90-DAY INHALATION TOXICITY STUDY OF CF_3I IN FISCHER 344 RATS

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This study was designed to determine and evaluate the potential toxic effects of a 90-day inhalation exposure of rats to CF_3I in nose-only exposure chambers. The repeated, short-duration, multiconcentration inhalation study is designed to permit the demonstration of a no-observable-effect level and toxic effects associated with repeated exposure to the test substance over a period of time. The study will provide information on health hazards likely to arise from repeated exposures by the inhalation route as well as identify target organs associated with exposure. It will provide information necessary for establishing safety criteria for human exposure.

Four groups of 15 male and 15 female Fischer 344 rats were exposed to 8, 4, 2, and 0% CF_3I daily, 2 h/day, excluding weekends, for 90 days. *An* interim sacrifice occured at four weeks at which time five male and five female rats were removed from each group for evaluation. The remainder of the rats were sacrificed at the end of 90 days. Body weights were measured preexposure and weekly thereafter. A clinical examination was made each exposure day. Signs of toxicity were recorded. A complete blood assay (including thyroid hormone evaluation) was conducted on blood samples taken at sacrifice from all animals. Thirty eight tissues were taken for histopathologic examination with wet tissue weights being determined from 11 major organs. Full histopathology was performed on tissues from the control and high-dose exposure groups. In addition, all gross lesions and all target organs of all animals were examined.

INTRODUCTION

A modified acute inhalation toxicity test was performed in which rats were exposed in a nose-only chamber to 12% CF_3I for 15 min (Skaggs et al., 1993). Salivation was observed in the rats upon removal from the chamber, however, all rats appeared to be fully recovered by two hours postexposure. A 15-min, nose-only inhalation $LC_{,}$, has been established at 27% CF_3I (Ledbetter, 1994). No deaths occurred following an acute four-hour nose-only inhalation exposure to either 0.5 or 1.0% CF_3I (Kinkead et al., 1994). Additionally, no treatment-related signs of toxic stress were noted immediately following exposure. Histopathologic examination of tissues from animals examined immediately following exposure, three-days postexposure, or after a 14-day postexposure observation period showed no lesions of pathologic significance.

Military personnel exposure to CF_3I during routine use or repeated use is anticipated to be approximately 1%. Because of the high vapor pressure of CF_3I and resultant volatility, actual exposure concentrations could be less. The EPA Health Effects Guidelines suggest that if an anticipated exposure level is known, the lowest concentration tested should exceed that concentration. The high concentration level selected for this study is 8%, a level that is anticipated to produce some toxic effects but no fatalities. The intermediate level is 4%, which might result in minimal observable effects. The low level target is 2%, which is slightly higher than the anticipated human exposure level, and is expected to be a no-observable-effect level (NOEL).

METHODS

TEST MATERIAL:

Trifluoroiodomethane is a fluoroalkane. Decomposition products are likely to include hydrogen fluoride and hydrogen iodide. The test material used in this study will be supplied by Pacific Scientific, Tulsa, OK. Pertinent physical and chemical properties follow:

CAS No.	2314-97-8
Systematic Name	Iodotrifluoromethane
Molecular Weight	195.91

Empirical Formula	CF_3I
Physical State	Colorless gas
Specific Gravity	2.3608 g/mL (-42 °C)
Melting Point	Not applicable
Boiling Point	-22.5 °C
Flash Point	Not flammable
Vapor Pressure	85 psi @ 20 °C
Solubility in H_2O	Insoluble

The test material was analyzed for purity using a combined gas chromatograph/mass spectrometer. The mass spectra were compared to the library spectra of CF_3I.

TEST ANIMALS:

Sixty male and sixty female Fischer 344 (F-344) rats were purchased from Charles River Breeding Laboratories, Raleigh, NC. The rats were 6 weeks of age upon arrival and 8 weeks of age at the initiation of exposures. All rats were identified by tail tattoo and were subjected to a two-week quarantine period. Water and feed (Purina Formulab #5002) were available ***ad*** *libitum,* except during exposure. Animal room temperatures were maintained at 21 to 25°C, and the light/dark cycle was set at 12-h intervals. The animals were single housed in clear plastic cages with wood chip bedding (Betta-Chip, Northeastern Products Corp., Warrensburg, N.Y.). The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use **of** Laboratory ***Animals,*** prepared by the **US** Department of Health and Human Services (1985).

