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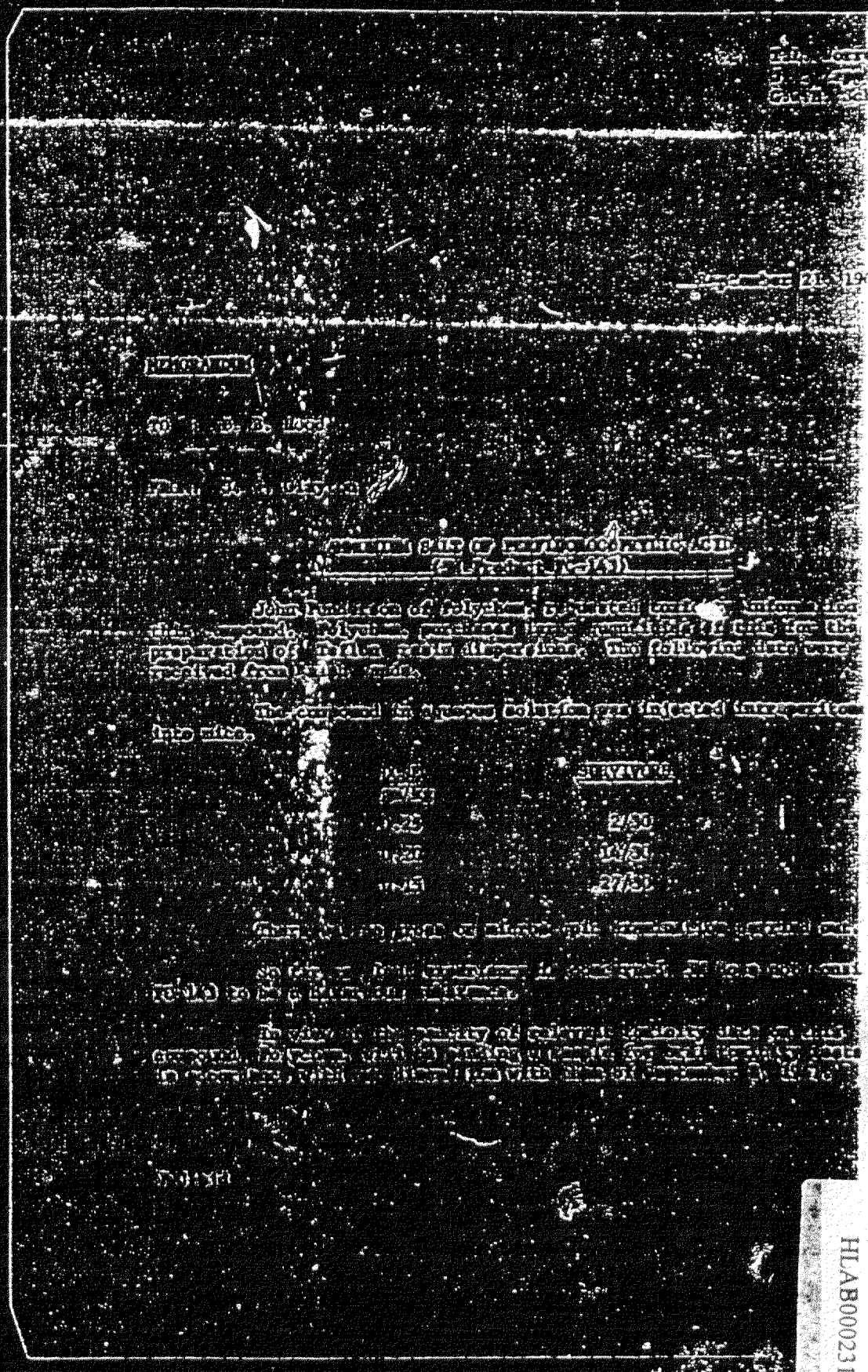

November 9, 1961

GERALD J. ARENSON
POLYCHEMICALS DEPARTMENT
RESEARCH & DEVELOPMENT DIVISION
EXPERIMENTAL STATION

TOXICITY OF TEFLON DISPERSING AGENTS

A brief summary of our toxicity work on AHT and other Teflon dispersing agents with emphasis on liver enlargement which seems to be the most sensitive sign of toxicity is given below. The detailed reports of work completed to date will be available within a few days.

AHT - (Ammonium 3,6 dioxa 2,5 di(trifluoro methyl undecafluorononanoate)

The oral ALD for rats was found to be 60 mg/kg. Survivors showed definite liver enlargement in doses down to 1.5 mg/kg and with possible changes at 0.45 and 0.13 mg/kg. Single doses of 12 mg/kg produced liver enlargement which tended to increase during the two months following the dose. One one-hundredth of the lethal dose or 0.6 mg/kg given daily 5 times a week for 2 weeks produced enlargement which was significant in those rats killed on the day of final treatment and in those killed 14 days later. Histological examination of the livers indicated that the enlargement was due to increase in cell size rather than an increase in the number of cells.

The lethal dose by skin absorption in rabbits was 430 mg/kg. Although the changes in liver weight in these rabbits are more difficult to evaluate, there was a tendency toward enlargement and similar signs of liver injury.

A 25% aqueous solution in contact with the eye caused damage which persisted through 8 days. Washing with water 20 seconds after instillation prevented permanent damage. Ten and twenty-five percent solutions were also irritating to guinea pig skin but did not cause skin sensitization.

Cg-APFC - (Ammonium perfluorocaprylate)

The oral ALD for rats was 670 mg/kg. Liver enlargement was definite down to a dose of 200 mg/kg with possible early signs down to 1.5 mg/kg.

C_9-APFC - (Ammonium ω-hydrohexa decafluorononanoate)

The oral ALD was 1500 mg/kg. Survivors showed enlargement which appears evident in doses as low as 12 mg/kg.

"Teflon" Feeding Tests with "Teflon" 7, "Teflon" 6 made with C_8-APFC, "Teflon" 6C made with C_9 and "Teflon" 6C made with [illegible]

The compounds were fed at a level of 25% in the [illegible] weeks. Rats were sacrificed 2, 3 and 5 weeks af[illegible] materials started.

Livers of rats sacrificed after two and three we[illegible] showed slight enlargement only in the group fed [illegible] After a two-week rest period the remaining rats [illegible] fed "Teflon" 6C with AHT and "Teflon" C_8 APFC sh[illegible] significantly different from the controls and t[illegible] The values of those fed "Teflon" 6C with C_9-AP[illegible] the controls and the others. Although the num[illegible] small and the time of feeding relatively short, [illegible] confirms the earlier liver enlargement observed in rat[illegible] resin in the diet for 90 days (H. Report No. 49-60). A direct comparison among these compounds is difficult to make in these feeding tests because we do not know the concentrations of the fluoro acid dispersing agents present.

Conclusions:

AHT is a very toxic compound. Not only does it have a low lethal dose but a single dose of 1/5 the lethal dose produced liver enlargement which increased with time. And 1/100 of the lethal dose fed 10 times produced definite liver enlargement. In addition, it was easily absorbed through the skin and produced liver damage in a second species. When "Teflon" containing less than 5 ppm AHT was fed to rats, it still produced enlargement which was apparent after 2 weeks.

The C_8 and C_9 acids have much lower acute toxicity, but they too have the ability to increase the size of the liver of rats at low doses. These short experiments may indicate differences in rate of development rather than qualitative differences but completion of microscopic examination of animals in the current series as well as dosing of greater numbers of rats at the critical levels and holding them for longer periods would be needed to establish the lowest effect level for each compound.

It is recommended that all of these materials, especially AHT, be handled with extreme care. Contact with the skin should be strictly avoided. Tests on a third species, e.g. dogs, should be carried out where changes in liver function could be studied over a long period of time. The results of such tests might also throw some light on any possible species differences in susceptibility.

DOROTHY B. WOOD
CHIEF, TOXICOLOGY SECTION

DBW:bch

COPIED FROM THE RESIN GROUP WEEKLY REPORT - JANUARY 29-FEBRUARY 2, 1962

Toxicity Behavior of Fluorocarbon Surfactants - Garrison

Recent data obtained from Haskell Lab (via C. J. Emerson) indicate that the toxicity of AHD (the ammonium salt of HFPO dimer acid) is very low (ca. half that of sodium chloride). We also know that AHD is a poor surfactant. These data indicate a possible relationship between toxicity and surface activity. A tabulation of available data follows:

Agent	CMC (%)	Surface Tension of a 0.2% Aqueous Solution	ALD**
ATQ*	0.27	23	60
ATT	0.42	31	60
C_8APFO	1.1	46	670
C_8AFO	1.5	54	1500
AHD	7	61	7500
H_2O	-	72	-

*ATQ = NH_4 salt of TFEO tetramer acid
**ALD values probably ± 50%

The CMC (critical micelle concentration) and surface tension data are indicative of surface activity. These data suggest that:

1. ATT (NH_4 salt of TFEO trimer acid), which is a slightly poorer surfactant than C_8AFO, should be of lower toxicity than AHT. Semiworks trials have shown that ATT may be used as a dispersing agent.

