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EFFECT OF FLUOROCARBON DISPERSING AGENTS ON THE LIVERS OF RATS AND DOGS

Medical Research Project Nos. MR-639, 10-B, 10-C and 10-D

Report No. 123-65

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EFFECT OF FLUOROCARBON DISPERSING AGENTS ON THE LIVERS OF RATS AND DOGS

Medical Research Project Nos. MR-639, 10-B, 10-C and 10-D

Report No. 123-65

Ammonium perfluorocaprylate (C_8APFC), ω-hydrohexadecafluorononanoate (C_9AFC) and ammonium 3,6-dioxa-2,5-di(trifluoromethyl)-undecafluorononanoate (AHT) are powerful surfactants. In acute oral toxicity studies with these compounds, the most striking abnormality in animals surviving a single sublethal dose was gross enlargement of the liver (Haskell Laboratory Report Nos. 54-61, 55-61 and 70-61). A similar but less marked effect was observed in rabbits that had absorbed AHT through the skin. Although liver enlargement is not an uncommon finding in chemical intoxications, the effect of AHT was remarkable in that 56 days after a single dose of 12 mg/kg the liver was approximately three times larger than normal.

The experiments reported here were preliminary studies designed to determine more details concerning:

(1) the histological changes, that occur with increasing periods of time, in the livers of rats after a single oral dose of AHT;

(2) the biochemical changes during the period of rapid growth and recovery in the livers of rats treated with AHT;

(3) the combined effects of oral administration of ethanol and AHT in the rat;

(4) the effect of AHT upon the pentobarbital sleeping time in the rat; and

(5) the effect on liver function in dogs treated with AHT, C_8APFC and C_9AFC in order to find some clinical laboratory procedure that might be useful in detecting liver injury in personnel exposed to fluorocarbon dispersing agents.

I. HISTOLOGICAL CHANGES DURING THE TIME OF RAPID GROWTH

A. Procedure: In order to study the changes in size and structure of the liver following a single oral dose of AHT, 14 male rats were each given a dose of 12 mg/kg. A second group of animals of the same age and weight served as controls. One test and one control animal were killed at intervals over a period of 56 days and two test and two control animals at 75 and 128 days. The livers were removed, weighed and a section taken for microscopic examination.

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B. Results: The rapid growth of the liver, in terms of both weight and per cent of whole body mass, is shown in Figure 1. The increase in size was most marked during the first ten days, reached a maximum between 40 and 60 days, and then began to decrease in size, although some enlargement was still evident after 128 days.

Morphological changes in the liver were evident 24 hours after the single dose. The initial change was characterized by markedly enhanced mitosis of the hepatocytes. This was less evident by the second day; by the ninth day, the mitotic activity had decreased. After the 56th day, most of the features which characterized the response were less intense.

The structural changes in the cells were quite evident upon microscopic examination and appeared to result principally from an enlargement of the individual cells.

II. BIOCHEMICAL AND METABOLIC CHANGES IN RAT LIVER

1. Changes in the Liver of Intact Animals

A. Procedure: Thirty male ChR-CD rats were given 12 mg/kg of AHT as a single oral dose. Two to three weeks later, eight of the rats were sacrificed by decapitation, the livers removed, weighed, and analyzed. An equal number of untreated controls were examined to establish the normal range of values that might be expected in rats of this age and strain. Two AHT-treated rats and two controls were also sacrificed for microscopic examination of the tissue. Eight to ten weeks after the single oral dose of AHT, a second group of ten rats was sacrificed and the remaining ten rats three months later. Tissue slices were prepared from a portion of the median lobe for respiration and choline oxidase measurements in a Warburg respirometer. One portion of the left lateral lobe was homogenized for measurement of alkaline phosphatase activity (APase), esterase activity, and the nucleic acids. Another portion was used for glycogen determination. The remaining tissue was used to measure water, protein, fat, ash, cholesterol, phospholipids, and potassium. An average value was calculated for each group of animals and compared with the controls. Differences between groups were tested for significance with the "t" test.

B. Results: The results of the biochemical measurements are given in Table I. Two weeks after the single dose of AHT, the livers of the treated rats were quite large in size, more than twice that of the controls, and comprised approximately 9% of the body weight. An increase in the alkaline phosphatase activity and phospholipids and a decrease in glycogen were the most important changes. Small but statistically significant ($p < 0.05$) increases in oxygen consumption, non-protein nitrogen, water and protein and decreases in the respiratory quotient, choline oxidase, RNA and DNA occurred.

