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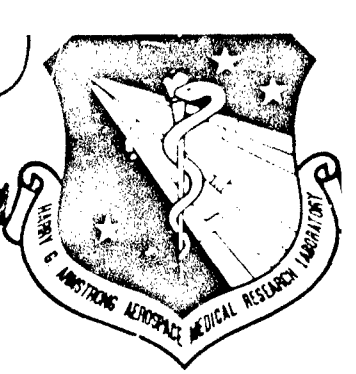

CHLOROTRIFLUOROETHYLENE OLIGOMER: EVALUATION OF ACUTE DELAYED NEUROTOXICITY IN HENS, AND STUDY OF ABSORPTION AND METABOLISM IN RATS FOLLOWING ORAL, DERMAL, AND INHALATION EXPOSURE

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TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-87-044

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Melvin E Andersen

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Acute toxicity studies including delayed neurotoxicity, single dose oral, 4- and 6-hour vapor inhalation, and single dose dermal were conducted with the chlorotrifluoroethylene (CTFE) oligomer MLO 83-322. A second sample, MLO 81-125 from the same lot as MLO 83-322, did not contain the additives and was used only during part of the inhalation study. Hens receiving five consecutive oral doses of up to 9.2 g CTFE/kg body weight remained neurologically asymptomatic through a 30 day observation period. CTFE was readily absorbed and converted to free fluoride following oral and inhalation exposure. Single oral doses of 9.2 g CTFE/kg failed to produce mortality in the test rats. Six-hour saturated vapor exposures to MLO 83-322 resulted in mortality while four-hour exposures caused no deaths. Four-hour inhalation exposures to MLO 81-125 produced deaths at both concentrations tested. CTFE absorption was not evident following dermal exposure.

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PREFACE

The research covered in this report began in April 1984 and was completed in January 1986. The work described in this report was performed by the University of California, Department of Community and Environmental Medicine, Toxic Hazards Research Unit, at Wright-Patterson Air Force Base, OH, under Air Force Contract Number F33615-80-C-0512. M.K. Pinkerton served as the Contract Technical Monitor for the Air Force Harry G. Armstrong Aerospace Medical Research Laboratory (AAMRL). The report was drafted by Northrop Services, Inc. – Environmental Sciences (NSI-ES), 101 Woodman Drive, Dayton, OH. NSI-ES has operated the Toxic Hazards Research Unit since 16 January 1986 under Air Force Contract Number F33615-85-C-0532. Dr. Melvin Andersen is presently the AAMRL Contract Technical Monitor. Although NSI-ES has no reason to question these data, the company makes no warranty, expressed or implied, and assumes no legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, products, or processes disclosed.

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SECTION 1

INTRODUCTION

Chlorotrifluoroethylene (CTFE) oligomer is an inert, nonflammable, saturated, and hydrogen-free chlorofluorocarbon oil. It is noncorrosive and has high thermal stability, good lubricity, and high dielectric strength. Recent dermal and inhalation exposure tests with CTFE indicate that it has a low degree of toxicity. Clayton (1977) reported a 4-h LC_{50} of 1000 ppm in rats, with the primary toxic effect at low to moderate concentrations being nephrotoxicity. There were no deaths among male and female rabbits exposed to 2 g CTFE/kg body weight (Gargus, 1983). Furthermore, there were no deaths among male and female rats exposed for a 4-h period to atmospheres containing saturated-vapor concentrations of CTFE (Coate, 1984).

The neurotoxic potential of CTFE was evaluated because current and other candidate hydraulic fluids are suspected neurotoxicants. Adult female chickens were used to determine whether delayed neurotoxic effects result from exposure to oral doses of CTFE. The U.S. Environmental Protection Agency (EPA Health Effects Test Guidelines, 1982) specifies the use of adult hens in studies of this type. Final determination of injury was based on a comparison of neurotoxic signs and histopathologic examination of nerve tissue from the test chickens with that of the triorthocresylphosphate (TOCP)-positive control chickens.

There is a possibility that free fluorides may be released during the *in vivo* metabolism of CTFE. Human ingestion of fluorides has been shown to result in vomiting, abdominal pain, diarrhea, and convulsions (Patty, 1967). Continuous exposure to fluorides at high concentrations also has detrimental effects on bone and teeth (Hodge, 1975). Therefore, a portion of this study was designed to determine whether CTFE exposure by the oral, inhalation, or dermal route could result in the metabolism of CTFE to free fluorides.

SECTION 2

MATERIALS AND METHODS

ANIMALS

The neurotoxicity study used leghorn hens (*Gallus domestica*, Carey Nick:300-320 hybrid) 5 to 7 months of age, weighing between 1.10 and 1.95 kg. They were purchased from Carey Farms in La Rue, OH. The debeaked hens were identified by leg bands and group housed in 3' x 6' pens to allow

free movement. Food (Carnation, Triple Duty 02250) and water were available *ad libitum*. A photoperiod of 12 h light/12 h darkness was employed.

The animals used in the oral, dermal, and inhalation exposures were male Sprague-Dawley [CD-Crl:CD(SD)BR] rats, weighing in excess of 200 g (Charles River Breeding Labs, Wilmington, MA). Quality control studies conducted during a 14-day quarantine period showed the animals to be in good health. Food (Purina, Formulab #5008) and water were available *ad libitum* except during inhalation exposure.

TEST MATERIAL

Two samples of CTFE were supplied by the U.S. Air Force One sample, labeled MLO 83-322, contained the following additives: 1.0% of a common antirust agent, neutral barium dinonylnaphthalene sulfonate, and 0.05% of a proprietary antiwear additive (at the manufacturer's request, no analysis was made). The second sample, MLO 81-125, was from the same lot as MLO 83-322 but did not contain the additives; it was used during part of the inhalation study only.

Chemically, halocarbon oils are saturated, low molecular weight polymers of CTFE that have the general formula $(CF_2CFCl)_n$. They are made using a controlled polymerization technique and are stable -- the terminal groups being completely halogenated and inert. The product is then separated by vacuum distillation into various fractions, from light oils to waxes.