2. The telomer acid salts higher than C_9 might possess toxicity on the order of that of AHT.

3. Fluorocarbon surfactants as a class may possess a high degree of toxicity corresponding to their surface activity.

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COMPANY [illegible]

CONFIDENTIAL

TOXICITY DATA

SOURCE: [illegible]
(VIA J.D. PENDERSON)

ANIMAL	MANNER OF ADMINISTERING DOSE		[illegible]	[illegible]	[illegible]	[illegible]
		AHT	mg/kg	[illegible]	[illegible]	
RAT	INGESTION		1.3	[illegible]	[illegible]	[illegible]
RABBIT	25% [illegible] ON SKIN		—	—	[illegible]	[illegible]
		C-8				
RAT	INGESTION		[illegible]	[illegible]	[illegible]	[illegible]
		C-9				
RAT	INGESTION		[illegible]	[illegible]	[illegible]	[illegible]

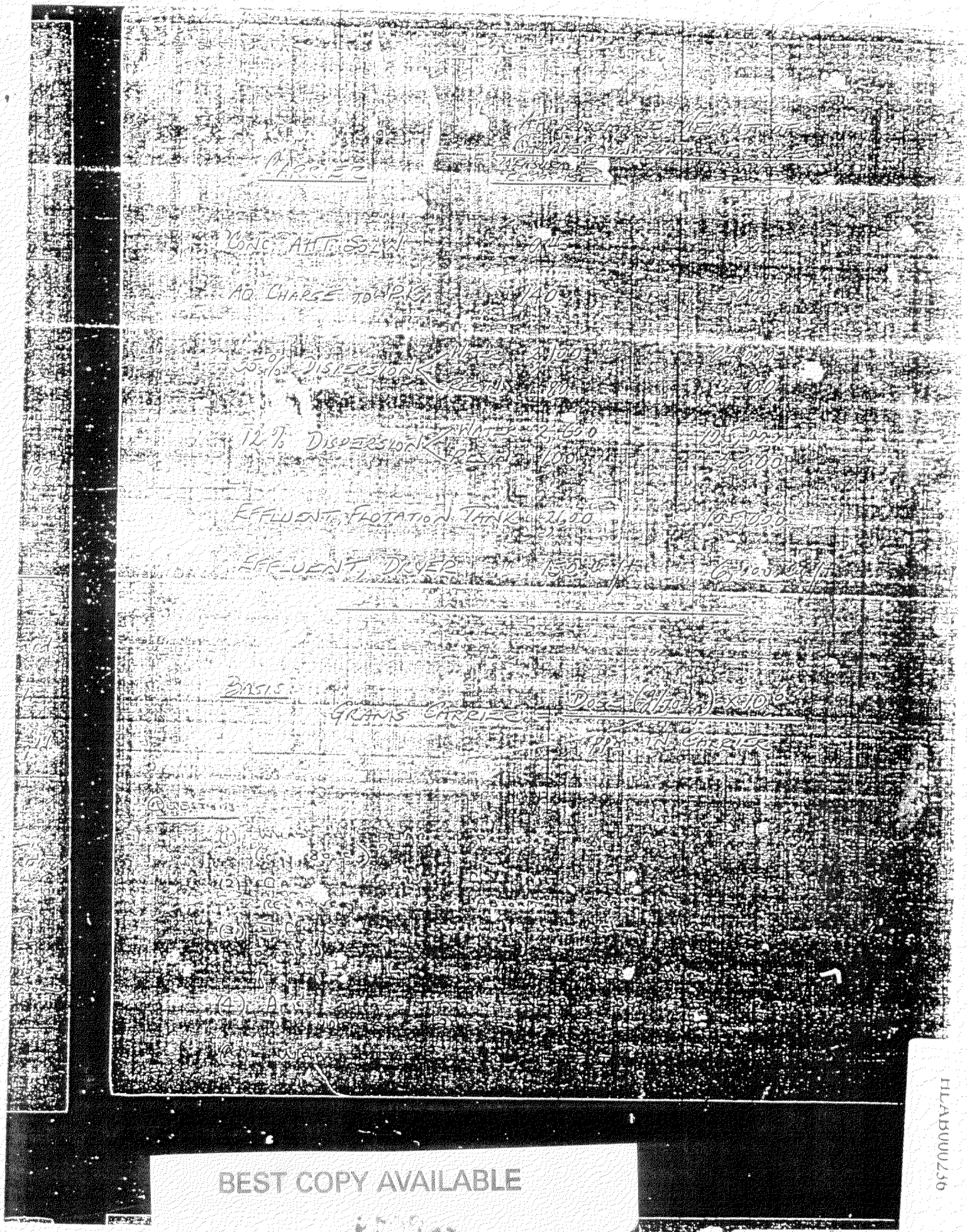
* LIVER ENLARGEMENT

FINE POWDER: Up to 100 ppm of AHT on resin – no [illegible]

DISPERSION ([illegible]): Splashing – Be reasonably careful, i.e. wash affected skin areas within a few hours after contact. Extreme precaution [illegible] [illegible]

FURTHER TOXICITY DATA

RABBIT	10% SOL'N IN EYE	[illegible]	= [illegible]
	25% SOL'N IN EYE	[illegible] NOT [illegible]	= [illegible]
GUINEA PIG	10% SOL'N ON SKIN		= [illegible]
	15% SOL'N ON SKIN		= [illegible]
	25% SOL'N ON SKIN		= [illegible] AFTER [illegible]



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ACUTE ORAL TOXICITY TEST

DATE [illegible]

MATERIAL Ammonium Perfluorocaprylate

MR 691 DEPT. [illegible] BK NO. [illegible]

DEPT. SAMPLE NO. [illegible] HASK NO. [illegible] DENSITY [illegible]

Solid ( ) Solution ( ) gms/100 ml ( )
Liquid ( ) ____ % Paste ( ) gms/100 gms ( )
Semi-Liquid ( ) Powder (X) ml/100 ml ( )

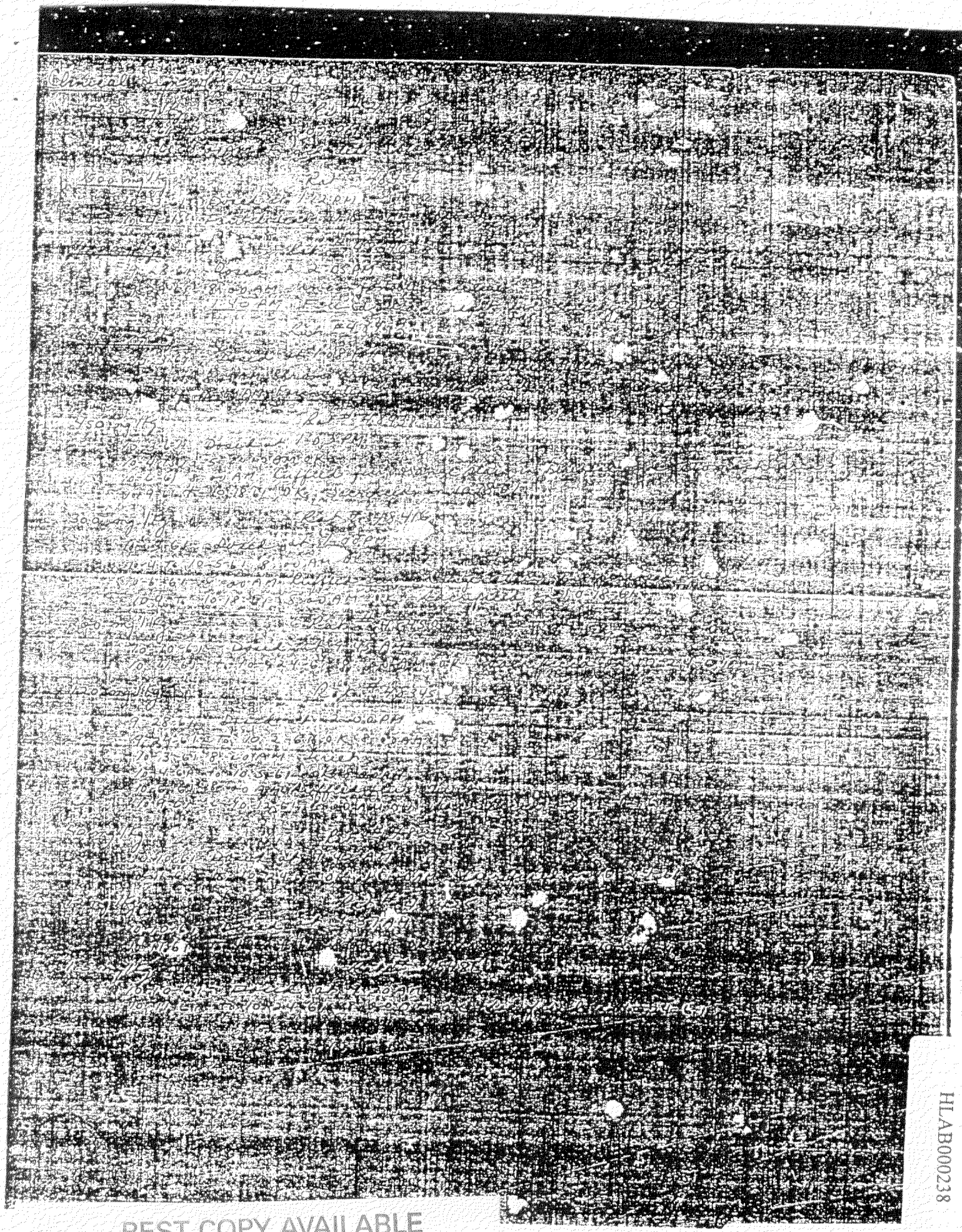
Material given by stomach tube or by [illegible] to [illegible] in original form or as a [illegible] % solution of active ingredient in [illegible]
oil suspension original sample