Two months after the single oral dose of AHT, the livers were no longer growing at an accelerated rate, for there was no further increase in the absolute size of the liver and its portion relative

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to the whole body had decreased. The increase in phospholipids, protein and non-protein nitrogen, and decrease in glycogen respiratory quotient, RNA and DNA again was observed.

Five and one-half months after the dose of AHT, the livers of the treated rats were still slightly larger than normal, but the small differences in the biochemical measurements were no longer significant.

These results indicate that AHT produces significant biochemical as well as morphological changes in the liver of rats. A number of these changes point to disturbances in the normal metabolic and synthetic functions of the liver. The lowered concentration of nucleic acids is consistent with the observed arrested mitotic activity. These effects are most evident when the liver is rapidly increasing in size. Later, when the accelerated growth has slowed down or ceased, a reversal of biochemical changes is apparent. Five or six months after the dose of AHT, when the liver is nearly normal in size, the recovery of the organ is virtually complete.

2. Biochemical Changes and Liver Enlargement in Hepatectomized Rats

A. Procedure: To study the effect of AHT on liver tissue already in a state of rapid growth or regeneration, six male ChR-CD rats were subjected to partial hepatectomy by removing 60 to 70% of the organ. Forty-eight hours after the operation, the rats were given a single oral dose of AHT of 12 mg/kg. The experiment was also conducted reversing the order of treatment by removing 60 to 70% of the liver of rats which had been treated with AHT 48 hours previously. Sham-operated animals dosed with AHT and hepatectomized animals served as controls. Fourteen days after the single oral dose of AHT, the livers of the rats were removed, weighed and analyzed for glycogen, phospholipids, DNA, RNA and alkaline phosphatase activity.

B. Results: The results of these experiments are shown in Table 2. Two weeks after the operation, the livers of the partially hepatectomized control animals were normal in size for rats of this age, sex, and strain, but the alkaline phosphatase activity and DNA were higher than in normal livers. When partially hepatectomized rats were treated with AHT, the livers grew very rapidly; two weeks after treatment with the trimer, the livers were more than twice those of the hepatectomized controls. The phospholipid content and alkaline phosphatase activity were increased while the glycogen content decreased. The DNA was again decreased as in the livers of intact AHT-treated rats, but the RNA had increased. Except for this increase in RNA, the results were in agreement with the previous measurements on livers from AHT-treated rats shown in Table 1. The biochemical changes in the liver occurred whether partial hepatectomy preceded or followed treatment with AHT, although the effects were somewhat less when hepatectomy followed the treatment with AHT.

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3. Effect of Chemical Injury on AHT-treated Rat Livers

A. Procedure: Six male ChR-CD rats were given a single oral dose of 12 mg/kg of AHT and then, 48 hours later, a single sublethal dose of carbon tetrachloride, equivalent to 0.1 ml per 100 gm of body weight, intraperitoneally. Animals which received only carbon tetrachloride served as controls.

B. Results: The results of this experiment are also shown in Table 2. One of the six rats dosed with carbon tetrachloride died eight days later, but all six rats treated 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, approximately 30 to 40 gm, and yellow in color, probably from acute fatty infiltration. The rapidly enlarging liver, with a greater than normal lipid:glycogen ratio, is more susceptible to the toxic effect of CCl_4 than normal liver.

Biochemical measurements on the surviving carbon tetrachloride rats showed no appreciable differences from controls two weeks after the treatment.

III. COMBINED EFFECTS OF ORAL ADMINISTRATION OF ETHYL ALCOHOL AND AHT IN THE RAT

A. Procedure: The study was designed as an additional evaluation of the effects of combining hepatotoxic agents. The objective was to determine, by oral administration to rats, whether repeated sublethal doses of ethyl alcohol would enhance the effect of a single hepatotoxic dose of AHT. The outline of the study, in tabular form, is presented in Table 3.

Male ChR-CD rats, weighing between 300-400 gm, were used in the study. They were offered water and Purina Laboratory Chow on an ad libitum basis and were weighed daily.

At the time of the prescribed sacrifices, the animals were subjected to gross pathological evaluation. The liver was removed, weighed, and preserved in appropriate fixatives.