A comparison of the chromatographic peak areas representative of the two samples analyzed at 0.1 ppm (v/v) in hexane demonstrated some differences (among the four major groups of peaks [A through D], Table 1); principally, the presence of more volatile materials in MLO 81-125 as indicated by the peaks with retention times of 1.45 and 1.61 min. The density of the materials used in this study was reported as 1.82 g/cm^3 at 37.7°C. The MLO 83-322 sample was measured in the laboratory at 1.85 g/cm^3 at 22°C (room temperature).

TABLE 1
COMPARISON BETWEEN CTFE (MLO 81-125) AND CTFE (MLO 83-322)
GAS CHROMATOGRAPHY RETENTION PROFILES

Group	Retention Time (min)	Percentage of Total Area[a]	
		MLO 81-125	MLO 83-322
	1.14	.10	.09
	1.27	.11	.09
	1.45	2.11	.41
A	1.61	8.29	3.14
	1.84	50.51	51.95
	2.11	13.30	11.79
	2.48	.59	1.51
	2.80	0.00	.43
	3.20	.53	.34
	3.78	4.18	4.36
B	4.24	13.47	17.47
	4.72	1.94	3.91
	5.31	0.00	.69
	6.20	.24	.02
C	7.20	.43	.71
	7.73	1.75	1.28
	8.27	.28	0.00
	10.83	.43	.36
	11.39	1.66	1.35
D	12.03	.07	0.00
	12.48	0.00	.09

a Values represent the mean percentage of total area represented in individual peaks from three separate chromatograms.

ACUTE DELAYED NEUROTOXICITY

Prior to the neurotoxicity testing, the acute oral toxicity of the hydraulic fluid in chickens was determined. Oral intubation was accomplished employing a syringe fitted with a 6" infant catheter The nonfasted hens were individually weighed to determine the proper dosage. All hens survived a single dose of 9.2 g/kg. To determine whether hens could survive this regimen for 5 consecutive days, doses of 9.2 g/kg were administered to 3 naive hens over a 5-day period. All of them survived the ensuing 14 days.

The CTFE (MLO 83-322) groups, TOCP-positive control group and vehicle control group, were tested concurrently. The hydraulic fluid was administered in an undiluted state to nonfasted hens. The positive control agent, TOCP, was diluted with corn oil to provide doses of 60, 75, and 90 mg/kg

in a total volume of 5 mL/kg. A negative control group received appropriate volumes of corn oil. Dosing was performed on 5 consecutive days, beginning on a Monday. The method follows that of Siegel *et al.* (1965), recommended for testing of US Navy materials. The dosing regimen was as follows.

CTFE: Groups of 4 hens each were treated with 9.2, 7.4, 5.5 or 3.7 g/kg/day for 5 days

TOCP: Groups of 4 hens each were treated with 90, 75, or 60 mg/kg/day for 5 days

Corn oil: Twelve hens were given the maximum total volume of fluid equal to that given test animals, 5 mL/kg/day for 5 days

Observations and grading by 3 observers began 7 days after the first dose and continued 3 times a week (Monday, Wednesday, and Friday) until 30 days after the first dose. The following scoring system was used.

Symptom-free	0 points
Doubtful or minor symptoms	2 points
Positive paralytic symptoms	8 points
Advanced paralytic symptoms	12 points
Death	16 points

During observation and grading, the chickens were removed from their enclosures and placed on a rubber mat to provide sure footing. Symptoms observed in test hens during the observation period were compared with those seen in the TOCP-treated hens.

All test and control hens were examined for gross pathology at death. Longitudinal and cross sections of the spinal cord (cervical, lumbar, and thoracic regions) and a section of the sciatic nerve were sampled from representative hens from each group for histopathologic examination. These specimens were processed for paraffin embedding, sectioned at 5 μ, and stained with hematoxylin and eosin. CNS sections were stained with Kluver-Barrera stain.

ORAL TOXICITY STUDIES

Blood and urine samples were collected from 3 groups of 10 rats orally dosed with CTFE. One group was used for the determination of blood concentrations and urine excretion of CTFE; the second group was used for assessing blood fluoride concentration and urinary fluoride excretion; and the third group served as controls. CTFE was administered using glass syringes equipped with ball-tipped oral dosing needles. A dose of 9.2 g/kg was administered. Five unanesthetized animals from each group were bled via the orbital sinus at 4, 24, and 48 h posttreatment. Blood samples were taken from the entire group one week posttreatment. All 10 rats from each of the three

groups were held in metabolism cages for the first 24 h posttreatment for urine collection. Additional 24-h urine samples were collected at 8 and 15 days posttreatment.

INHALATION TOXICITY STUDIES

Concentrated vapors of CTFE were generated in a gas washing bottle equipped with a fritted disk. Dried air was delivered at a controlled rate through the disk and bubbled through the bottle which contained known amounts of the test material. The resulting air-vapor mixture was conducted to a 60-L chamber that contained male Sprague-Dawley rats. Initial exposures were conducted without in-line filters between the generation system and the exposure chamber. Additional exposures were conducted utilizing in-line filter systems to eliminate the possibility of pulmonary edema from aerosol formation in the exposure atmosphere. All of the chamber air was supplied through the generator. The initial exposure period was 6 h; however, due to unexpected deaths, this period was shortened to 4 h in subsequent exposures. CTFE concentrations were not chemically analyzed during the 6-h exposures; however, nominal concentrations were calculated by material balance. Chamber concentrations of the 4-h exposures were analyzed using a Miran 80. Blood and urine for CTFE and fluoride ion analysis were collected from these animals in the same manner as that used in the oral exposure portion of these studies. A separate group of control animals was also maintained.

DERMAL TOXICITY STUDIES

A dose equivalent to 3.7 g CTFE/kg was applied to a clipped area on the backs of 10 rats and held in place with a gauze patch, 2 single layers thick. The entire area was covered with plastic wrap held in place with adhesive tape The patches and wrap were removed after 24 h. Blood and urine collections for fluoride determinations were conducted in the same manner as in the oral portion of the study. The same procedure was repeated using another group of rats for the CTFE determinations. A group of 10 rats, which were clipped and bandaged in the same manner as the treated groups, served as controls for the fluoride determinations.

SECTION 3

ANALYTICAL PROCEDURES

INHALATION VAPOR ANALYSIS

A scanning infrared analyzer (Beckman Acculab 4) was used to determine the optimal wavelength for the analysis of chamber atmospheres with a Miran 80. The wavelength chosen was 9.835 μ with a reference wavelength of 5.2 μ. A standard curve for the Miran 80 was prepared using

known CTFE concentrations in 70-L Mylar® bags. Gas chromatograph samples were drawn from the exposure chamber twice per hour to monitor any shifts in the relative distribution of the various CTFE oligomers in the chamber atmospheres.