GENERATION:

The CF_3I which was obtained in 60 lb cylinders was diluted with 10 L/min flow **of** laboratory air. Both flows were controlled and monitored with Matheson (600 series) rotameters. Fine concentration control was by minor adjustment of the CF_3I flow in response to analysis. The total air flow provided more than 300 mL/minute delivered at each exposure port. Target concentrations of CF_3I during the 90-day study were 8, 4, 2, and 0% (Vol/Vol). No oxygen was added to the exposure atmosphere.

A portion of the input air passed through a gas washing bottle (Ace Glass, Vinland, N.J. Model 7166-26) containing water. Humidity and temperature of the exposure atmosphere were

constantly monitored and recorded using H-CAL dual probes (Models CT830, HY-CALI,Atlanta, GA) and a data acquisition system. The RH ranged between 45 and 55%, while the temperature ranged from 68 to 74°F.

The nose-only chambers used are modified stainless-steel flow-past chambers as described by Cannon et al., 1983. The modification results in an efficient and continuous delivery of exposure atmosphere to the breathing zone of the rat. Fifteen of the 52 ports of each chamber were slected randomly for the rat exposures.

EXPERIMENTAL GROUPS:

Group	Target Concentration (%)	Number of Animals
I	8	15 male 15 female
II	4	15 male 15 female
III	2	15 male 15 female
IV	0 (control)	15 male 15 female

EXPOSURE REGIMEN:

All rats received one week (5days) of sham exposure to acclimate them to the daily treatment regimen. Animals were exposed daily, 2 h/day, excluding weekends, for 90 days. The exposure period was considered as starting when the chamber concentration reached T-99 and concluded one hour later. The rats remained in the restraining tubes until the chamber concentration returned to baseline values. An interim sacrifice occurred after four weeks (20 exposures) at which time five male and five female rats were removed from each group for evaluations as detailed later.

CHAMBER ANALYSIS:

Continuous analysis of the CF_3I was performed using infrared absorption spectrometers (Miran 1A, Foxboro Analytical, South Norwalk, CT). A 10 cm short path cell in combination with a low intensity absorption band at 9.6 to 9.7 microns facilitated the analysis **of** the high concentrations involved in these studies. Instrumental calibration was performed using known concentrations of CF_3I in air contained in Tedlar sample bags **(231** series, SKC, Eighty Four, PA).

TOXICITY ASSESSMENT:

Records were maintained of body weights (BW), signs of toxicity, and mortality. Euthanasia was via CO, inhalation overdose. At sacrifice, gross pathology was performed on all animals. Wet tissue weights were determined on:

Adrenals	Lungs
Brain	Ovaries (females)
Heart	Spleen
Kidneys	Testes (males)
Liver	Thymus
Thyroid w/parathyroids	

Tissues were fixed in 10% neutral buffered formalin, trimmed, and further processed via routine methods for hematoxylin-eosin-stained paraffin-embedded sections (Luna, **1968).** Additionally, blood was drawn for the following hamatology and clinical chemistry assays:

Whole Blood	*Serum*	
Hematocrit	Calcium	Total Protein
Hemoglobin	Phosphorus	Albumin
Red blood cell count	Chloride	Globulin
Platelet count	Potassium	Urea Nitrogen
Total and Differential leukocyte count	Creatinine	Total Bilirubin
	Glucose	Sodium
	Magnesium	CO_2
	Triglycerides	Thyroxine (T_4)
	Alkaline phosphatase	
	Alanine aminotransaminase (ALT)	
	Aspartate aminotransaminase (AST)	
	Thyroxine-binding globulin (TBG)	

Gamma-Glutamyl transferase (GGT)
Triiodothyronine (T_3)
Thyroid stimulating hormone (TSH)
Reverse T_3 (RT_3)

Erythrocytes were enumerated on a Coulter Counter (Coulter Electronics, Hialeah, FL), and sera for clinical chemistry evaluations were assayed on a Ektachem 700 XR (Eastman Kodak, Rochester, NY). Thyroxine and thyroxine-binding globulin (TBG) assays were performed using a DuPont ACA Analyzer (DuPont Co., Wilmington, DE).