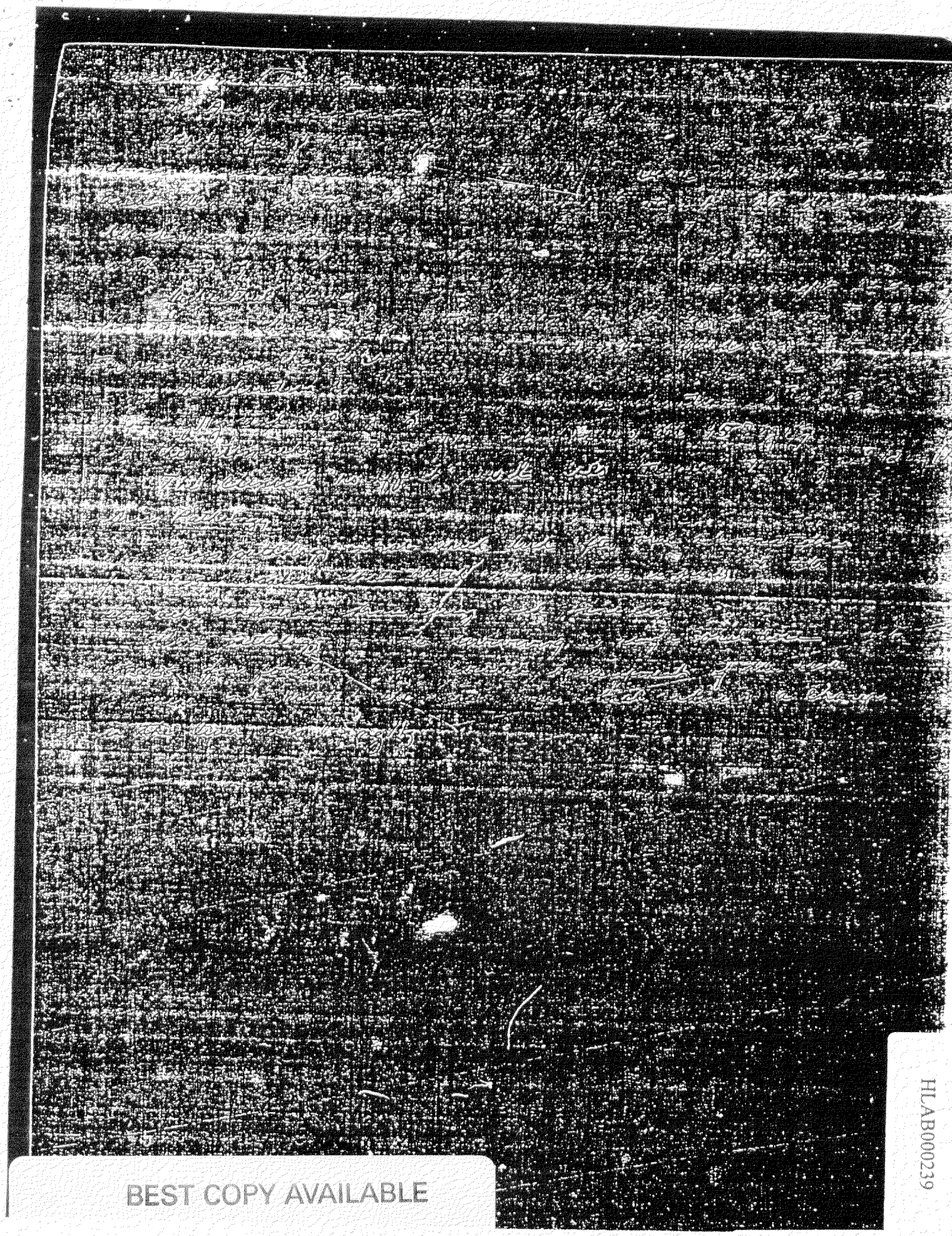
Date	Animal Number	Init Wt.	Dose (ml)	Dose (mg/kg)	% Sol	Weight Change (grams)	Results	TS
9-28	48405	373	2.80	2250	30	[illegible]	[illegible] (18 hours)	yes
10-3	48456	425	[illegible]	1500	[illegible]	[illegible]	[illegible]	No
9-28	48390	398	1.33	1000	30	[illegible]	[illegible]	yes
10-3	48453	430	[illegible]	670	10	[illegible]	[illegible]	No
10-3	48438	435	[illegible]	450	10	[illegible]	[illegible]	yes
10-3	48416	440	1.32	300	10	[illegible]	[illegible]	yes
10-10	48450	512	1.02	200	10	490 [illegible] 512 518 523 531	540 550 [illegible]	[illegible]
9-28	48345	345	[illegible]	129	[illegible]	[illegible]	[illegible]	[illegible]
10-10	48395	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10-10	48425	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10-10	48486	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

ALD: [illegible] mg/kg

REMARKS:

* Grams per 100 ml





AMMONIUM PERFLUOROCAPRYLATE
(C8APFC)

MR-004 - H-2[illegible] - P-62-172

SUMMARY

Oral administration of C8APFC, in three experiments involving forty-one rats, proved lethal in high dose concentrations through injury to the stomach, intestine, brain, lung and pancreas. At lower dose concentrations the chemical induced enlargement of the liver, pancreas and kidney, the least dose which induced a change in the liver being 1.5 mg/kg.

G. W. H. Schepers, M.D.
Pathologist

GWHS/ah
2-14-62

ML '04

C8 APPC

AMMONIUM PERFLUOROCAPRYLATE (C8APFC) (FC-143)

[illegible] Single Oral Administration

Male CRCD Rats

[illegible]

[illegible] is soluble in water, acetone, and ethanol. The [illegible] active agent, which has the formula $CF_3(CF_2)_6COONH_4$. It [illegible] as a dispersing agent in PTFE polymerizations.

[illegible] treatment as an aqueous solution in concentrations ranging from 1.2 to 3%. Quantities of these solutions varying from 1 to 2 ml were administered by intragastric incubation. Initially eleven dose levels were administered, ranging from 2250 mg/kg through 1500, 1000, 670, 450, 300, 200, 130, 85, 12 to 1.5 mg/kg. Subsequently two confirmatory tests were conducted. In the first, three rats received 12 mg/kg of the C8APFC and were killed 14 days later. In the second, groups of 3 rats received 130, 85, 26, 12, 5.1, 2.3 and 1 mg/kg and were killed 14 days later. The incidence and severity of lesions found at necropsy are listed in Table 1. Organ weight data are presented in Tables 2 to 5.

Four rats died within one day after administration of the 2250, 1500, 1000 and 670 mg/kg doses. These deaths were due primarily to injury to the stomach and intestine, but there were secondary effects on the lungs, brain, and pancreas. Post-mortem autolysis partially obscured the effects of the chemical in these four instances.

The principle change was in the liver which showed morphological deviations and weight changes. The 12 mg/kg dose level appears to be the transition dose for this effect.

The chemical also produced changes in the kidneys down to the 12 mg/kg level, which were accompanied by an increase in weight at 26 mg/kg and higher.

While no morphological changes were found, the glandular weight showed significant deviations from those of the test rats.