CTFE ANALYSES

CTFE was extracted from the blood and urine samples by using hexane. Gas chromatography of the hexane extract using electron capture detection gave maximum sensitivity for quantitative analysis while CTFE in hexane served as a standard. The chromatograms of the parent material contained more than 20 peaks in 5 groupings and differed in varying degrees from the chromatograms of the CTFE extracted from blood and urine samples. To facilitate the interpretation of the data, analysis of the chromatograms was limited to the first two major groups of compounds to elute. These peaks contained approximately 90% of the total integrated peak area of the parent materials. Quantitation was based on the electron capture response for the sample, corrected for any area determined to be due to coincident peaks found in control samples.

FLUORIDE ANALYSIS: PLASMA

The method of Singer and Ophaug (1979) was used to determine unbound or ionic fluoride in plasma. A fluoride-specific electrode directly measured the concentration of the ionic form following dilution of the plasma in a simple buffer system (Singer and Ophaug, 1979). The method was standarized using known concentrations of fluoride ion in the buffer.

FLUORIDE ANALYSIS: URINE

A fluoride-specific electrode was used to determine fluoride ion in urine, using the method of Neefus *et al.* (1970). The method utilized synthetic urine as well as a buffer for standardization. The buffer corrected for pH and the necessary ionic strength for linear electrode response when analyzing urine.

STATISTICAL METHODS

Repeated Measures Analysis (Barcikowski, 1983) was used to examine the data from the oral and dermal toxicity studies with rats. The inhalation toxicity studies were statistically analyzed by using the Independent T-test (Zar, 1974).

SECTION 4

EXPERIMENTAL RESULTS

ACUTE DELAYED NEUROTOXICITY

Neurotoxic signs were observed in 11 of the 12 hens that received TOCP. One hen in the lowest TOCP dosage group (60 mg/kg) was symptom-free throughout the 30-day observation period. The corn oil control group showed no signs of neurotoxicity. Two of four hens dosed at 9.2 g CTFE/kg/day died within 1 week; neither animal showed neurotoxic symptoms prior to death. Of the remaining CTFE-treated hens, none showed neurotoxic signs during the 30-day period.

Histopathologic examination of the nerve tissue revealed no remarkable lesions in the corn oil- or CTFE-treated chickens. The positive control group, exposed to TOCP, had minimal to moderate numbers of swollen and degenerating axons in the lateral and ventral funiculi. These lesions were most prominent in the thoracic and lumbar spinal cord.

ORAL TOXICITY STUDIES

Summaries of the urine and plasma fluoride concentrations in male rats following oral administration of CTFE are presented in Figures 1 and 2. These results indicate that CTFE had been absorbed after oral dosing and conversion to free fluoride occurred. The fluoride levels were still elevated in plasma at 7 days postadministration and in urine at 16 days postadministration.

The summaries of CTFE concentrations in blood and CTFE excretion in the urine following oral administration are presented in Figure 3 and Table 2. The blood CTFE concentrations peaked at 48 h, followed by a decline at 1 and 2 weeks. Excretion of CTFE in the urine demonstrated the same general trend. During the first 24-h period, 2.6 mg of CTFE was excreted via the urine. During days 7 and 14 posttreatment, 24 and 2 µg, respectively, were excreted via this route.

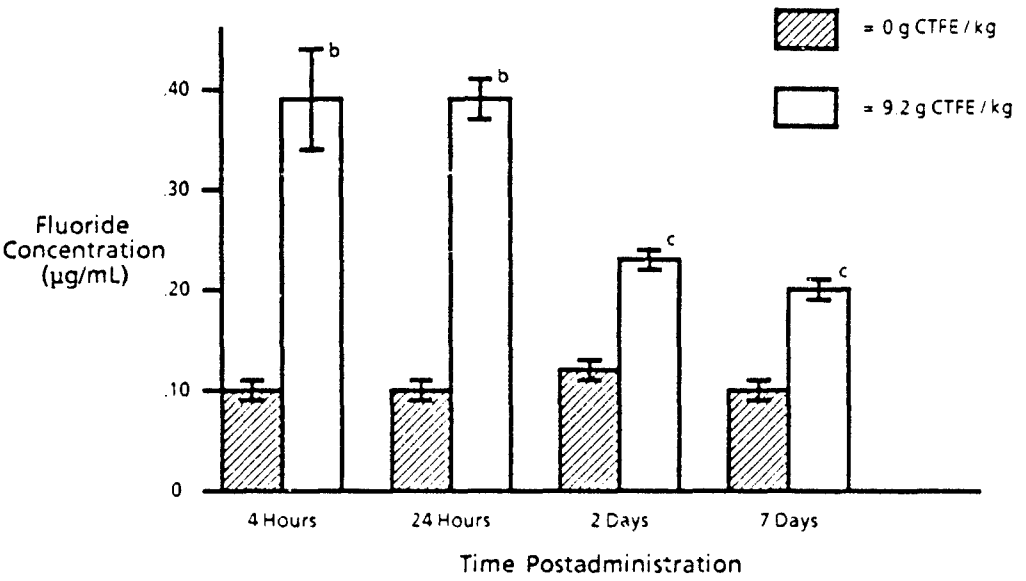
INHALATION TOXICITY STUDIES

The urine and plasma fluoride data from male rats following a 6-h exposure to CTFE-saturated vapor (MLO 83-322) are summarized in Figure 4. However, all 10 male rats in this exposure group (~2.77 mg CTFE/L) died within 3 days following exposure. Therefore, only the first urine sample and the first two blood samples were collected. Fluoride concentrations in both urine and blood samples from treated animals were increased over controls, although not to the extent observed following oral exposure. Pulmonary edema was the only apparent lesion found during gross necropsy. Tissues were not examined histopathologically. A summary of rat mortality following exposure to saturated CTFE vapor is shown in Table 3. Mortality also occurred following 6-h exposures utilizing a glass

wool in-line filter to remove aerosol particles. Again, pulmonary edema was the only lesion observed.