B. Results: A summary of the liver weights obtained at the various scheduled autopsies is given in Table 4. The preliminary results of this study, wherein AHT was administered to rats in a single dose of 12 mg/kg, simultaneously with, or after, their exposure to ethyl alcohol, indicate that the increase in liver weights observed in this experiment was not any greater than that produced by AHT alone at a dose of 12 mg/kg.

IV. THE EFFECT OF AHT UPON PENTOBARBITAL SLEEPING TIME IN THE RAT

A. Procedure: Changes in liver function or liver morphology have served as traditional indicators of hepatotoxicity. More recently, a quick and simple pharmacologic test has been developed

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which has been used to determine liver damage - Pentobarbital Sleeping Time (PST).

Pentobarbital is metabolized in the liver; a change in particular liver enzymes is reflected by a prolongation, or shortening, of the sleeping time induced in animals by this compound's normal anesthetic action. Therefore, alteration in the PST has been correlated with changes in liver function and liver morphology. When young adult male ChR-CD rats are given 30 mg/kg Na pentobarbital in an aqueous solution intraperitoneally, their average sleeping time was found to be 58 ± 19 minutes (for 110 rats).

In studying the effect of AHT administration upon PST, groups of ten rats were employed. A control value for PST was obtained for each group of ten rats, usually 24 hours before the test began. Twenty-four hours later, the rats were given a single oral dose of AHT at the rate of 12 mg/kg. PST was determined on each group of rats four hours, 24 hours, 48 hours, and at other time intervals after the oral administration of the AHT. Control groups were similarly examined for PST at the same time intervals. The results of two of these tests are summarized in Table 5.

B. Results: The PST of rats that received AHT was first prolonged; it then became shorter and shorter until, at approximately six days after dosing, none of the rats could be anesthetized by a dose of Na pentobarbital that still affected untreated rats. Approximately seven weeks later, the PST of AHT-treated rats could again be determined, but it was still much shorter than that observed in control animals. The same observations were made for the next two weeks, at the end of which time the experiment was terminated.

V. EFFECT OF FLUOROCARBON SURFACTANTS ON LIVER FUNCTION IN DOGS

A. 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. The dog that received 4 mg/kg was later given doses of 26 and 60 mg/kg. Four other male beagles were given a single oral dose of either C_8APFC or C_9AFC, equivalent to 450 mg/kg and a similar series of measurements made. Since both dogs receiving the C_8APFC died within 48 hours, the experiment was repeated with two other dogs which were given a 200 mg/kg dose of this dispersing agent. The two dogs that survived the exposure to the C_9AFC were given an additional oral dose, equivalent to 670 mg/kg, at this time.

The following biochemical measurements were made routinely on the blood: sugar, urea nitrogen, total cholesterol and alkaline phosphatase. When the 60 mg/kg dose of AHT, 470 mg/kg dose of C_8APFC or the 670 mg/kg dose of C_9AFC were administered, the level of activity of lactic dehydrogenase (LDH), isocitric dehydrogenase (ICDH), aldolase, glutamic oxalacetic (GOT) and glutamic pyruvic (GPT) transaminase were also measured. A routine hematological

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examination and an analysis of a 24-hour urine specimen were 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.

B. Results: The significant biochemical and clinical findings are summarized briefly in Table 6.

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. The effects on cholesterol and APase are shown in Figures 2 and 3. These began to change within one week and became "abnormal", i.e., exceeded an arbitrary limit of two standard deviations from the pre-exposure mean within three weeks after the dose was administered. The increase in BSP retention occurred during the first 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 in plasma APase from the pre-exposure mean. The activity of a number of enzymes considered to be sensitive indicators of liver injury was measured in the plasma when a 60 mg/kg dose of AHT was administered. The results of these biochemical measurements are presented in Table 7. All the plasma enzymes measured were elevated within the first three days after the dose of 60 mg/kg of AHT was administered. The greatest increases were in the GPT and APase. 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 as occurred in the dogs receiving 6 or 9 mg/kg.

With the 200 mg/kg dose of C_8APFC, the GPT and GOT were elevated in both dogs within 48 hours. One week later, they were in the normal range. When 450 mg/kg of this compound was administered, all of the enzymes measured were markedly elevated 24 to 48 hours later. The greatest change occurred in the GPT and ICDH. Both animals expired within 48 hours after dosing.