The sporadic occurrence of changes in the thymus and stomach may be related to the moderately severe chronic pneumonitis in these rats. These changes were not clearly dose-related, but may represent indirect effects of the chemical.

Comment

The effect of this chemical on the liver is of special interest since similar chlorinated aliphatics have produced persistent increases in liver weight. It is of interest to note that the liver weight was increased even at the 1.3 mg/kg level in rats which had a relatively high body weight at the start of the experiment, whereas in rats with the standard initial body weight, liver enlargement was not noted for doses below 26 mg/kg. The same observation applies for the kidney. The weight response of the pancreas was more irregular but suggests a dose relationship to a degree, especially in the second confirmatory test (Table 5). A decisive weight increase for the pancreas was demonstrated even at the low dose of 5.1 mg/kg. It would seem, therefore, that the pancreas is more responsive to this compound than is the liver.

It would be of interest to see if the change in liver weight progresses, as occurred with AHT.

Carl Johnson

Carl Johnson, D.V.M.

G. W. H. Schepers

G. W. H. Schepers, M.D.
Pathologist
2-8-62

TABLE 1

GROSS PATHOLOGY SUMMARY

Haskell No: 3035 Material: Ammonium Perfluorocaprylate Excipient: Water

MR No: 60[illegible] Test: ALD Species: Male CRCD Rats Procedure: Gastric Intubation

Report No: P-62-172 Experiment Period: 9-28-61 - 12-12-61 T.I. A.D., B.G. Prosectors: [illegible]

Group	Dose mg/kg	Animal No.	Survival Days Test	Survival Days After	Cause of Death	1	2	3	4	5	6	7	8	9	10	11		Pathology Possible
	2250	48[illegible]5		3/4	D_1	-	-	-	-	-	-	-	-	-	-	-	-	No
	1500	48450		3/4	D_2	-	-	-	-	-	-	-	-	-	-	-	-	"
	1000	48390		1	D_1	-	-	-	-	-	-	+	++	++	-	-	a,b,f,g	"
	670	48453		2	D_2	-	-	-	-	-	-	-	-	-	-	-	-	"
	450	48438		15	K	+	-	++	-	-	-	-	-	++	-	-	a	Yes
	300	48416		15	K	+	-	-	-	++	+	-	-	++	++	++	-	"
	200	48450		14	K	+	+	+	-	-	-	-	-	++	++	-	a	"
	[illegible]0	48345		14	K	++	+	-	++	+	+	++	-	-	++	++	-	"
	[illegible]0	46295		14	K	+	+	-	+	++	-	-	+	+	+	-	a,d	"
	12	48425		14	K	++	+	+	-	+	++	-	+	-	+	-	-	"
	12	48486		14	K	-	-	-	-	-	-	-	-	++	++	-	e	"
	12	48685		14	K	-	-	-	-	+	-	-	-	+++	-	-	a,b,c	"
Comment:	12	48665		14	K	-	-	-	-	-	-	-	-	-	++	-	-	"
	12	48693		14	K	-	-	-	-	-	-	-	-	++	++	-	-	"

Prepared By: Carl Johnson
Carl Johnson, D.V.M.

Approved By: C. W. H. Schepers
C. W. H. Schepers, M.D.
Pathologist
2-8-62

LEGEND FOR TABLE 1

1. Liver - edema
2. Liver - pale or brownish
3. Liver - soft
4. Liver - lobular markings prominent
5. Kidneys - surface speckled
6. Kidneys - pale
7. Thymus - petechiated
8. Lungs - edema
9. Lungs - irregular congestion
10. Lungs - focal atelectasia
11. Lungs - semi-consolidation

D_1. Chemical gastroenteritis with pneumonitis
D_2. Advanced autolysis
D_3. Hemorrhagic gastritis
K. Killed

a. Diffuse congestion of lung
b. Hyperinflation of lung
c. Subpleural plaques +++ (lung)
d. Adrenals: pale
e. Spleen: enlarged
f. Pancreas: pale
g. Brain: congested and edematous

\+ = Slight reaction
++ = Moderate reaction
+++ = Marked reaction

Prepared By: Carl Johnson
Carl Johnson, D.V.M.

Approved By: G. W. T. Schepers
G. W. T. Schepers, M.D.
Pathologist
2-8-62

TABLE 2

ORGAN WEIGHTS

Haskell No: 3035 Material: Ammonium Perfluorocaprylate Excipient:

MR No: 644 Species: Test: Procedure:

Report No: P-62-122 Experiment Period: T.I. A.D. Prosector: G.S.S.D.W.A.S.

Group Dose	Animal No.	Survival Days	Body Weight Init.	Body Weight Final	Age Days	Brain	Heart	Lungs	Liver	Pancreas	Stomach	Spleen	Kidneys	Testes	Adrenals	Thymus	Lung/Body Ratio	Body Ratio
2250	48405																	
1500	48456		NO															
1000	48390		ORGAN															
670	48453			WEIGHTS														
450	48438	15	435	452		1.93	1.30	1.80	28.20	0.73	1.83	0.83	3.85	3.49	.058	0.56		
300	48416	15	440	474		2.00	1.35	1.60	25.17	0.52	2.15	0.82	3.78	3.72	.056	0.75		
200	48450	14	512	542		2.10	1.52	2.00	30.61	0.82	2.07	0.86	4.73	3.09	.063	0.72		
120	46345	14	340	374		1.85	1.05	1.39	16.18	0.35	1.76	0.61	2.71	3.48	.051	0.86		
40	48295	14	515	530		2.06	1.40	1.51	24.10	0.89	1.92	0.75	4.28	3.31	.054	0.69		
12	48425	14	550	598		2.15	1.75	2.15	25.20	0.75	2.20	0.90	4.85	3.75	.063	0.65		
1.5	48486	14	535	592		2.14	1.73	2.16	24.01	.96	2.18	0.92	4.58	3.88	.062	0.75		

Comments:

Prepared by: August H. Stahnholm
August H. Stahnholm

Approved by:
G. W. H. Schepers, M.D.
Pathologist
2-8-62

TABLE 3

ORGAN WEIGHTS

Haskell No: 3535 Material: Ammonium Perfluorocaprylate Excipient:

AR No: 604 Species: Male CRCD Rats Test: Oral - 1 dose 12 mg/kg Procedure:

Report No: P-62-172 Experiment Period: T.I. B.G. Prosectors: O.J.A.S.

Group	Animal No.	Survival Days	Body Weight Init.	Body Weight Final	Age Days	Brain	Heart	Lungs	Liver	Pancreas	Stomach	Spleen	Kidneys	Testes	Adrenals	Thymus	Liver - Body Wt. Ratio
	49685	14	333	403		1.82	1.18	2.36	17.51	0.63	1.76	0.88	3.25	3.04	.053	0.57	4.34
	49686	14	308	363		1.83	1.02	1.48	15.03	0.73	1.38	0.61	3.22	3.48	.047	0.61	4.14
	49693	14	325	405		1.96	1.43	1.62	18.03	0.68	1.72	0.76	3.37	2.73	-	0.78	4.45
	Average	14	319	390		1.87	1.21	1.82	16.52	0.68	1.62	0.75	3.28	3.08	.050	0.65	4.22
	Control			390		2.02	1.25	1.55	15.51	0.83	1.76	0.76	3.03	3.48	.053	0.71	
	Deviation from normal			0		-2.5	-3.2	+17	0	-18	-8	0	+8	-11	-6	-8	

Comments:

Prepared by: August H. Stenholm
August H. Stenholm

Approved by: O. W. H. Schlepers
G. W. H. Schlepers, M.D.
Pathologist
2-8-62

TABLE [illegible]

[illegible] (C8A-FC, FC-113) [illegible]

[illegible] Following Single Oral [illegible] Intubation

Male [illegible] Rats

[illegible]

Comparison of Organ Weights With Those of Controls on the Basis of Body Weight

[illegible]	[illegible]	Body Weight	LIVER R	LIVER D	KIDNEY R	KIDNEY D	PANCREAS R	PANCREAS D
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	+16	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	+11	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	.87	+21	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	+11	[illegible]	–	[illegible]	–
[illegible]	[illegible]	[illegible]	[illegible]	+17	.81	+12	[illegible]	+54
[illegible]	[illegible]	[illegible]	1.21	+ 8	.81	[illegible]	[illegible]	+16
[illegible]	[illegible]	[illegible]	[illegible]	+ 4	.77	+ 7	[illegible]	+36
Controls (?)		[illegible]	[illegible]		.72		[illegible]	

R = Organ/body weight ratio %

D = Deviation from control value %

Prepared by: Carl Johnson
Carl Johnson, D.V.M.