FIGURE 1. PLASMA FLUORIDE CONCENTRATIONS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION OF CTFE

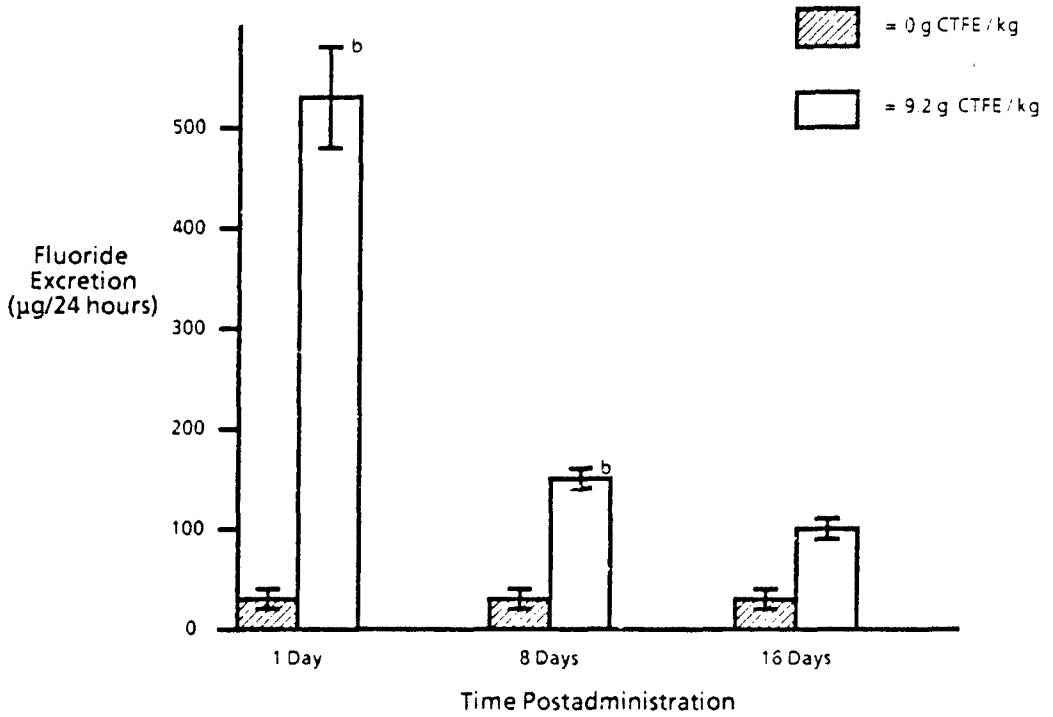
Dose CTFE (g/kg)	Fluoride Concentration (µg/mL)			
	4 Hours	24 Hours	2 Days	7 Days
0	0.10 ± 0.008 (5)	0.10 ± 0.012 (5)	0.11 ± 0.009 (5)	0.10 ± 0.004 (10)
9.2	0.39 ± 0.049 (5)[b]	0.39 ± 0.022 (5)[b]	0.23 ± 0.001 (5)[c]	0.20 ± 0.008 (10)[c]



[a] Mean ± SEM (N)
[b] Statistically different from control at $p < 0.01$ using the Repeated Measures Analysis
[c] Statistically different from 4- and 24-h-treatment groups as well as corresponding control groups at $p < 0.05$ using the Repeated Measures Analysis

FIGURE 2. URINARY FLUORIDE CONCENTRATIONS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION OF CTFE

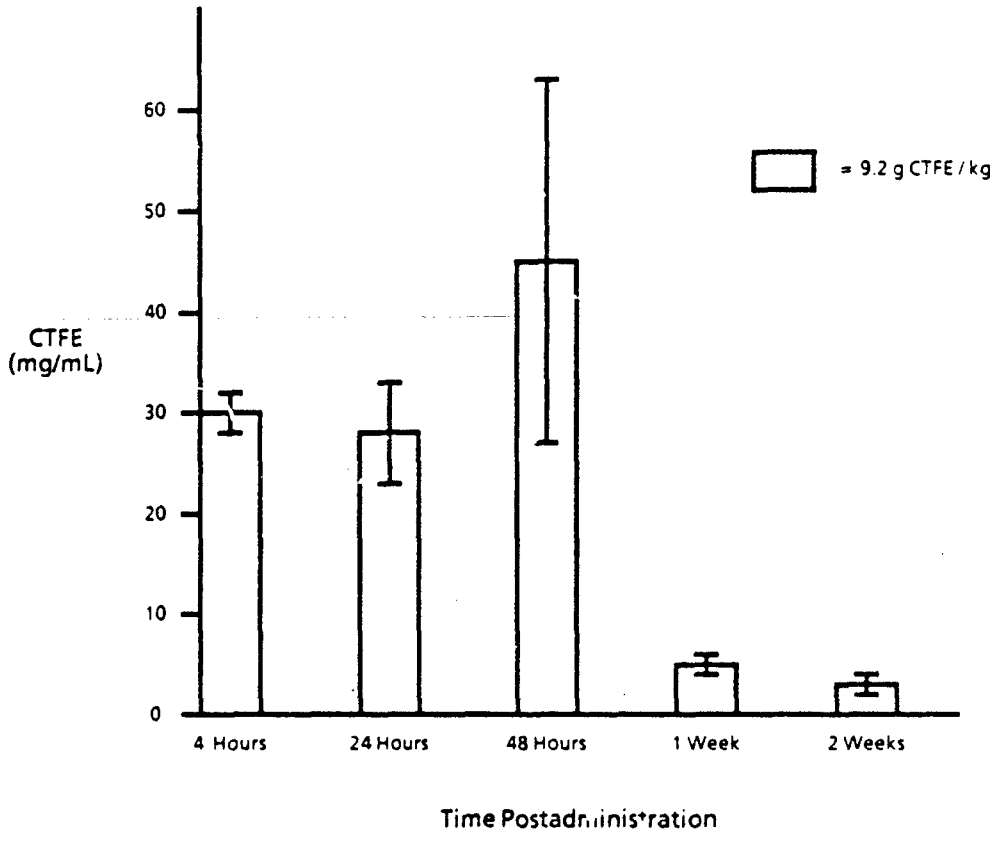
Dose CTFE (g/kg)	Fluoride Concentration (µg/24 hours)		
	1 Day	8 Days	16 Days
0	22 ± 2.1 (10)	19 ± 2.1 (5)	20 ± 0.8 (5)
9.2	526 ± 43.0 (10)[b]	146 ± 9.4 (5)[b]	92 ± 9.0 (5)