The 450 mg/kg dose of C_8APC caused elevated levels of all the enzymes measured within 48 hours in one (No. 2) of the two dogs exposed. On the tenth day after the dose was administered these were within the normal range. The other dog showed no effect from the treatment. At the 670 mg/kg dose of this compound,

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Dog No. 2 showed a rise in GOT, GPT, and APase during the first 48 hours, and a return to normal within two weeks. There was a slight rise in the APase, GPT and GOT in Dog No. 1.

SUMMARY

Single oral doses of 12 mg/kg of AHT, administered to rats, cause a rapid growth of the liver that continues for as long as two months after the treatment. Morphologically, the change is characterized by an enlargement of the hepatocyte. Changes in the biochemical composition, enzyme activity and respiration, accompany this enlargement and indicate a disturbance in the normal metabolic functions of the organ. The changes in nucleic acid content and morphology of the cell suggest an interference in mitotic activity. Reversal of these changes begins in two months and is nearly complete in five to six months.

Rats that have been subjected to excision of 60 to 70% of the liver 48 hours before or after a single oral dose of 12 mg/kg of AHT regenerate larger than normal livers.

Carbon tetrachloride is more toxic for rats that have been given a single oral dose of 12 mg/kg of AHT. This may result from the accumulation and retention of the CCl_4 in the enlarging liver which has more fat and less glycogen than normal liver.

When AHT is administered to rats in a single dose of 12 mg/kg simultaneously with or after their exposure to repeated sublethal oral doses of ethyl alcohol, the increase in liver weight observed is not any greater than that produced by AHT alone at the same dose.

Following an initial depressant effect by AHT on enzymes that metabolize sodium pentobarbital, there is apparently an increase in the rate of metabolism of sodium pentobarbital so that it cannot exert its anesthetic action at usually anesthetic dose levels.

Liver function studies in dogs have shown that AHT, C_8APFC and C_9APC dispersing agents affect the liver. On the basis of mg/kg dose, AHT is most, and C_9APC least, toxic to the liver. Doses of 26 mg/kg of AHT or 200 mg/kg of C_8APFC produce elevated plasma levels of enzyme activity indicative of cellular damage. Only AHT lowers the cholesterol level in dogs given 4 mg or more per kg of body weight in a single oral dose. Of all the measurements made, alkaline phosphatase and GPT are the most sensitive in detecting an effect on the liver in dogs from all three dispersing agents.

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EFFECT OF FLUOROCARBON DISPERSING AGENTS ON THE LIVERS OF RATS AND DOGS

Medical Research Project Nos. MR-639, 10-B, 10-C and 10-D

Report No. 123-65

HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE

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TABLE 1

BIOCHEMICAL CHANGES IN RAT LIVER

	2-3 Weeks		2 Months		5 1/2 Months	
	Control	AHT	Control	AHT	Control	AHT
Body Weight gm	345	324	546	476	640	602
Liver Weight gm	13.5	28.5	18.0	27.9	18.8	21.4
Liver % Body	3.94	8.80	3.30	5.86	2.93	3.52
O_2 µL/hr.	7.23	7.97	7.00	6.99	6.10	5.93
CO_2 µL/hr.	5.84	5.73	5.67	5.08	4.68	4.50
RQ	0.81	0.72	0.81	0.72	0.77	0.76
APase BU/gm	2.45	3.60	2.66	2.86	3.41	3.04
Esterase U/gm	245	180	210	245		
Choline Oxidase	69	61	65	78	74	78
Water %	68.8	69.8	68.0	68.2	68.3	68.3
Protein %	18.1	19.6	17.2	18.3	16.7	16.9
Fat %	4.14	4.18	5.03	5.31	4.87	4.81
Glycogen %	4.54	2.60	3.86	2.93	4.44	4.05
Ash %	1.38	1.36	1.36	1.32	1.36	1.37
Cholesterol %	0.43	0.40	0.44	0.40		
NPN %	0.23	0.26	0.21	0.23	0.29	0.30
Phospholipids %	3.15	3.96	3.29	4.17	3.08	3.20
Potassium %	0.34	0.33	0.42	0.40	0.[illegible]	0.42
RNA %	0.907	0.872	0.834	0.786	0.824	0.815
DNA %	0.160	0.124	0.176	0.158	0.185	0.176
Plasma APase $\frac{BU}{100\ ml}$			51	51		
Plasma Cholesterol mg%			93	144		

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TABLE 2

BIOCHEMICAL CHANGES IN RAT LIVER TISSUE 2 WEEKS AFTER HEPATECTOMY AND/OR TREATMENT WITH AHT AND AFTER TREATMENT WITH AHT AND/OR CCl_4