Approved by: G. W. H. Schepers
G. W. H. Schepers, M.D.
Pathologist
2-8-62

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TABLE 5

AMMONIUM PERFLUOROCAPRYLATE

MR-604 - H-3035 - F-62-172

Group	Animal No.	Dose* mg/kg	Body Wt.	Liver Wt.	R	D	Kidney Wt.	R	D	Pancreas Wt.	R	D
1	50555	13	322	21.11	-	-	3.23	-	-	.70	-	-
	50556	13	316	18.15	-	-	2.72	-	-	.88	-	-
	50557	13	329	21.23	-	-	3.03	-	-	.71	-	-
	Average		322	20.83	6.2	+35	2.98	.96	+8	.76	.25	+22
2	50561	6	308	22.30	-	-	3.19	-	-	1.01	-	-
	50562	6	345	20.24	-	-	3.01	-	-	.78	-	-
	50563	6	335	17.78	-	-	3.02	-	-	.61	-	-
	Average		329	21.11	6.4	+38	3.07	.93	+3	.83	.24	+18
3	50564	26	345	20.93	-	-	3.13	-	-	1.02	-	-
	50565	26	336	17.51	-	-	3.03	-	-	.51	-	-
	50566	26	315	15.33	-	-	2.96	-	-	.91	-	-
	Average		332	17.91	5.4	+17	3.03	.91	+5	.81	.24	+17
4	50567	12	319	15.76	-	-	2.62	-	-	.80	-	-
	50568	12	340	16.50	-	-	2.85	-	-	.76	-	-
	50571	12	326	15.35	-	-	2.91	-	-	.72	-	-
	Average		328	16.18	4.9	+ 7	2.79	.85	-2	.76	.26	+2

* Dosed on 1-15-62
(Survival time - 14 days)
D = Deviation %
R = Organ weight/body weight ratio %

Carl Johnson
Carl Johnson, D.V.M.

G. W. H. Schepers, M.D.
Pathologist
2-13-62

TABLE 5 (Continued)

AMMONIUM PERFLUOROOCTANOATE

MR-60[illegible] - H-3035 - P-62-72

Group	Animal No.	Dose* mg/kg	Body Wt.	Liver Wt.	R	D	Kidney Wt.	R	I	Pancreas Wt.	R	D
[illegible]	[illegible]	[illegible]	329	12.80	-	-	2.72	-	-	.64	-	-
	[illegible]	[illegible]	321	14.85	-	-	3.20	-	-	.77	-	-
	[illegible]	[illegible]	294	11.62	-	-	2.52	-	-	.90	-	-
	average		315	13.09	4.1	-10	2.81	.70	+4	.77	.24	+17
[illegible]	[illegible]	[illegible]	302	11.65	-	-	2.77	-	-	.60	-	-
	[illegible]	[illegible]	329	15.03	-	-	2.85	-	-	.91	-	-
	[illegible]	[illegible]	305	14.45	-	-	2.52	-	-	.66	-	-
	Average		312	14.16	4.5	- 2	2.71	.87		.72	.23	+ 9
7	[illegible]	[illegible]	336	15.[illegible]	-	-	2.86	-	-	.85	-	-
	[illegible]	[illegible]	284	14.[illegible]		-	3.10	-	-	.71	-	-
	[illegible]	[illegible]	315	11.[illegible]	-	-	2.63	-	-	.62	-	-
	Average		311	13.78	4.1	-11	2.93	.88	+1	.73	.22	+ 5
8	[illegible]	Control	321	15.48	-	-	2.56	-	-	.62	-	-
	[illegible]	Control	321	13.72	-	-	2.72	-	-	.52	-	-
	[illegible]	Control	333	14.78	-	-	3.17	-	-	.95	-	-
	Average		325	14.66	4.5	-	2.83	.87	-	.68	.21	-

* Dosed on 1-18-62
(Survival time = 17 days)

I = Inflection %

R = Organ weight to body weight ratio

Carl Johnson, D.V.M.

G. W. H. Schepers, M.D.
Pathologist
2-13-62

GUS REV. 11-58

ENGINEERING DUPONT COMPUTATION SHEET

SHEET NO. _____

TITLE OF PROJ. OR STUDY _____ PROJ. OR STUDY NO. _____

SUBJECT H.R. 3035 WORKS _____

_____ COMPUTER _____ DATE _____ 19 _____

Gross pathology on rats dosed at levels of 130, 60, 26, 12, 5.1, 2.3, and 1 mg/kg of H.R. 35 and killed fourteen days later revealed increased pancreatic weight at dose of 5.1 mg/kg and above, and enlarged livers and kidneys in rats at dose of 26 mg/kg and above. The liver weight were increased even at the 1.5 mg/kg level in rats which had a relatively high body weight at the start of the experiment, whereas in rats with the standard initial body weight, liver or kidney enlargement was not found for doses below 26 mg/kg.

SUBACUTE ORAL TOXICITY TEST

HASKELL NO. 3035 MATERIAL [illegible]

DEPT. [illegible] CODE NO. [illegible] MR- 604 BOOK [illegible] PAGE [illegible]

ANIMAL [illegible] SEX [illegible] STRAIN [illegible] ALD 670 mg/kg

Material given by stomach tube or ________ ~~in origina~~

form or as a [illegible] %* solution ~~suspension~~ of ~~active ingredient~~ original sample in [illegible]

DOSE [illegible] mg/kg NO. TREATMENTS 10 MORTALITY RATIO 0/6

WEIGHT RECORD

Date	[illegible]	11-28	11-29	11-30	12-1	12-4	12-5	12-6	12-7	12-8	12-11	12-15	12-18	12-22	FATE OF ANIMALS	T S *
Day																
Tr. No.	1	2	3	4	5	6	7	8	9	10	3	7	10	14		
D. of Ob.																
Animal Number	Weight of Animals (grams)															
[illegible]	215	258	261	276	313	295	303	306	309	325	333	343	348	364	[illegible] 12-22-61	[illegible]
[illegible]	[illegible]	253	[illegible]	[illegible]	289	310	309	313	315	325	340	355	378	377	[illegible] 12-22-61	[illegible]
[illegible]	222	258	[illegible]	[illegible]	314	319	309	306	309	321	341	359	383	402	[illegible] 12-22-61	[illegible]
[illegible]	255	252	238	261	266	294	302	303	312	312					[illegible] 12-8-61	**
[illegible]	[illegible]	251	[illegible]	[illegible]	[illegible]	285	310	290	299	303					[illegible] 12-8-61	**
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	281	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]					[illegible] 12-8-61	**
Avg. Wt. (Test)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	381		
Avg. Wt. (control)	251	258	265	273	[illegible]	318	[illegible]	306	[illegible]	315	329	347	354	372		

* grams/100 ml

** tissues saved

[illegible signature]
Investigator

[illegible]
Date

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DBH:emh
11-10-60

000032

HLAB000251

USEPA 16168

Clinical Signs of Toxicity

Treatment Period

First Week: Five oral 6.7 mg/kg doses of H# 3035 administered over a one week period produced no clinical signs of toxicity in all the treated animals.

Second Week: Five oral 6.7 mg/kg doses of H# 3035 administered during the second week produced no clinical signs of toxicity in all the treated animals.

Observation Period

First Week: Three of the rats were sacrificed four hours after the tenth treatment. The remaining three animals exhibited no clinical signs of toxicity.

Second Week: The remaining three animals exhibited no clinical signs of toxicity during the second week of observation.

Summary and Conclusion

Clinical

H# 3035 produced no observable clinical signs of toxicity during the treatment or observation period.

Conclusion: Based upon its effect upon weight gain, Ammonium Perfluorocaprylate is not considered cumulatively toxic.

Table of Liver Weights

Test Group

Day of 10th Treatment

Rat No.	Body Wt. gm.	Liver Wt. gm.	Liver Wt. / Body Wt. x 100
49952'	312	19.91	6.38
49954'	303	18.00	5.94
50011	305	18.25	5.99

14 Days After 10th Tr.

49955	364	21.60	5.93
49962'	402	20.80	5.52
49989	377	18.84	4.69

Control Group

Day of 10th Treatment

50006'	316	13.50	4.27
50009	305	12.26	4.02
49988'	332	14.43	4.43

14 Days After 10th Tr.

49964'	356	16.10	4.47
49989	354	14.95	4.04
50002'	375	18.63	4.82

AMMONIUM PERFLUOROCAPRYLATE (C_8APFO)

MR-604 - II-3035 - P-62-1.2

SUMMARY

Administration of ten successive doses of 6.1 mg/kg to six rats caused moderate enlargement of the liver and slight enlargement of the kidneys, adrenals and testes. Simultaneously there was slight depression of pancreatic weight and the lungs of the rats showed slightly enhanced pneumonitis.

