THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48640

December 9, 1982

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Dear Sir or Madam:

As required by 40 CFR 716, we herewith submit copies of reports which meet the requirements of the referenced rule on Health and Safety Studies. As noted in the statement enclosed with the reports, some contain confidential business information.

The reports are separated into three categories for your convenience.

Package 1. Reports which contain no Confidential Business Information.

Package 2. Reports which contain Confidential Business Information.

Package 3. Reports from which Confidential Business Information has been deleted. (Public File Copy of reports in Package 2).

Each report is marked with an identifying number at the top of the first page of the report, e.g., D-155. Use of this identification number in future correspondence regarding this submission will facilitate handling of questions.

In order to expedite the completion of our search and submission, no attempt was made to determine whether or not we manufactured or processed the chemicals which formed the subject of submitted reports. (40 CFR 716.6). Thus, submission of a report for any given material should not be construed as indicative of Dow's status as a manufacturer or processor of the material.

Many of the submitted reports contain information which is not relevant to Health or Safety Studies of listed chemicals, e.g., references to unlisted chemicals, marketing or process data, account numbers, internal document identification codes or distribution lists. Such information has been deleted from all copies submitted.

The index required by 40 CFR 716.6(b) is enclosed. It lists the Dow identification number and title of each report submitted in CAS number order. Please note that the index contains Dow Confidential Business Information.

We have also included a reprint of a recently published article dealing with methylene chloride.

Very truly yours,

Robert L. Hagerman
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THE DOW CHEMICAL COMPANY
BIOCHEMICAL RESEARCH LABORATORY

TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF

AEROTHENE® TT SOLVENT

FILE
CASE NO.
REF.
VOUCHER
SUB. BY Mark M. Rutsky
DEPT. BY NLitchfield-JMNorris
CHECKED BY T. R. Torkelson
DATE August 25, 1969

INTERPRETIVE SUMMARY WITH CONCLUSIONS STATED ON THE SAMPLE RECEIVED. ADDITIONAL INTERPRETIVE INFORMATION THE EFFECT OF DIFFERENT EXPOSURE MAY BE PROVIDED AS SPECIFIC USES AND FORMULATIONS ARE DEVELOPED OR IF PROCESS CHANGES OCCUR.

A sample of Aerothene® TT Solvent was submitted to the Biochemical Research Laboratory for toxicological studies and definition of industrial handling hazards. Aerothene® TT Solvent is a specially inhibited grade of 1,1,1-trichloroethane used by the aerosol industry.

The test material has a very low acute oral toxicity. The single dose oral LD_{50} for female rats is between 7.95 and 15.8 gm/kg body weight. The test material should present no problem from ingestion incidental to industrial handling.

Instillation of the undiluted material in the eye of a rabbit caused moderate pain, slight redness and swelling of the conjunctival membranes, and slight transient corneal injury. Washing of a similarly exposed eye for 2 minutes within 30 to 60 seconds after contact did not lessen the response.

Minimal eye protection is recommended for industrial handling whenever the likelihood of eye contact exists.

Skin irritation tests conducted on rabbits demonstrated that repeated and prolonged contact of the undiluted material with the inner surface of the rabbit ear resulted in no more than slight skin redness and slight exfoliation. A confined, prolonged contact with intact and abraded shaved belly skin of a rabbit caused slight redness and slight swelling. Subsequent exposures caused slight increase in the intensity of the skin redness and moderate exfoliation.

Brief human skin exposure with Aerothene® TT Solvent would not be likely to result in a skin response other than superficial defatting of the skin. Prolonged exposure could result in slight redness and, when confined to the skin by clothing, shoes or gloves, slight blistering may occur. Frequent and prolonged skin exposure would probably cause scaling of the skin. Skin exposure would not result in adverse effects due to absorption of the material through the skin.

(over)

®Aerothene is the registered trademark of The Dow Chemical Company for its specially inhibited grades of chlorinated solvents for the aerosol industry.

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The effects of vapor inhalation of Aerothene TT will be reported separately.

OFFICE OF TOXIC SUBSTANCES

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Chemical Name (100 per name)	25	CAS No. (10)	26
METHYL CHLOROFORM		71-55-6	

A.K. 12/21/82

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Extra copy

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

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REPORT TO

DOW CHEMICAL U.S.A.

30-DAY SUBACUTE VAPOR INHALATION TOXICITY STUDY WITH SIX STABILIZED METHYL CHLOROFORM FORMULATIONS IN ALBINO RATS, ALBINO RABBITS AND BEAGLE DOGS

P. O. NO. MM-917244

MARCH 25, 1976

IBT NO. 663-06354

REPORT TO

DOW CHEMICAL U.S.A.