Treatment 1	Treatment 2	No. of Rats	Mortality	Liver Weight	Liver % Body	Glycogen	APase	Phospholipids	RNA	DNA
0	Hepatectomy	4	0	12.6	3.15	4.60	3.80	3.3	0.95	0.20
AHT[a]	Sham Operated	6	0	30.5	8.24	2.47	3.94	3.8	1.15	0.12
Hepatectomy	AHT[a]	6	0	29.2	7.29	2.07	4.55	4.3	1.04	0.12
AHT[a]	Hepatectomy	6	0	24.6	7.18	1.74	3.82	4.0	0.93	0.15
CCl_4[b]	0	6	1/6	13.6	3.71	4.16	2.69	3.0	0.96	0.19
AHT[a]	CCl_4[b]	6	6/6	All animals died within 48 hours after receiving AHT						

a) 12 mg AHT/kg body weight

b) 0.1 ml CCl_4/100 gm body weight

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TABLE 3

DOSING AND SACRIFICE SCHEDULE OF ANIMALS GIVEN ETHYL ALCOHOL AND AHT

Group	Total No of Animals	Number of Animals Sacrificed			
		4 Hrs After 10th Dose	14 Days After 10th Dose	28 Days After 10th Dose	2 Months After 10th Dose
Control (no dosing)	8	2	2	2	2
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks)	8	2	2	2	2
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks) + AHT (12 mg/kg on day of first alcohol dose)	6	2	2		2
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks) + AHT (12 mg/kg on day of 10th alcohol dose)	6		2	2	2
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks) + AHT (12 mg/kg on 14th day after 10th alcohol dose)	4			2	2

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TABLE 4

LIVER WEIGHTS OF ANIMALS RECEIVING REPEATED DOSES OF ETHYL ALCOHOL AND A SINGLE DOSE OF AHT

Group	Liver Weights (gm) of Animals Sacrificed			
	4 Hrs. After 10th Dose	14 Days After 10th Dose	28 Days After 10th Dose	After 10th Dose
Control (no dosing)	16; 15	16; 20	19; 23	22; 16
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks)	13; -*	24; 19	25; 21	16; 22
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks) + AHT (12 mg/kg on day of first alcohol dose)	23; 30	42; 32		40; 30
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks) + AHT (12 mg/kg on day of 10th alcohol dose)		39; 44	36; 33	36; 36
C_2H_5OH (2250 mg/kg/day, 5 x week for 2 weeks) + AHT (12 mg/kg on 14th day after 10th alcohol dose)			30; 41	37; 36

* 1 animal died after the fourth dose

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TABLE 5

AVERAGE SLEEPING TIMES OF RATS GIVEN Na PENTOBARBITAL INTRAPERITONEALLY AT A DOSE LEVEL OF 30 mg/kg

Treatment	Pre-Exposure 24 Hours PST ± SD	R	Post-Exposure 4 Hours PST ± SD	R	24 Hours PST ± SD	R	48 Hours PST ± SD	R	6 Days PST ± SD	R	7 Days PST ± SD	R
Control	49 ± 14	10/10	52 ± 8	10/10	56 ± 11	10/10	40 ± 9	9/10			41 ± 12	8/10
AHT (12 mg/kg)	47 ± 6	10/10	121 ± 14	10/10	36 ± 8	7/10	25 ± 6	8/10			-	0/10
Control	50 ± 12	10/10	47 ± 8	10/10	47 ± 6	10/10	46 ± 11	8/10	56 ± 12	10/10		
AHT (12 mg/kg)	56 ± 15	10/10	143 ± 18	10/10	33 ± 12	7/10	22 ± 6	7/10	-	0/10		

PST ± SD = Average Pentobarbital Sleeping Time ± Standard Deviation (minutes)

R = Number of rats which went to sleep / Total number of rats dosed

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TABLE 5 (Cont'd)

AVERAGE SLEEPING TIMES OF RATS GIVEN Na PENTOBARBITAL INTRAPERITONEALLY AT A DOSE LEVEL OF 30 mg/kg

Treatment	Post-Exposure 9 Days PST ± SD	R	16 Days PST ± SD	R	27 Days PST ± SD	R	49 Days PST ± SD	R	56 Days PST ± SD	R	64 Days PST ± SD	R
Control AHT (12 mg/kg)	----------Discontinued after seven days----------											
Control	72 ± 10	9/10	65 ± 18	8/10	80 ± 25	9/10	81 ± 20	10/10	76 ± 16	10/10	82 ± 17	10/10
AHT (12 mg/kg)	-	0/10	-	0/10	-	0/10	18 ± 4	7/10	22 ± 6	6/10	18 ± 2	7/10