G. W. H. Schepers, M.D.
Pathologist

GWHS/ah
2-9-62

000035

14 day rat

[illegible] PERFLUOROCAPRYLATE (C8AMFC) (FT-185)

[illegible] Action Following Repeated Oral Administration

Male CRCD Rats

MR-64 - H-3135 - F-62-172

The chemical was administered by intragastric intubation as an aqueous solution. Each of 6 rats received 10 daily doses of 6.7 mg/kg over a period of 12 days. Three rats were killed 4 hours after receiving the 10th dose, and the remainder were killed 14 days later. Six undosed rats served as controls. The incidence and severity of lesions are supplied in Table 1 and organ weights are presented in Tables 2 and 3.

As could be predicted from the experiment with single doses (Report 62-172) the most prominent effect of the chemical again was enlargement of the liver, which, at the end of the dosage regimen, was about 45% heavier than the average liver weight of the control rats. This change persisted after cessation of dosage, but the weight discrepancy was somewhat less since 14 days later the livers were only 20% heavier than those of control rats. The increase in liver size was accompanied by slight discoloration in one rat in the group killed after the 10th dose and in one rat in the group killed 14 days later.

The renal weights were 20% heavier than those of the corresponding control rats, and this increase persisted after cessation of dosage, being 22% above that of the control rats 14 days later. These weight changes were not accompanied by morphological changes.

The pancreatic weights were slightly depressed, being 8% and 12% lower than those of controls at the end of the test and recovery phases, respectively. The adrenals and testes were slightly increased

in weight after the last dose, +14% and +11%, respectively, but returned to normality 14 days later.

Comment

It is not clear why a dose of 6.7 mg/kg was selected for this repeated dosage experiment since decisive liver injury was demonstrable for mature rats after a single dose of 1.5 mg/kg . However, the experiment served the purpose of showing that cumulative liver, kidney and pancreatic changes can be induced in young rats by relatively low doses of C8APFC.

It may be worthwhile to extend the test to include rats killed at 30, 60, and 90 days after dosage, to determine the remote consequences of the various organ changes.

Carl Johnson, D.V.M.

G. W. H. Schepers, M.D.
Pathologist

CJ:mfs
2-7-62

TABLE 1

GROSS PATHOLOGY SUMMARY

Haskell No: 3035 Material: Ammonium Perfluorocaprylate Excipient: Water

MR No: 604 Test: Oral Repeated Dosage Species: Male CRCD Rats Procedure: Gastric Intubation

Report No: P-62-173 Experiment Period: 11-26-61 - 12-22-61 T.I. B.G. Prosector: [illegible]

Group	Dose mg/kg x 10	Animal No.	Survival Days Test	Survival Days After	Cause of Death	1	2	3	4	5		Pathology Possible
	0	49988	12		K	-	++	-	+++	++	Hydronephrosis, right ++	[illegible]
	0	50006	12		K	-	+++	-	++	++	-	"
	0	50009	12		K	-	++	++	+	++	-	"
	6.7	49952	12		K	+	++	+	++	-	-	"
	6.7	49954	12		K	-	++	+	++	-	-	"
	6.7	50011	12		K	-	+	++	-	+	-	"
	0	49964	12	14	K	-	++	++	-	-	-	"
	0	49989	12	14	K	-	++	-	-	-	Hydronephrosis, bilateral ++	"
	0	50002	12	14	K	-	+	+	-	-	-	"
	6.7	49955	12	14	K	-	-	-	-	-	Hydronephrosis, right +++	"
	6.7	49968	12	14	K	+	++	-	-	-	-	"
	6.7	49987	12	14	K	-	++	++	-	-	-	"

Comment:
1. Liver pale or brownish
2. Lungs irregular congestion
3. Lungs focal detelectasia
4. Lungs subpleural plaques
5. Lungs mediastinal lymph nodes enlarged

\+ = Slight reaction
++ = Moderate reaction
+++ = Marked reaction

Prepared by: Carl Johnson
Carl Johnson, D.V.M.

Approved by: G. W. H. Schepers
G. W. H. Schepers, M.D.
Pathologist
2-7-62

TABLE 2

ORGAN WEIGHTS

Haskell No: -- Material: Controls for H-3035 & 3036 Excipient:

MR No: 604 Species: Male CRCD Rats Test: (Control) - Subacute Procedure:

Report No: P-62-17 Experiment Period: T.I. B.G. Prosector:

XXXXX No Exposure	Animal No.	Survival Days	Body Weight Init.	Body Weight Final	Age Days	Brain	Heart	Lungs	Liver	Pancreas	Stomach	Spleen	Kidneys	Testes	Adrenals	Thymus	Liver
Test Phase	49988	12	253	332		1.91	1.18	1.55	14.72	0.62	1.70	0.65	2.60	2.75	.048	0.75	
	50006	12	254	316		1.90	1.00	1.72	13.50	0.38	1.35	0.58	2.72	2.90	.039	0.85	
	50009	12	245	305		1.80	1.22	1.38	12.20	0.50	1.58	0.70	2.48	2.80	.051	0.64	
	Average	12	251	318		1.87	1.13	1.55	13.47	0.50	1.54	0.64	2.60	2.82	.046	0.75	
Recovery Phase	49964	26	249	370		1.91	1.35	1.50	16.10	0.82	1.80	0.54	2.85	2.88	.054	0.48	
	49989	26	256	360		1.99	1.30	1.83	14.95	0.79	1.65	0.75	2.81	3.30	.052	0.70	
	50002	26	250	385		2.09	1.14	1.75	18.63	0.79	1.65	0.63	3.29	3.13	.052	0.60	
	Average	26	252	372		2.00	1.26	1.69	16.56	0.80	1.70	0.64	2.98	3.10	.053	0.60	

Comments:

Prepared by: August H. Stenholm

Approved by: G. W. H. Schepers, M.D.
Pathologist
24-7-62

TABLE I

ORGAN WEIGHTS

Haskell No: 3035 Material: Ammonium Perfluorocaprylate

MR No: 604 Species: Male CD(R) Rats Test: Oral - Subacute Procedure:

Report No: R-62-173 Experiment Period:

Dose Group 6.7 mg/kg	Animal No.	Survival Days	Body Weight Init.	Body Weight Final	Age Days	Brain	Heart	Lungs	Liver	Pancreas	Stomach	Spleen	Kidneys	Testes	Adrenals	Thymus	Lab.
Test Phase	49952	12	255	312		1.80	0.98	.72	19.91		1.54	0.59	2.85	3.00	.044	0.26	64
	49954	12	250	303		1.85	1.02	1.51	18.00	0.45		0.50	3.70	3.15			74
	50011	12	248	305		1.89	1.08	1.42	18.25	0.42	1.51	0.72	3.51	2.91	.040		55
	Average	12	251	307		1.85	1.03	1.55	18.72	0.44	1.53	0.57	3.02	3.02	.048	0.62	66
Recovery Phase	49955	26	255	364		1.89	1.15	1.58	21.60	0.73	1.67	0.72	3.60	2.28	.049	0.61	55
	49968	26	252	402		2.03	1.51	1.80	20.80	0.81	1.91	0.68	3.94	3.23	.052	0.70	55
	49987	26	245	377		1.91	1.34	2.11	18.84	0.63	1.79	0.53	3.61	3.49	.056	0.50	55
	Average	26	251	381		1.94	1.33	1.83	20.41	0.72	1.79	0.64	3.72	3.00	.056	0.60	55

Comments:

Prepared by: August H. Stenholm

Approved by: W. H. Schepertheil, M.D.
Pathologist
7-27-62

ACUTE ORAL TOXICITY TEST

DATE [illegible] 19[illegible]

MATERIAL [illegible]

MR- [illegible] DEPT. [illegible] BK.NO. [illegible] P. [illegible] TECH. [illegible]

DEPT. SAMPLE NO. [illegible] HASK. NO. [illegible] DENSITY [illegible]

Solid	(X)	Solution	( )	gms/100 ml	( )
Liquid	( ) ___%	Paste	( )	gms/100 gms	( ) in [illegible]
Semi-Liquid	( )	Powder	( )	ml/100 ml	( )

Material given by stomach tube ~~or by~~ [illegible] to [illegible] ~~in original form~~ or as a [illegible]% solution ~~suspension~~ of ~~active ingredients~~ in [illegible] original sample

Date	Animal Number	Init Wt.	Dose (ml)	Dose (mg/kg)	% Sol	Weight Change (grams)	Results	TS
11-29	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
11-29	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
11-29	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

ALD: [illegible] mg/kg

REMARKS: [illegible]

000041

*Grams per 100 ml

Sherman

TOXICITY STUDIES ON FLUOROCARBON DISPERSING AGENTS

Medical Research Project Nos. MR-639 and 10-D-1

BIOCHEMISTRY

Ammonium perfluorocaprylate (C_8FDA), ammonium ω-hydrohexadecafluoro-nonanoate (C_9FDA) and ammonium-3,6-dioxa-2,5-(trifluoromethyl)-undecafluoro-nonanoate (AHT) are dispersing agents used in the polymerization of tetrafluoroethylene. In acute toxicity studies with these compounds, the most striking abnormality in animals surviving a single sublethal dose was gross enlargement of the liver. A similar but less marked effect was observed in rabbits that had absorbed AHT through the skin.