HISTOPATHOLOGY:

Animals were not fasted prior to necropsy. The following tissues were taken for histopathologic examination:

Gross lesions	Heart
Brain	Liver
Lungs (perfused)	Spleen
Trachea	Duodenum
Prostate	Spinal cord
Epididymides	Urinary bladder
Jejunum	Cecum
Ileum	Salivary glands
Esophagus	Stomach
Mandibular lymph nodes	Colon
Mesenteric lymph nodes	Rectum
Thymus	Sternum w/bone marrow
Kidneys	Sciatic nerve
Adrenals	Skeletal muscle (thigh)
Ovaries/Testes	Bone (femur including stifle)
Pituitary	
Thyroid w/parathyroid	Nasal turbinates
Zymbal gland	Pancreas
Lachrymal gland	uterus

Table 1 **Absolute and Relative Organ Weights[a] of Male Rats Treated with CF_3I for 30 Days**

Oreans	Control	Low	Medium	High
Body	2 18.48± 8.21		2 13.46± 4.99	187.24± 7.39'
Brain	1.70± 0.02		1.71± 0.01'	1.65± 0.03'
Ratio[b]	0.78 ± 0.03		0.80 ± 0.02	0.89 ± 0.03[d]
Liver	7.37 ± 0.41		7.72 ± 0.31	6.94 ± 0.39
Ratio'	3.37 ± 0.08		3.61 ± 0.07	3.70 ± 0.11[d]
Kidneys	1.81± 0.18		1.63± 0.05	1.46± 0.09[c]
Ratio	0.83 ± 0.10		0.76 ± 0.01	0.78 ± 0.02
Spleen	0.43 ± 0.02		0.39 ± 0.01[d]	0.35 ± 0.02[d]
Ratio	0.19 ± 0.01		0.18 ± 0.00	0.19 ± 0.00
Thymus	0.23 ± 0.02		0.18 ± 0.01[d]	0.19 ± 0.02[d]
Ratio	0.10 ± 0.01		0.08 ± 0.01'	0.10 ± 0.01'
Heart	0.72 ± 0.04		0.67 ± 0.02	0.67 ± 0.03
Ratio	0.33 ± 0.01		0.32 ± 0.01	0.36 ± 0.01
Adrenal Gland	0.05 ± 0.01		0.04 ± 0.01	0.05 ± 0.01
Ratio	0.02 ± 0.00		0.02 ± 0.00	0.03 ± 0.00
Lungs	1.38± 0.04		1.19± 0.06	1.16± 0.09[d]
Ratio	0.63 ± 0.01		0.56 ± 0.02	0.62 ± 0.03
Thyroid	0.02 ± 0.00		0.02 ± 0.00	0.01 ± 0.00[e]
Ratio	0.01 ± 0.00		0.01 ± 0.00	0.01 ± 0.00[ce]
Testes	2.53 ± 0.07		2.34 ± 0.08	1.63± 0.07[d]
Ratio	1.16 ± 0.02		1.10± 0.03	0.87 ± 0.02[d]

[a]Mean ± SEM, N=5.

[b]Organ weight/body weight x 100.

'Significantly different than Control at P<0.05.

[d]Significantly different than Control at P<0.01.

[e]N=4.