[a] Mean ± SEM (N)
[b] Statistically different from control at $p < 0.01$ using the Repeated Measures Analysis

FIGURE 3. PLASMA CTFE CONCENTRATIONS[a] IN MALE RATS FOLLOWING ORAL ADMINISTRATION OF CTFE

Dose CTFE (g/kg)	CTFE Concentration (µg/mL)				
	4 Hours	24 Hours	48 Hours	1 Week	2 Weeks
9.2	29.5 ± 1.39 (5)	28.2 ± 6.35 (5)	46.1 ± 19.40 (5)	3.7 ± 0.31 (5)	1.4 ± 0.28 (5)



[a] Mean ± SEM (N)

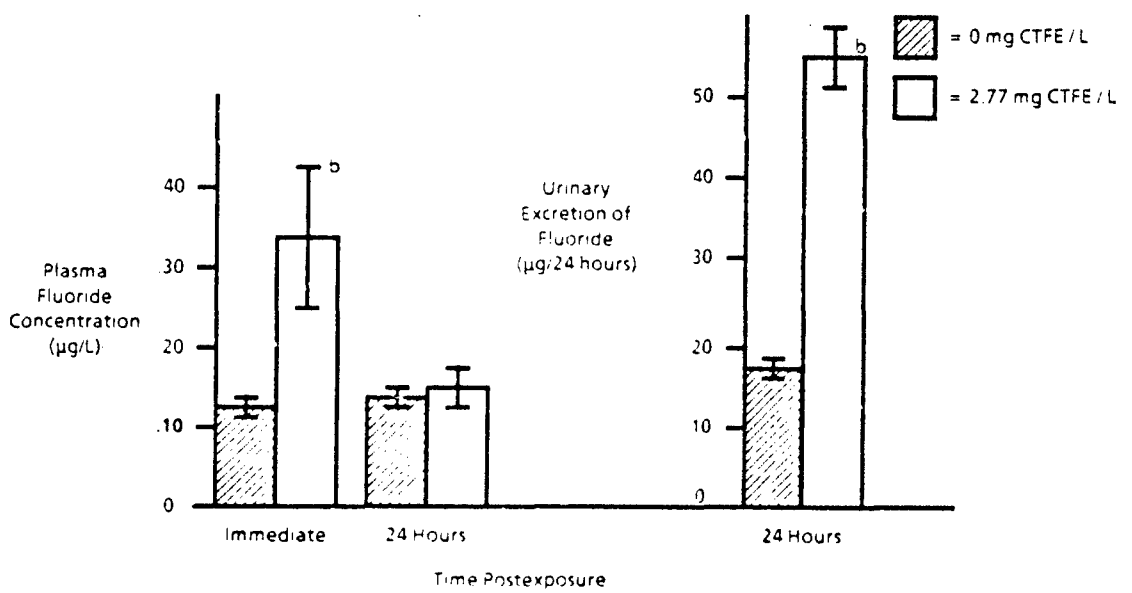
TABLE 2
URINARY CTFE EXCRETION BY MALE RATS FOLLOWING ORAL ADMINISTRATION

Dose CTFE (g/kg)	CTFE (µg/24 hours)		
	1 Day	7 Days	14 Days
9.2	2610 ± 200 (4)	24 ± 6 (5)	2 ± 0.7 (5)

[a] Mean ± SEM (N)

FIGURE 4. PLASMA CONCENTRATION AND URINARY EXCRETION OF FLUORIDE FOLLOWING A 6-HOUR EXPOSURE TO SATURATED VAPOR OF CTFE (MLO 83-322)

CTFE Conc. (mg/L)	Plasma Conc.[a] (µg/mL) Time Postexposure		Urinary Exc.[a] (µg/24 hours)	Mortality (N Dead/ N Exposed)
	Immediate	24 Hours	24 Hours	
0	0.11 ± 0.004 (5)	0.13 ± 0.011 (5)	17.9 ± 1.1 (5)	0/10
2.77	0.33 ± 0 84 (5)[b]	0.15 ± 0.016 (5)	55.7 ± 4.0 (5)[b]	10/10



[a] Mean ± SEM (N)
[b] Statistically different from control at $p < 0.01$ using Independent T-Test

TABLE 3
SUMMARY OF MORTALITY FOLLOWING EXPOSURE OF MALE SPRAGUE-DAWLEY RATS TO SATURATED VAPOR OF CTFE (MLO 83-322)

Exposure	Exposure Duration (Hours)	Filter	Nominal Conc. (mg/L)	Mortality (N Dead / N Exposed)
1	6	None	2.77	10/10
2	6	Glass Wool	2.86	4/4
3	6	Glass Wool	3.51	1/5

Additionally, 4-h inhalation exposures were conducted with the MLO 83-322 CTFE material as well as the MLO 81-125 sample. Chamber concentration, temperature, and mortality data from four 4-h saturated-vapor exposures are presented in Table 4. The saturated-vapor concentrations obtained in the exposure chamber were both material- and temperature-dependent. Exposure concentrations to MLO 81-125 were higher than MLO 83-322, and, in general, higher temperatures resulted in higher concentrations. Deaths occurred only during the MLO 81-125 exposures: 100% mortality at the higher concentration (5.68 mg/L) exposure and 20% at the lower (4.74 mg/L). In the two MLO 83-322 exposures, no mortality was observed even though both of the exposure concentrations were higher than those used in earlier 6-h exposures, which resulted in mortality.

TABLE 4
SUMMARY OF 4-HOUR CTFE INHALATION EXPOSURES WITH MALE RATS

Test Material	Mean Bubbler Temp. (°C)	Mean Chamber Temp. (°C)	Nominal Conc. (mg/L)	Analyzed Conc. (mg/L)	Mortality (N Dead/N Exposed)
MLO 83-322	23.9	25.6	4.47	4.37	0/5
MLO 83-322	24.5	26.0	4.92	4.22	0/5
MLO 81-125	23.1	25.0	5.33	4.74	1/5
MLO 81-125	26.5	27.5	6.57	5.68	5/5

Urine excretion and plasma concentration of fluoride following the two MLO 83-322 exposures are summarized in Figure 5. Fluoride levels were elevated at 24 h postexposure in both urine and plasma. However, only in the urine was the increase statistically significant ($p < 0.05$). The fluoride levels were lower than those following oral administration. However, they were elevated sufficiently to indicate absorption and conversion of CTFE to free fluoride. The CTFE concentrations in urine and plasma are summarized in Table 5. These data again indicate that absorption of CTFE does occur following exposure to atmospheres containing saturated-vapor concentrations.