The preliminary experiments reported here were designed to measure some of the biochemical changes that accompany the accelerated growth of rat liver; to determine whether functional impairment of the liver occurs as well; and whether there is a species difference in susceptibility. The liver function studies were undertaken to find some clinical laboratory procedure that might be useful to medical supervision in detecting fluorocarbon liver injury. The latter phase of the work was supported by the Plastics Department under Medical Research Project No. MR-639.

1. DOGS

Procedure: Three male beagles from the stock colony were given a single oral dose of AHT, equivalent to 4, 6, or 9 mg/kg. Samples of blood were taken at frequent intervals for three weeks and then weekly thereafter until the animals were sacrificed or given an additional dose of the compound. Four other male beagles were given a single oral dose of either C_8FDA or C_8FDA, or C_9FDA, equivalent to 450 mg/kg and a similar series of measurements made. Since both dogs receiving the C_8FDA died within 48 hours, the experiment was repeated later with a lower dose (200 mg/kg) of this dispersing agent. The two dogs that survived the exposure to the C_9FDA were given an additional oral dose, equivalent to 670 mg/kg,

at this time. Blood sugar and amylase activity were measured on these dogs in an effort to detect an effect on the pancreas.

The following biochemical measurements were made routinely on the blood: sugar, urea nitrogen, total cholesterol, and alkaline phosphatase APase). When high doses of the dispersing agents were administered, the level of activity of lactic dehydrogenase (LDH), isocitric dehydrogenase (ICDH), aldolase, glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminases were also measured. The free and esterified cholesterol, phospholipids, demasterol (24-dehydrocholesterol), thymol turbidity, albumin and globulin were also determined on one or more samples of blood from the dogs given low doses of AHT. In addition a routine hematological examination and analysis of a 24-hour urine specimen was made at intervals on these animals.

The level of the various components of the blood following the dose of fluorocarbon dispersing agents was compared with an average value observed prior to the exposure and with a similar measurement made at the same time on specimens from stock colony dogs.

For the enzyme activities, a normal range was established from measurements made on a number of stock dogs. The activity was also measured at least once, prior to treatment, on the dogs dosed with the dispersing agents.

Results: The significant biochemical and clinical findings are summarized briefly in Table 1.

The principal finding indicative of some injury or dysfunction in the dogs that received AHT was a decrease in the plasma cholesterol and an increase in bromsulfalein retention (BSP) and APase activity. These

changes began to occur within one week after the dose was administered. The changes in cholesterol and APase became "abnormal", i.e. exceeded an arbitrary limit of two standard deviations from the pre-exposure mean, one or two weeks later. The increase in dye retention occurred during the first week or ten days and then began to return to normal. The depression of plasma cholesterol in all three dogs occurred earlier and began to return to normal sooner than the rise in plasma APase activity. The dog that received the highest dose of AHT showed the greatest change from the pre-exposure mean. For doses of AHT up to 26 mg/kg, however, the actual values observed for all three dogs did not differ greatly. (See figures 1 and 2.)

Some qualitative and quantitative changes in the serum proteins of one of the three dogs were noted. All three showed decreases in the plasma phospholipids. There were also some changes in red cell morphology and a decrease in the number of red blood cells of the dogs receiving low doses of AHT. All other measurements made on the blood or urine showed no significant deviation from the normal.

When high doses of AHT, C_8FDA or C_9FDA were administered the activity of a number of enzymes, reported to be sensitive indicators of liver injury, were measured in the plasma. The results of the biochemical measurements are presented in Table 2.

When a high dose (60 mg/kg, equivalent to the rat lethal dose) of AHT was administered to the one dog that had previously received 1, and 26 mg/kg, all the plasma enzymes measured were elevated within the first three days after the dose was given. The greatest changes were in the GPT (25X) and APase (10X). Within one week all but these were within the

normal range. The depression of the plasma cholesterol occurred more slowly, but there was no evidence of a return to normal after three weeks. Bilirubinuria was also observed.

With the lower dose of the CgFDA (30% of rat lethal dose) the transaminases were elevated in both dogs within 48 hours and continued to rise during the first week after the exposure. Thereafter there was a return to normal. The dog that appeared to have retained the largest portion of the dose showed the greatest effect. The APase was also elevated in this dog and paralleled the GPT. The cholesterol was normal or only slightly elevated within one week after the treatment. There was no effect on the blood sugar and a decrease in amylase activity. When a higher dose of this compound was administered (2/3 of the rat lethal dose) all of the enzymes measured were markedly elevated within 24 to 48 hours. The greatest changes occurred in the GPT and ICDH. There was no change in the plasma cholesterol. Both animals expired within 48 hours after dosing.

With a dose of the CgFDA equivalent to 30% of the rat lethal dose, elevated values of all enzymes measured occurred within 48 hours in one of the two dogs exposed. These returned to the normal range within seven to ten days. The plasma cholesterol was normal or only slightly elevated after after one week. The other dog showed no effect from the treatment. At the higher dose (45% of the rat lethal dose) of this compound the more susceptible dog again showed a rise in GOT and GPT during the first 48 hours and a return to normal within two weeks. There was a slight rise in APase and in the plasma cholesterol. Similar, but less marked changes occurred in the other dog. The amylase activity was lower than normal during the first 48 hours, but a rise did occur during the week following the exposure. There was also a rise in the blood sugar at this time. Within three weeks after the exposure all measurements were normal.

The biochemical data have shown that the three fluorocarbon dispersing agents affect the liver. Very high doses of AHT or the C_8FDA that are lethal or near lethal for dogs, produce elevated plasma levels of enzyme activity indicative of cellular destruction. The depression of cholesterol seems to be peculiar to AHT. The C_9 dispersing agent may also affect the pancreas but this follows the liver injury. Low doses of the C_8 compound do not show this affect. Of all the measurements made the APase and GPT seem to be the most sensitive in detecting an effect from all three dispersing agents. Of these, the latter may be more useful in that there is a narrower range of normal values. Depressed cholesterol levels may be useful in detecting an exposure to AHT.

2. RATS

The experiments with rats were conducted with only one of the three fluorocarbon dispersing agents, AHT, and were divided into two parts. The first part was a study of the biochemical changes in liver tissue which might reflect functional impairment; the second dealt with the effects of AHT on degenerating or regenerating liver tissue.

1. Biochemical Changes

Procedure: Thirty young albino rats (ChR-CD) were given a single oral dose, equivalent to 12 mg/kg of body weight, of AHT by stomach tube. Fifteen to twenty-three days later eight of these were sacrificed and their livers examined for biochemical evidence of abnormality. A similar number of untreated control animals were examined to establish a normal range of values for the various biochemical measurements in rats of this age and strain.

Sixty to sixty-eight days after the single dose, a second group of eight AHT-treated rats and eight controls were sacrificed and the same measurements made. At this time, samples of blood were also taken for measurement of the plasma alkaline phosphatase activity (APase) and cholesterol concentration. Approximately 5 1/2 months after the AHT treatment the remaining animals were sacrificed.

Results: The results of the biochemical examination of the livers are summarized in Table 3. In the group of rats sacrificed fifteen to twenty-three days after a single oral dose of AHT, the principal changes in the liver, in addition to the relative and absolute increase in liver size, were an increase in alkaline phosphatase activity, phospholipids and a decrease in glycogen, non-protein nitrogen (NPN), and deoxyribonucleic acid (DNA). Small but significant increase in oxygen consumption, water and protein occurred and a slight but significant decrease in respiratory quotient (RQ), choline oxidase and ribonucleic acid (RNA). All other measurements were in the range of normal.

Sixty days after the single dose, the livers of the AHT-treated rats were still quite large, although they were apparently no longer growing at the accelerated rate. The growth depression as measured by live body w however, was greater than observed previously.

The increase in phospholipid and decrease in glycogen were still the most outstanding change. Small but significant decreases in RQ, RNA and DNA again occurred. The alkaline phosphatase was no longer significantly different from the controls, while the choline oxidase had actually increased. Unlike the dogs these rats showed no increase in plasma alkaline phosphatase, and a rise rather than a fall in plasma cholesterol.