Table 2 **Absolute and Relative Organ Weights[a] of Male Rats Treated with CF_3I for 90 Days**

Organs	Control[e]	Low[f]	Medium[g]	High[e]
Body	269.84 ± 10.25	261.56 ± 11.62	250.32 ± 7.91	222.77 ± 3.44[c]
Brain	1.91 ± 0.03	1.83 ± 0.05	1.78 ± 0.04[f]	1.82 ± 0.06[f]
Ratio[b]	0.71 ± 0.03	0.71 ± 0.03	0.72 ± 0.02	0.82 ± 0.02[d]
Liver	8.78 ± 0.40	8.53 ± 0.44	8.23 ± 0.34	7.40 ± 0.19
Ratio[f]	3.25 ± 0.06	3.26 ± 0.05	3.28 ± 0.05	3.32 ± 0.05[d]
Kidneys	1.95 ± 0.07	1.82 ± 0.08	1.18 ± 0.06	1.66 ± 0.05[c]
Ratio	0.72 ± 0.02	0.70 ± 0.01	0.72 ± 0.01	0.74 ± 0.01
Spleen	0.58 ± 0.03	0.70 ± 0.18	0.48 ± 0.01[d]	0.44 ± 0.03[d]
Ratio	0.21 ± 0.01	0.26 ± 0.06	0.19 ± 0.01	0.20 ± 0.01
Thymus	0.25 ± 0.02	0.22 ± 0.02[f]	0.21 ± 0.01[d]	0.18 ± 0.02[d]
Ratio	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.00[f]	0.08 ± 0.01[f]
Heart	0.87 ± 0.03	0.85 ± 0.04	0.89 ± 0.10	0.77 ± 0.04
Ratio	0.32 ± 0.01	0.33 ± 0.02	0.36 ± 0.05	0.35 ± 0.02
Adrenal Gland	0.11 ± 0.02	0.10 ± 0.03	0.07 ± 0.01	0.09 ± 0.02
Ratio	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01
Lungs	1.71 ± 0.06	1.62 ± 0.05	1.52 ± 0.07	1.42 ± 0.05[d]
Ratio	0.63 ± 0.01	0.63 ± 0.05	0.61 ± 0.02	0.64 ± 0.02
Thyroid	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Ratio	0.01 ± 0.00	0.01 ± 0.00[c]	0.01 ± 0.00[f]	0.01 ± 0.00[f]
Testes	2.85 ± 0.04	2.59 ± 0.12	2.52 ± 0.08	2.06 ± 0.14[d]
Ratio	1.07 ± 0.03	1.00 ± 0.06	1.01 ± 0.02	0.92 ± 0.06[d]

[a]Mean ± SEM.

[b]Organ weight/body weight x 100.

[f]Significantly different than Control at $P<0.05$.

[d]Significantly different than Control at $P<0.01$.

[e]N=9.
[f]N=8.
[g]N=10.

Table 3 **Absolute and Relative Organ Weights[a] of Female Rats Treated with CF_3I for 30 Days**

Organs	Control	Low	Medium	High
Body	152.47 ± 2.11	147.74 ± 3.78	150.16 ± 1.82	138.16 ± 2.74'
Brain	1.72 ± 0.03	1.64 ± 0.04	1.65 ± 0.02	1.60 ± 0.01'
Ratio[b]	1.12 ± 0.02	1.11 ± 0.02	1.09 ± 0.02	1.16 ± 0.03[d]
Liver	5.14 ± 0.11	4.76 ± 0.19	5.16 ± 0.14	5.14 ± 0.14
Ratio'	3.35 ± 0.07	3.23 ± 0.05	3.42 ± 0.08	3.72 ± 0.07[d]
Kidneys	1.12 ± 0.02	1.16 ± 0.03	1.19 ± 0.03	1.11 ± 0.02'
Ratio	0.79 ± 0.01	0.78 ± 0.01	0.79 ± 0.02	0.81 ± 0.03
Spleen	0.39 ± 0.01	0.34 ± 0.02	0.32 ± 0.02[d]	0.30 ± 0.01[d]
Ratio	0.25 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
Thymus	0.23 ± 0.02	0.20 ± 0.03'	0.17 ± 0.02[d]	0.15 ± 0.01[d]
Ratio	0.15 ± 0.01	0.13 ± 0.02	0.11 ± 0.02'	0.11 ± 0.01'
Heart	0.56 ± 0.01	0.54 ± 0.02	0.54 ± 0.01	0.56 ± 0.01
Ratio	0.37 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.40 ± 0.01
Adrenal Gland	0.09 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
Ratio	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Lungs	1.10 ± 0.04	1.05 ± 0.05	1.08 ± 0.03	1.08 ± 0.04
Ratio	0.72 ± 0.03	0.71 ± 0.02	0.72 ± 0.01	0.78 ± 0.03
Thyroid	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Ratio	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00'	0.01 ± 0.00'
Overies	0.11 ± 0.02	0.11 ± 0.01	0.08 ± 0.01'	0.08 ± 0.01'
Ratio	0.07 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0.06 ± 0.01

[a]Mean ± SEM, N=5.