PST ± SD = Average Pentobarbital Sleeping Time ± Standard Deviation (minutes)

R = Number of rats which went to sleep / Total number of rats dosed

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TABLE 6

CLINICAL AND BIOCHEMICAL OBSERVATIONS IN DOGS ADMINISTERED FLUOROCARBON DISPERSING AGENTS

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 retention
	1	4	7	None	0	Hypocholesterolemia, elevated APase, BSP retention
		26	43	None	0	Hypocholesterolemia, elevated APase, GPT, BSP retention
		60	100	Vomiting, anorexia, polydipsia, weakness, wt. loss, black tarry feces for 4 days	0	Hypocholesterolemia, elevated APase, GPT, LDH, ICDH, aldolase, jaundice, bilirubinuria
C_8FDA	2	200	30	Vomiting, polydipsia	0	Elevated APase (1/2), GOT, GPT (2/2), lowered amylase
	2	450	67	Vomiting, polydipsia, blood, feces and vomitus, tremors, convulsions, anorexia, death 1-3 days	2	Elevated APase, GPT, ICDH, LDH, aldolase, jaundice
C_9FDA	2	450	30	Vomiting, 2-4 hours	0	Normal or slightly elevated cholesterol, APase, GPT, ICDH, LDH (1/2)
	2	670	45	Vomiting	0	Normal or sl. elevated cholesterol, elevated GOT, GPT (2/2), sl. elevated APase (1/2), amylase and sugar (2/2), elevated after 1 week

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TABLE 7

LIVER FUNCTION TESTS IN DOGS - AHT

Dose (mg/kg)	Days After Dose	Cholesterol mg %	APase Bod. Units	GPT Units	ICDH Units	LDH Units	Aldolase Units
	0*	133	2.3	35	230	150	< 20
	8	66	5.5				
26**	15	49	5.5				
	22	47	7.3				
	37	86	7.2				
	3	74	21.7	850	3900	360	47
	7	78	15.7	270	145	60	11
60	10	64	13.4	135		40	10
	14	50	13.1	91	290	100	13
	21	48	11.4	77	145		

* Average pre-exposure

** Previously received 4 mg/kg 61 days before a dose of 26 mg/kg

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TABLE 7 (Cont'd)

Dose (mg/kg)	Days After Dose	Cholesterol mg % 1	Cholesterol mg % 2	APase Bod. Units 1	APase Bod. Units 2	GPT Units 1	GPT Units 2	GOT Units 1	GOT Units 2	ICDH Units 1	ICDH Units 2	LDH Units 1	LDH Units 2
	0*	101	112	2.8	1.7	42	36	23	21				
	1	102	93	2.6	2.1	38	50	20	36				
	2	109	111	2.6	6.9	56	845	38	880				
C_8APFC 200	5	126	126	2.5	10.1	108	425	34	52				
	7	106	112	1.8	8.0	56	166	18	22				
	12	100	127	1.4	5.4	40	70	12	14				
	21	92	109	1.8	3.7	36	44	16	30				
	27	89	106	2.4	3.3	42	40	16	16				
C_8APFC 450	1	118	164	7.3	20.5	280	8500			2100	114,000	300	302
	2		145		38.7		8600				3860		1320
	0*	112	112	1.2	2.4	25	33	14	16	153	150	72	43
	2	126	126	1.6	3.8	30	116			116	595	74	200
	4	102	124	2.0	4.4	33	84						
C_9AFC 450	7	128	152	1.7	3.8								
	10	100	123	1.3	2.3	27	43			145	189	68	48
	14	121	135	1.7	2.1		40						
	22	142	156	1.1	1.4		25						
	2	142	124	1.9	2.4	66	52	38	22				
	4	159	149	1.9	2.8	70	170	44	172				
C_9AFC 670	5	144	134	1.7	3.6	58	190	18	54				
	7	124	133	1.4	3.5	42	100	14	22				
	12	102	135	1.2	2.4	34	48	16	16				
	21	127	133	1.0	2.0	36	50	18	24				
	27	140	136	1.2	2.1	36	46	14	16				

* Average pre-exposure

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