Approximately five and one-half months after the exposure to AHT, the livers were still slightly larger than in untreated rats, but absolute

size of the liver had decreased appreciably. Although small differences were still observed between the control and treated animals, all of these were within the range of normal for rats of this age, sex and strain, as established by the untreated controls.

The results indicate that the enlarged livers resulting from a single dose of AHT are biochemically as well as anatomically abnormal. The rise in O_2 consumption or decrease in CO_2 production and RQ reflect some change in the metabolic behavior of the liver, ~~for these are a measure of the integrated reactions that go on in living tissue.~~ The difference in respiratory quotient was small and after two months greater variation was seen in the AHT-treated group as more of the animals approached the normal range.

Some individual enzymes were affected to a greater degree than the RQ between 15 and 23 days. After two months, however, two of the enzyme activities were in the normal range, and the third was actually more active than the controls. These changes, together with the respiratory changes in the tissue, suggest that some modification in the effect of AHT had occurred.

The liver was depleted of glycogen at both two weeks and two months. The depletion became less marked, however, with time. ~~The glycogen was replaced by water and protein in the early stages of the liver enlargement, but later by protein and fat.~~

The decrease in DNA and RNA probably resulted from a decrease in cell density as the number of cells in a given amount of tissue decreased. This would agree with the observation that the early enlargement is due principally to cellular hypertrophy rather than proliferation. After two

months there was less difference between control and AHT-treated rats, suggesting either a decrease in the size of the cells or an increase in mitosis.

The results reported seem to indicate that the livers of rats stimulated by a single oral dose of AHT undergo alteration in their biochemical behavior and metabolism during the period of rapid growth. Later, when the accelerated growth has slowed down or ceased, the biochemical aberrations also tend to disappear. The greatest changes occur during the first few weeks or month after the exposure. By the end of two months some evidance of a reversal of these effects is already apparent; between five and six months recovery of the organ is virtually complete.

2. Regenerating and Degenerating Liver

Procedure: Regenerating liver tissue was induced by partial hepatectomy (60-70%) in six male rats from the stock colony. Forty-eight hours after the operation a single oral dose of AHT, equivalent to 12 mg/kg body weight was administered by intubation. Fourteen days later the animals were sacrificed, the livers removed, weighed and analyzed. The experiment was repeated a second time, reversing the procedure by performing the partial hepatectomy forty-eight hours after the dose of AHT had been administered.

Degenerating liver tissue was induced chemically by administering, intraperitoneally, 0.1 ml of carbon tetrachloride per 100 gm of body weight to rats 48 hours after intubating them with 12 mg/kg of AHT. Animals which received only carbon tetrachloride served as controls.

Results: The results are summarized in Table 4. Hepatectomy alone produced an accelerated growth of the liver tissue for within two weeks all, or nearly all of the tissue was restored. The DNA, RNA and [illegible] were higher than in normal tissue.

AHT caused an accelerated growth of the liver so that it was more than twice normal size two weeks after the exposure. It also caused a decrease

in DNA and glycogen as well as an increase in APase and phospholipid. These changes occurred regardless of whether two-thirds of the liver was removed before or after the dose of AHT, although there was somewhat less of an effect on the nucleic acids, phospholipids and APase when hepatectomy followed treatment with AHT.

This remarkable growth of the liver even after two-thirds of it has been surgically removed, is quite interesting. Apparently removal of much of the affected tissue does not appreciably alter the unusual growth rate of that remaining. From another point of view, AHT would appear to have an even greater stimulus on regenerating tissue. If one assumes that the amount of liver remaining after partial hepatectomy was between 4 and 5 grams, it increased 2 to 3 fold within two weeks after hepatectomy; about the same accelerated growth that occurred in normal tissue after treatment with AHT. The 4 or 5 grams of tissue remaining after partial hepatectomy, however, had increased 5 or 6 fold when treated with AHT.

One of the six rats dosed with carbon tetrachloride died eight days later, but all six rats dosed with AHT prior to the carbon tetrachloride died within 48 hours. No analysis of the liver was possible for all of the animals were found dead and post-mortem changes had begun. When examined grossly, however, the livers were massive in size (i.e. 30 to 40 grams) and yellow in color, probably from acute fatty infiltration.

Biochemical measurements on the surviving carbon tetrachloride rats showed no appreciable differences from controls aside from small changes in the nucleic acids.

The biochemical data suggest that there are probably qualitative differences in the effect of AHT fluorocarbon dispersing agent on rats and

dogs. Liver enlargement occure in rats but in dogs, hypocholesterolemia is the most unusual finding. It would be useful to investigate these in several other species -- mice, guinea pigs, rabbits. The combined effect of carbon tetrachloride and AHT may be of practical significance since such multiple exposures could conceivably be experienced by personnel handling AHT. Further study of this problem is recommended.

TABLE 1.

Compound	No. Dogs	Dose mg/kg	Dose % Rat ALD	Clinical	Mortality	Biochemical
AHT	1	6	10	None	0	Hypocholesterolemia, elevated APase
	1	9	15	None	0	Hypocholesterolemia, elevated APase, BSP
	1	4	7	None	0	Hypocholesterolemia, elevated APase, BSP
		26	43	None	0	Hypocholesterolemia, elevated APase, GPT BSP retention
		60	100	Vomiting; anorexia, polydipsia, weakness, weight loss, black tarry feces for 4 days	0	Hypocholesterolemia, elevated APase, GPT LDH, HCDA, aldolase, jaundice, bilirubinuria
C_8	2	200	30	Vomiting, polydipsia	0	Elevated APase (1/2), GOT, GPT (2/2) lowered amylase
C_8	2	450	67	Vomiting, polydipsia, blood feces and vomitus, tremors, convulsions, anorexia, death, 1-3 days	2	Elevated APase, GPT, HCDH, LDH, aldolase, jaundice
C_9	2	450	30	Vomiting, 2-4 hours	0	Normal or slightly elevated cholesterol APase, GPT, HCDH, LDH, (1/2)
	2	670	45	Vomiting	0	Normal or slightly elevated cholesterol elevated GOT, GPT (2/2); slightly elevated APase (1/2); amylase and sugar (2/2) elevated after 1 week

TABLE 2

PLASMA LEVELS OF BLOOD CONSTITUENTS AFTER EXPOSURE TO FLUOROCARBON DISBURSING AGENTS

1. AHT

Dose	Days After Dose	Cholesterol mg %	APase Bod. Units	GPT Units	ICDH Units	LDH Units	Aldolase Units
	0*	133	2.3	3.5	230	150	<20
26 mg/kg	8	66	5.5				
	15	49	5.5				
	22	47	7.3				
	37	86	7.2				
	3	74	21.7	850	3900	360	47
60 mg/kg	7	78	15.7	270	145	60	11
	10	64	13.4	135	-	40	10
	14	50	13.1	91	290	100	13
	21	48	11.4	77	145	-	-

* Average pre-exposure

Table 2 (Continued)

2. C_8 FDA

There are also necropsy records

Dose mg/kg	Days after Dose	Cholesterol mg%		APase Bod. Units		GPT Units		GOT Units		ICDH Units		LDH Units		Sugar mg%		Amylase Units	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
200	0*	101	112	2.8	1.7	42	36	23	21					80	95	997	626
	1	102	93	2.6	2.1	38	50	20	36					89	89	530	240
	2	109	111	2.6	6.9	56	845	38	880					85	85	670	350
	5	126	126	2.5	10.1	108	425	34	52					94	94	760	610
	7	106	112	1.8	8.0	56	166	18	32					92	92	780	560
	12	100	127	1.4	5.4	40	70	12	14					90	90	860	580
	21	92	109	1.8	3.7	36	44	16	30					96	96	890	410
	27	89	106	2.4	3.3	42	40	16	16					83	83	850	440
450	0*	114	148	1.2	2.1	19	22			117	138	70	60				
	1	138	164	7.3	20.5	280	8500			2100	114000	300	302				
	2	-	145	-	38.7	-	8500			-	3860	-	1320				

* = average pre-exposure

JRB/jh

Summary and Conclusion

Procedure:

Single oral 12 mg/kg doses of H# 3035 were given to three(3) rats, which observed for 14 days and sacrificed. This procedure was employed so as to enable investigators to compare results with other dispersing agents administered in a similar manner.

Clinical

H# 3035 given in single oral 12 mg/kg doses produced no clinical signs of toxicity.

Conclusion

H# 3035 may be considered relatively harmless when administered to rats in single oral doses of 12 mg/kg, having produced no clinical signs of toxicity.

Table of Liver Weights

Rat No.	Body Wt. gm.	Liver Wt. gm.	Liver Wt. / Body Wt. x 100
49685	403	17.51	4.34
49686	363	14.03	3.86
49693	405	18.03	4.45

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