30-DAY SUBACUTE VAPOR INHALATION TOXICITY STUDY WITH SIX STABILIZED METHYL CHLOROFORM FORMULATIONS IN ALBINO RATS, ALBINO RABBITS AND BEAGLE DOGS

MARCH 25, 1976

I. Introduction

Six clear liquid samples were received from Dow Chemical U.S.A. for the purpose of conducting a 30-day subacute vapor inhalation toxicity study using albino rats, albino rabbits and beagle dogs as experimental animals. Three of the samples were labeled Methyl Chloroform Formulation XN-6054.1-L with either 1X inhibitor, 2.5X inhibitor or 5X inhibitor. The remaining 3 samples were labeled Methyl Chloroform Formulation XN-6080 with either 1X inhibitor, 2.5X inhibitor or 5X inhibitor.

II. Summary

A 30-day subacute vapor inhalation toxicity study was conducted with 6 methyl chloroform formulations wherein each of the 6 materials was to be generated at a concentration of 1750 ppm. A separate group of animals was exposed to each of the materials. Each test group consisted of 20 albino rats, 6 albino rabbits and 4 beagle dogs. Exposures were conducted 6 hours per day, 5 days per week, for a period of 4 weeks (a total of 21 exposures). An additional group of dogs, rats and rabbits served as untreated controls. These animals remained in their cages and received no exposures. Below is an outline of group organization, as well as target and actual chamber concentrations.

Group	Test Material	Chamber Concentrations (ppm) Target	Actual (Mean)
Untreated Control	-	-	-
T-I	XN-6056.1-L 1X inhibitor	1750	1743
T-II	XN-6056.1-L 2.5X inhibitor	1750	1708
T-III	XN-6056.1-L 5X inhibitor	1750	1777
T-IV	XN-6060, 1X inhibitor	1750	1847
T-V	XN-6060, 2.5X inhibitor	1750	1853
T-VI	XN-6060, 5X inhibitor	1750	1741

Observations were made with respect to incidence of mortality, body weight effects and reactions displayed. Hematologic and clinical chemistry studies as well as urinalyses were conducted on all dogs, 10 male and 10 female rats and all rabbits in the untreated control, T-III and T-VI groups prior to the first exposure and at the end of the testing period.

All surviving animals were sacrificed upon completion of the test and subjected to gross pathologic examinations. In addition, those from the control, T-III and T-VI groups were examined histologically. A complete set of tissues and organs were preserved in 10 percent buffered formalin. The weights of major organs were recorded and the organ weights and organ to body and organ to brain weight ratios of test animals were statistically compared to those of the untreated controls.

There were 3 deaths during the investigational period. Two male rats, 1 untreated control and 1 T-VI, died during the final blood sampling. One male T-VI rabbit died on day 21.

The only untoward reaction noted among all test animals was hypoactivity. Ruffled fur was observed among all test rats and rabbits.

There were no significant weight gain differences between test and untreated control animals.

Hematologic and clinical chemistry studies, as well as urinalyses did not reveal any noteworthy differences between the test and control animals. Organ weight data, gross pathologic examinations and histopathologic examinations revealed no treatment-related effects.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Investigational Procedure

A. Experimental Animals

Six groups, each consisting of 20 albino rats*, 6 SWF:(NZW) (A) rabbits** and 4 beagle dogs were used for the study. The dogs, which were acquired from our own colony, were purebred beagles. They were eligible for AKC registration and had been immunized against distemper, canine hepatitis and leptospirosis. An additional group, consisting of 40 albino rats, 10 SWF:(NZW) (A) rabbits and 6 beagle dogs served as an untreated control group which received no inhalation exposure. Each species was equally divided by sex in each group.

Below is an outline of group organization, as well as target chamber concentrations.

Group	Test Material	Target Chamber Concentration (ppm)
Untreated Control	-	-
T-I	XN-6058.1-L, 1X inhibitor	1750
T-II	XN-6058.1-L, 2.5X inhibitor	1750
T-III	XN-6058-1-L, 5.0X inhibitor	1750
T-IV	XN-6060, 1.0X inhibitor	1750
T-V	XN-6060, 2.5X inhibitor	1750
T-VI	XN-6060, 5.0X inhibitor	1750

* CDRS/Charles River strain albino rats, Charles River Breeding Laboratories, Inc., Wilmington, Mass.
** Sweetwater Farm Inc., Hillsboro, Ohio.

The animals were selected after having been under observation for 10 days to insure their general health and suitability for testing. All animals were housed individually in stainless steel cages. Animals were fed a standard laboratory diet* plus water ad libitum, except during inhalation exposures.

B. Method of Exposure

Test animals were exposed in stainless steel and glass chambers having a capacity of 1.0 cubic meters. These animals were exposed for 6 hours per day, 5 days per week, for a period of 4 weeks to the test atmosphere. A total of 21 exposures resulted. All chambers were aerodynamically designed for inhalation purposes and allowed observation of animals during the exposures. Chamber temperature was maintained at 25°C ± 3°.