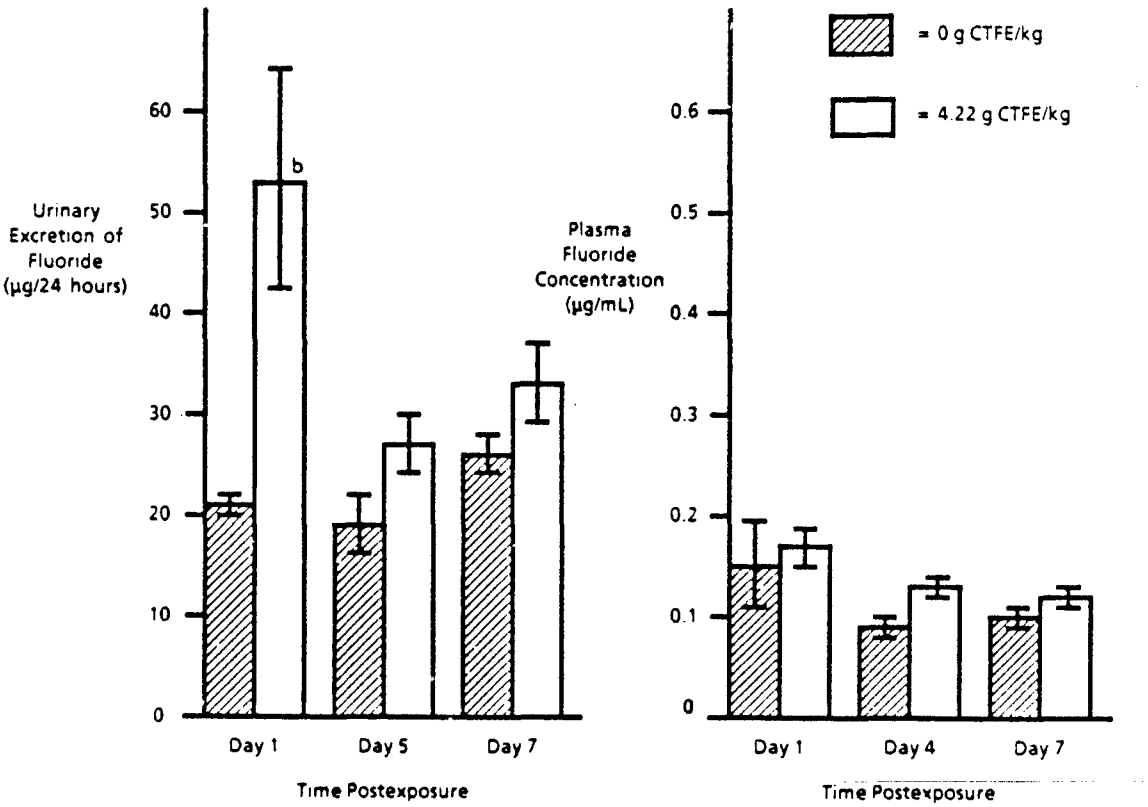
DERMAL TOXICITY STUDIES

Summaries of the urinary excretion and plasma concentration of fluoride from male rats following 24-h dermal contact with CTFE are presented in Figure 6. Overall, the plasma fluoride concentrations were higher than in the controls; however, there was not a time-related significance using the Repeated Measures Analysis (Barcikowski, 1983). There were no differences in the urine fluoride values from control and treated animals at any sampling period.

Results of the CTFE analysis of urine and blood following a 24-h dermal exposure of CTFE are shown in Table 6. These data indicate that only a small amount of CTFE was absorbed through dermal contact.

FIGURE 5. FLUORIDE EXCRETION[a] BY MALE RATS FOLLOWING A 4-HOUR EXPOSURE TO SATURATED-VAPOR CONCENTRATIONS OF CTFE (MLO 83-322)

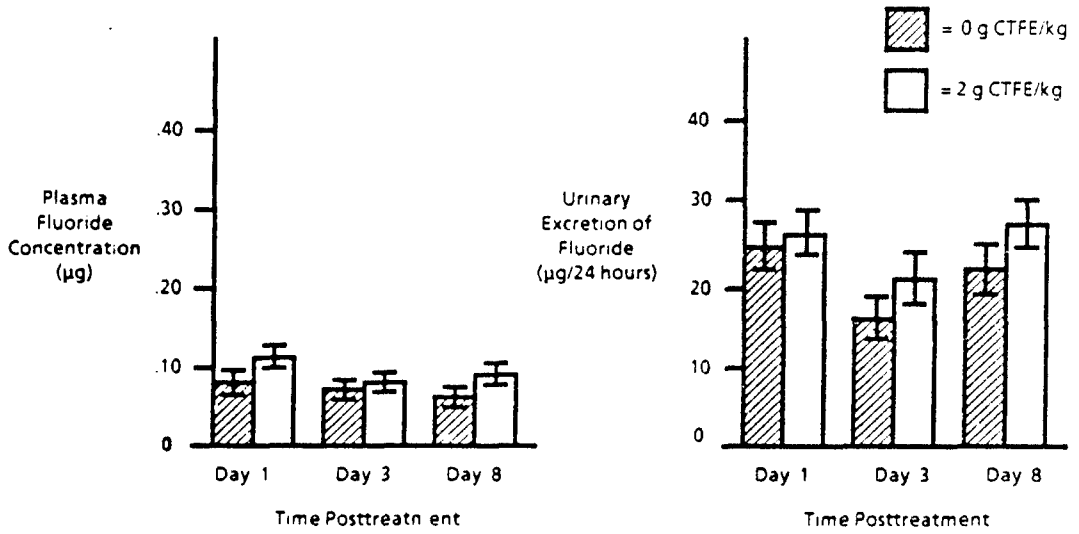
CTFE Conc. (mg/L)	Fluoride			
	Day 1	Day 4	Day 5	Day 7
	Urine (µg/24 hours)			
0	21.6 ± 0.9 (5)		19.5 ± 3.2 (5)	26.1 ± 1.5 (5)
4.22	53.8 ± 10.6 (5)[b]		26.6 ± 2.0 (5)	32.3 ± 3.2 (5)
	Plasma (µg/mL)			
0	0.150 ± 0.043 (5)	0.092 ± 0.007 (5)		0.096 ± 0.004 (5)
4.22	0.165 ± 0.020 (5)	0.119 ± 0.008 (5)		0.116 ± 0.007 (4)