[b]Organ weight/body weight x 100.

'Significantly different than Control at P<0.05.

[d]Significantly different than Control at P<0.01.

Table 4 **Absolute and Relative Organ Weights' of Female Rats Treated with CF_3I for 90 Davs**

Organs	Control	Low	Medium	High
Body	170.61 ± 3.51	172.22 ± 2.53	164.95 ± 2.28	147.43 ± 1.91'
Brain	1.81 ± 0.03	1.77 ± 0.03	1.71 ± 0.05'	1.68 ± 0.02'
Ratio[b]	1.07 ± 0.03	1.04 ± 0.02	1.04 ± 0.02	1.14 ± 0.02[d]
Liver	5.32 ± 0.13	5.40 ± 0.16	5.28 ± 0.22	4.81 ± 0.20
Ratio'	3.11 ± 0.03	3.16 ± 0.09	3.19 ± 0.09	3.26 ± 0.12[d]
Kidneys	1.32 ± 0.03	1.28 ± 0.02	1.32 ± 0.07	1.22 ± 0.02'
Ratio	0.78 ± 0.01	0.75 ± 0.01	0.80 ± 0.04	0.83 ± 0.01
Spleen	0.46 ± 0.02	0.40 ± 0.01	0.39 ± 0.02[d]	0.33 ± 0.01[d]
Ratio	0.27 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
Thymus	0.22 ± 0.02	0.18 ± 0.01'	0.18 ± 0.01[d]	0.15 ± 0.01[d]
Ratio	0.13 ± 0.01	0.11 ± 0.01'	0.11 ± 0.01'	0.10 ± 0.01[c]
Heart	0.64 ± 0.03	0.60 ± 0.01	0.61 ± 0.02	0.57 ± 0.02
Ratio	0.37 ± 0.02	0.35 ± 0.01	0.37 ± 0.01	0.38 ± 0.01
Adrenal Gland	0.12 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Ratio	0.07 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
Lungs	1.30 ± 0.05	1.24 ± 0.03	1.22 ± 0.04	1.13 ± 0.03[d]
Ratio	0.76 ± 0.02	0.73 ± 0.01	0.74 ± 0.02	0.77 ± 0.03
Thyroid	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Ratio	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00[d]
Overies	0.16 ± 0.02	0.11 ± 0.01	0.10 ± 0.01'	0.10 ± 0.01'
Ratio	0.10 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01

[a]Mean ± SEM, N=10

[b]Organ weight/body weight x 100.

'Significantly different than Control at P<0.05.

[d]Significantly different than Control at P<0.01.

DISCUSSION

Discussion and interpretation awaits receipt of clinical chemistry and histopathology information. Further evaluation of the toxicological status of CF_3I cannot be provided without analysis of these results.

REFERENCES

Cannon, W.C., E.F. Blanton, and K.E. McDonald. 1983. The Flow-Past Chamber: *An* Improved Nose-Only Exposure System for Rodents. *Am. Ind. Hyg. Assoc. J.* **44**(12):923-933.

Kinkead, E.R., S.A. Salins, R.E. Wolfe, H.F. Leahy, and J.H. English. *Acute Toxicity Evaluation of Halon Replacement Trifluoroiodomethane (CF_3I).* AL-TR-1994-____, Armstrong Laboratory, Wright-Patterson Air Force Base, OH.

Ledbetter, A. 1994. Personal communication.

Luna, L.G., ed. 1968. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.* 3rd Ed. New York: McGraw Hill.

Skaggs, S.R., D.S. Dierdorf, and R.E. Tapscott. 1993. *Update on Iodides as Fire Extinguishing Agents.* International CFE and Halon Alternatives Conference, Washington, DC. pp. 800-808.