 26 13 2 2 8	S 26 13 2 2 9	S 53 41 2 3 1	S 53 41 2 3 2	S 53 41 2 3 4	S 53 41 2 3 5	S 53 41 2 3 6
Skin (Untreated Site):																														
Head and Neck, Parakeratosis, Hyperkeratosis, Infiltration by Neutrophils		N	N	N	N	N	N	N	N	N	N	N	N	L 3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin (Treated Site):																														
Dermatitis, Acute Necrotizing		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L P	L P	N	N	N	N	N	N	N	N	N	N
Small Intestine (Duodenum):		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Spleen:		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Stomach:		L	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L	N	L	N	N	N	N	N	N	N	N	N
Glandular/Nonglandular, Lamina Propria/ Submucosa, Eosinophilic Infiltration/a		2																												
Glandular, Necrosis with Yellow Pigment Deposition, Focal/Multifocal																		P												
Nonglandular Portion, Lamina Propria Edema																														
Testes:																														
Spermatic Cell Atrophy, Unilateral		N	N	N	N	N	N	N	N	N	N	N	N	L 3	N	N	N	N	N	2 N	N	N	N	N	N	N	N	N	N	N
Thymus:																														
Cortex, Lymphoid Atrophy		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L 3	L 1	N	N	N	N	N	N	N	N	N	N
Cortex, Hemosiderin Deposition, Multifocal																			I											
Thyroid:																														
Cyst, Squamous Cell Lined		L P	O	N	N	N	N	N	L P	N	N	N	N	N	N	N	N	N	N	L P	O	S	N	N	N	N	L P	N	L P	N
Trachea:																														
Lamina Propria, Lympho-histiocytic Infiltration		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	L 2	L 4	N	N
Lamina Propria, Lymphocytic Infiltration/a																														

TABLE III

HISTOMORPHOLOGIC OBSERVATIONS OF TISSUES
FROM MALE ChR®-CD RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

		Group III - 200 mg/kg														Group IV - 20 mg/kg													
	Mode of Death*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	Days on Test	12	12	12	12	12	26	26	26	26	26	53	53	53	53	53	12	12	12	12	12	26	26	26	26	26	53	53	53	53	53
	Recovery Days	0	0	0	0	0	13	13	13	13	13	41	41	41	41	41	0	0	0	0	0	13	13	13	13	13	41	41	41	41	41
Tissue** and Observation***	Animal Number 263-	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
		3	3	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	6	7	7	7	7
		8	9	0	1	2	3	4	5	6	8	9	0	1	2	3	4	6	7	8	9	0	1	2	5	8	9	0	1	6	8
Liver:		L	L	L	L	N	N	N	L	N	N	L	N	N	N	N	N	N	N	L	L	N	L	N	L	N	N	N	D	L	N
Hepatocellular Necrosis With Kupffer Cell Proliferation, Focal/Multifocal		2	2	1																											
Periportal Fibrosis, Multifocal			2																	1	1		2						2		
Periportal Lymphocytic Infiltration/a									1			1																			
Centrolobular Sinusoidal Ectasia					1																									2	
Extramedullary Hematopoiesis																									2						
Skin (Untreated Site):		N	N	N	L	N	N	N	N	N	N	N	N	N	N	N	N	L	N	N	N	N	L	N	N	L	N	N	N	N	N
Ulceration with Acute Dermatitis					P																										
Neck, Ulcerative, Chronic-Active Dermatitis, Focal																		4					4			4					
Skin (Treated Site):		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

* Mode of Death: E = Sacrificed in extremis, F = Found Dead, S = Sacrificed.

** Tissue Accounting Code: A = Autolysis prevented complete evaluation; K = Autolysis present, tissue evaluation adequate; D = Fragmentation or disruption of tissue prevented complete evaluation; L = Lesion observed; N = No lesion observed; O = Tissue not available for histomorphological evaluation.

*** Severity of Lesion Code: 1 = Minimal change; 2 = Mild change; 3= Moderate change, Marked change; P = Change present, severity not graded; B = Bilateral change present, severity not graded.

TABLE IV

SUMMARY OF INCIDENCES OF HISTOMORPHOLOGIC OBSERVATIONS OF TISSUES FROM MALE ChR®-CD RATS TREATED BY DERMAL APPLICATION WITH OCTANOIC ACID, PENTADECAFLUORO- AMMONIUM SALT [redacted]

Group Designation	I			II			III			IV		
Dose	0 mg/kg			2000 mg/kg			200 mg/kg			20 mg/kg		
Recovery Days	0	13	41	0	13	41	0	13	41	0	13	41
Number in Treatment Group	5	5	5	5	5	5	5	5	5	5	5	5
Tissue/Observations												
Liver:	5	5	5	5	5	5	5	5	5	5	5	5
Hepatocellular Necrosis with Kupffer Cell Proliferation, Focal/Multifocal	0	0	0	3	3	1	3	0	0	2	1	1
Skin (Treated Site):	5	5	5	5	5	5	5	5	5	5	5	5
Dermatitis, Necrotizing	0	0	0	2	0	0	0	0	0	0	0	0

APPENDIX III

AVERAGE ORGANOFLUORINE VALUES FOR GROUPS I, II, III AND IV

Group No.	Dose mg/kg	Total Nonvolatile Fluorine (ppm) Exposure Day 10	Recovery Day 14	Recovery Day 42
I	0	10.2	2.6	0.6
II	2,000	117.8	44.8	8.2
III	200	80.8	26.2	3.7
IV	20	52.4	9.5	1.2

INDIVIDUAL ORGANOFLUORIDE VALUES (PPM) FOR GROUPS I, II, III AND IV

	Exposure Day 10	Recovery Day 14	Recovery Day 42
Group I	14.0	3.4	0.3
	8.0	3.3	0.8
	16.0	3.1	0.4
	11.0	2.3	0.4
	2.0	1.2	0.8
Group II	104.0	44.0	5.0
	133.0	41.9	4.2
	144.0	36.7	7.0
	116.0	67.2	13.9
	92.0	34.2	10.7
Group III	91.0	26.8	2.8
	41.0	25.9	2.1
	72.0	18.2	5.9
	122.0	29.1	5.9
	70.0	30.8	1.8
Group IV	52.0	17.6	0.9
	58.0	6.5	1.0
	53.0	3.1	0.9
	51.0	10.8	1.9
	48.0	9.5	*

* Insufficient blood sample