[a] Mean ± SEM (N)
[b] Different from control, $p < 0.05$ using Independent T-Test

FIGURE 6. PLASMA CONCENTRATIONS AND URINARY EXCRETION OF FLUORIDE[a] IN MALE RATS FOLLOWING 24-HOUR DERMAL EXPOSURE TO CTFE (MLO 83-322)

Dose (g/kg)	Fluoride		
	Day 1	Day 3	Day 8
		Urine (µg/24 hours)	
0	24.6 ± 2.2 (5)	17.8 ± 1.3 (5)	23.8 ± 4.4 (5)
2.0	26.1 ± 1.9 (5)	21.2 ± 1.7 (5)	28.1 ± 1.3 (5)
		Plasma (µg/mL)	
0	0.085 ± 0.011 (4)	0.074 ± 0.004 (5)	0.066 ± 0.005 (5)
2.0	0.120 ± 0.004 (5)	0.084 ± 0.005 (5)	0.091 ± 0.008 (5)



[a] Mean ± SEM (N)

TABLE 5
URINARY EXCRETION AND PLASMA CONCENTRATIONS OF CTFE IN MALE RATS FOLLOWING A 4-HOUR EXPOSURE TO SATURATION CONCENTRATIONS OF CTFE (MLO 83-322)

CTFE Conc. (mg/L)	CTFE Level			
	Day 1	Day 3	Day 7	Day 8
		Urine (µg/24 hours)		
4.37	6.42 ± 1.34 (5)[a]	4.41 ± 1.17 (5)	[b]	0.89 ± 0.18 (5)
		Plasma (µg/mL)		
4.37	1.42 ± 0.15 (5)	0.99 ± 1.14 (5)	0.38 ± 0.05 (5)	[b]

[a] Mean ± SEM (N)
[b] Not examined

TABLE 6
URINARY EXCRETION AND PLASMA CONCENTRATIONS[a] OF CTFE IN MALE RATS FOLLOWING 24-HOUR DERMAL EXPOSURE TO CTFE (MLO 83-322)

Dose mg/kg	CTFE Level			
	Day 1	Day 3	Day 7	Day 8
		Urine (µg/24 hours)		
2.0	0.141 ± 0.054 (5)	0.189 ± 0.090 (5)	[b]	0.059 ± 0.032 (5)
		Plasma (µg/mL)		
2.0	0.149 ± 0.028 (5)	0.072 ± 0.017 (5)	0.054 ± 0.010 (5)	[b]

[a] Mean ± SEM (N)
[b] Not examined

SECTION 5

SUMMARY/DISCUSSION

The EPA (1982) recommended a maximum dose limit of 5 g/kg body weight for oral administration of test compounds. If no deaths occur during the subsequent 14 days, the test agent is considered to be nonhazardous by the route of administration used. A group of 3 hens survived for 14 days following 5 consecutive days of oral administration of 9.2 g CTFE/kg body weight. Similar treatment to a group of 4 hens resulted in 2 deaths. Other groups of hens received 5 consecutive daily doses of 7.4, 5.5, and 3.7 g CTFE/kg body weight, and all survived throughout the 30 days. A group of 10 rats survived a single oral dose of 9.2 g CTFE/kg body weight. The dose levels of both the chickens and the rats greatly exceeded the EPA upper test limit and demonstrate that CTFE is nonhazardous via the oral route of administration.

Past studies (for example, see Abou-Donia, 1981) have shown the adult female chicken to be the preferred laboratory animal for evaluating the delayed neurotoxic potential of organophosphorus esters. In order to maintain equilibrium and locomotion, the chicken, a biped, must have well developed neuromuscular control. Surviving hens receiving 5 consecutive oral doses of up to 9.2 g CTFE/kg body weight remained neurologically asymptomatic throughout a 30-day observation period. Histopathologic examination of nerve tissue from these animals revealed no treatment-related lesions, while hens dosed with TOCP demonstrated spinal cord lesions consistent with organophosphate toxicity. Under the conditions of this test, CTFE would not be considered a neurotoxic agent. The dose level tested (9.2 g/kg) would be equivalent to a 70-kg man drinking more than 10 oz of the fluid each day for 5 consecutive days, a highly unlikely accidental event. If the human response to CTFE parallels that of hens, no neurotoxic hazard would be expected for military or civilian personnel involved in the manufacture or handling of the compound.

Four-hour inhalation exposures to atmospheres containing saturated vapors (approximately 4.5-4.9 mg/L) of MLO 83-322, the CTFE sample containing additives, caused no deaths among the exposed rats. These data are in agreement with the study by Coate (1984) in which rats were exposed for 4 h to a concentration of 3.3 mg/L and no mortalities resulted. However, extending the time period from 4 to 6 h resulted in mortality among the exposed animals.

Four-hour inhalation exposures to the CTFE sample lacking additives (MLO 81-125) produced deaths at both concentrations tested. The exposure at the higher temperature was also at a higher chamber concentration and resulted in greater mortality. It is possible that the additives in MLO 83-

322 decreased the vapor pressure of the material, which might be responsible for the lower analyzed concentrations and lack of mortality following exposure.

CTFE was readily absorbed and converted to free fluoride following oral and inhalation exposure. Plasma and urine fluoride levels remained elevated for more than one week following oral exposure and for at least 24 h following inhalation exposure. Because histopathologic evaluation of tissues from the animals exposed to CTFE was not performed, the hazard presented by the free fluorides to teeth, bone, or internal organs cannot be evaluated. CTFE absorption was not evident following dermal exposure.

A summary of acute toxicity results for a wide variety of hydraulic fluids tested in this laboratory is presented in Table 7. Whereas these hydraulic fluids have different base chemical compositions, they are basically fire-resistant fluids that meet various military specifications. The acute toxicity of CTFE compares favorably with the other hydraulic fluids. However, the irritation and sensitization potential of CTFE has not been investigated.

TABLE 7. ACUTE TOXICITY TEST RESULTS OF MILITARY HYDRAULIC FLUIDS

Hydraulic Fluid	Oral LD_{50}	Dermal LD_{50}	Inhalation LC_{50}	Irritation Skin	Irritation Eye	Sensitization	Neurotoxicity	Reference
Chlorotrifluoroethylene (CTFE)	>9.22 g/kg	>3.72 g/kg	>2.00 mg/L[a]	NT	NT	NT	Neg.	
Cyclotriphosphazene	>5.00 g/kg	>2.00 mL/kg	>5.00 mg/L[b]	Neg.	Neg	Neg	NT	(1)
Plurosafe 200	NT	NT	NT	Neg.	Min.	Weak	NT	(2)
WGF-200D (Sample #1)	NT	NT	NT	Neg.	Neg.	Severe	NT	(3)
WGF-200D (Sample #2)	NT	NT	NT	Neg.	Neg.	Severe	NT	(3)
Houghto-Safe 271	NT	NT	NT	Neg.	Neg.	Slight	NT	(3)
Houghto-Safe 273	>5.00 mL/kg	>5.00 mL/kg	>5.00 mg/L[b]	Neg	Neg	Neg	NT	(4)
Bel-Ray Syncom 1400	NT	NT	NT	Mild	Min	Neg	NT	(5)
MLO 82-233	>5.00 mL/kg	>2.00 mL/kg	>1120 mg/m^3[a]	Neg.	Neg.	Neg.	Neg.	(6)
MLO 82-585	>5.00 mL/kg	>2.00 mL/kg	>1140 mg/m^3[a]	Mod.	Neg	Neg	Neg	(6)
Polyalphaolefin-based Fluids								
N448*	>5.00 mL/kg	>2.00 mL/kg	>10.00 mg/L[b]	Neg.	Neg	Neg	NT	(7)
N501	>5.00 mL/kg	>2.00 mL/kg	167-2.39 mg/L[b]	Mild	Neg.	Neg	NT	(7)
N517	>5.00 mL/kg	>2.00 mL/kg	>5.00 mg/L[b]	Mild	Neg.	Neg	NT	(7)
N518	>5.00 mL/kg	>2.00 mL/kg	>5.00 mg/L[b]	Neg.	Neg.	Neg	NT	(7)
N525	>5.00 mL/kg	>2.00 mL/kg	>5.00 mg/L[b]	Neg.	Neg	Neg	NT	(7)
N527	>5.00 mL/kg	>2.00 mL/kg	>5.00 mg/L[b]	Neg.	Neg.	Neg	NT	(7)
885-174	>5.00 g/kg	>2.00 g/kg	1.39-1.62 mg/L[b]	Neg.	Neg.	Mod.	NT	(8)
R-1061-3*	>5.00 g/kg	>2.00 g/kg	0.85-1.23 mg/L[b]	Neg.	Neg	Neg.	NT	(8)
MLO 76-48	NT	NT	NT	NT	NT	NT	Neg	(9)
MLO 78-80	NT	NT	NT	NT	NT	NT	Neg	(9)
Fyrquel 220	>5.00 mL/kg	>5.00 mL/kg	>5.00 mg/L[b]	Neg.	Neg	Neg	Neg.	(4)
Durad MP280	>5.00 mL/kg	>5.00 mL/kg	>5.00 mg/L[b]	Neg.	Neg.	Neg	Pos	(4)
Hydraulic Fluid HF-20	NT	NT	NT	Neg	Neg	Neg	NT	(10)
Water-in-Oil Emulsions								
Quintolubric 958 30w	>5.00 g/kg	>2.00 g/kg	>180 mg/m^3[b]	Neg.	Mild	Weak	NT	(11)
Pyrogaud A-443	>5.00 g/kg	>2.00 g/kg	>110 mg/m3b	Neg	Mild	Neg	NT	(11)
Houghto-Safe 5047F	>5.00 g/kg	>2.00 g/kg	>210 mg/m3b	Neg	Mild	Neg	NT	(11)
Sunsafe F	>5.00 g/kg	>2.00 g/kg	>180 mg/m3b	Neg	Mild	Neg	NT	(11)

[a] Vapor
[b] Aerosol
Neg Negative
* Base fluid (no additives)
NT Not Tested
Min Minimal
Pos Positive

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(1) Kinkead and Bowers, Letter Report, (July) 1985a
(2) Kinkead and Bowers, Letter Report, (September) 1985b
(3) Kinkead, Letter Report, August 1979
(4) Gaworski, *et al.*, AAMRL-TR-86-030, 1986
(5) Gaworski and Horton, Letter Report, September 1985
(6) Kinkead, *et al.*, AAMRL-TR-85-070, 1985
(7) Kinkead, *et al.*, Report in preparation, 1987
(8) Kinkead and Henry, Report in preparation, 1987
(9) Kinkead, *et al.*, Letter Report, December 1983
(10) Kinkead, Letter Report, February 1981
(11) Kinkead and Culpepper, Report in preparation, 1987

SECTION 6

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APPENDIX

QUALITY ASSURANCE

The study "Chlorotrifluoroethylene Oligomer: Evaluation of Acute Delayed Neurotoxicity in Hens, and Study of Absorption and Metabolism in Rats Following Oral, Dermal, and Inhalation Exposure" was conducted by the University of California, Irvine (UCI), Toxic Hazards Research Unit under recognition of the U.S. Food and Drug Administration's Good Laboratory Practices Guidelines. The various phases of this study were inspected by members of the Quality Assurance Group. Results of these inspections were reported directly to the Technical Manager (Study Director) at the close of each inspection.

DATE OF INSPECTION	ITEM INSPECTED
Conducted under UCI	
July 31, 1984	Study records
August 19, 1985	Study records
January 8, 1986	Laboratory notebooks
January 13, 1986	Chemistry report
Conducted under NSI-ES	
January 24, 1986	Laboratory notebooks
December 22, 1986	Study records
January 6, 7, 1987	Final report

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QA Coordinator
Northrop Services, Inc. - Environmental Sciences
Toxic Hazards Research Program

Date June 19, 1987

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