Vapors of the undiluted test material were generated with gas wash bottles (impingers) having a capacity of 1,000 ml. A stream of clean, dry air (-40°C dewpoint) was passed through the impinger down into the undiluted test material and the resulting vapor was then introduced into the exposure chamber at the top center. The desired final vapor concentrations were achieved by varying rates of air flow through the impingers. An increase or decrease in these rates resulted in an increase or decrease in the output of vapor from the impinger. Air flow rates through each system were measured with rotameters connected to the air supply lines upstream of vapor contamination. The rotameters were calibrated with a bubble meter to assure a constant day to day air flow.

* Wayne LAB BLOX for Rats, Wayne Dog Feed and Wayne Rabbit Ration, Allied Mills, Inc., Chicago, Ill.

All chambers received clean, dry additional air at the rate of 250 l/min at the top center of each chamber. The air circulated through the chamber and was exhausted at the bottom. Main supply air was measured with an orifice meter which was calibrated with a TSI Linear Signal Conditioner (Model 67).*

C. Analysis of Chamber Atmosphere

1. Equipment

A Hewlett-Packard 5730 gas chromatograph** was used for the analysis. The gas chromatograph (GC) was equipped with a dual-flame ionization detector. It utilized a 6' x 1/8" O.D. stainless steel spiral column packed with 10% Carbowax 20M on 60/80 Chromosorb W, AW, DMSC. The temperature conditions were as follows: injection port 150°C, oven 126°C and flame ionization detector 250°C. The N_2 carrier flow was 51 ml/minute.

2. Calibration

Duplicate stock standards of methyl chloroform were made by injecting 200 µl of methyl chloroform into each of 2 1.0 liter evacuated gas collecting flasks. The flasks were allowed to sit to volatilize the methyl chloroform and they were vented to the atmosphere. The stock standards were diluted to the concentration ranges desired by the following method: 10.2, 15.4 and 20.46 ml samples were removed from each of the stock methyl chloroform flasks with a gas tight syringe.*** These were each injected into a 500 ml evacuated gas collecting flask and vented to the

* Thermo-Systems Inc., St. Paul, Minnesota.
** Hewlett-Packard, Skokie, Illinois.
*** Precision Sampling Corporation, Baton Rouge, Louisiana.

atmosphere. These dilutions were equivalent to the following methyl chloroform concentrations: 1000, 1500 and 2000 ppm. A 0.5 ml sample from each of these dilutions was injected into the GC with a gas-tight syringe. The concentration of methyl chloroform was plotted against the area count of each peak for the calibrating standards. The method was found to be linear in these ranges. A calibrating factor was then determined using a Hewlett-Packard 3352 computer system. The factor was used for calculating ppm of chamber air.

3. Sampling the Chamber

Actual methyl chloroform chamber concentrations were determined each hour by drawing chamber atmosphere into gas-tight syringes and then injecting the contents into the gas chromatograph for analysis. An injection volume of 0.5 ml was used and the ppm was calculated and recorded by the computer system.

The target methyl chloroform concentration for all test levels was 1750 ppm. A summary of actual test concentration is presented in Table I. Daily test conditions and concentrations for T-I through T-VI are presented in Tables II through VII.

TABLE I

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

A Summary of Concentrations (ppm)

Parameter	Group T-I	T-II	T-III	T-IV	T-V	T-VI
Mean	1743	1708	1777	1847	1863	1741
Standard Deviation	190.1	182.1	298.2	285.9	286.3	123.8

TABLE II

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Test Conditions and Concentrations - T-1

Exposure Day	Air Flow (L/Minute) Generator	Additional	Total	Concentration (ppm)
1	0.60	250.00	250.60	1872
2	0.62	250.00	250.62	1743
3	0.60	250.00	250.60	1747
4	1.25	250.00	251.25	1768
5	1.25	250.00	251.25	1836
6	1.50	250.00	251.50	1362
7	2.00	250.00	252.00	1552
8	2.00	250.00	252.00	1697
9	1.90	250.00	251.90	1919
10	2.20	250.00	252.20	1549
11	2.10	250.00	252.10	1582
12	2.10	250.00	252.10	2197
13	2.10	250.00	252.10	1923
14	-	-	-	-
15	-	-	-	-
16	2.10	250.00	252.10	1754
17	2.10	250.00	252.10	1862
18	2.10	250.00	252.10	1836
19	2.10	250.00	252.10	1575
20	2.60	250.00	252.60	1626
21	2.60	250.00	252.60	1738
Mean	1.56	250.00	251.56	1743

- = Data not available.

TABLE III

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Test Conditions and Concentrations - T-II

Exposure Day	Air Flow (L/Minute) Generator	Additional	Total	Concentration (ppm)
1	0.90	250.00	250.90	1693
2	0.75	250.00	250.75	1570
3	1.00	250.00	251.00	1908
4	1.00	250.00	251.00	1710
5	1.00	250.00	251.00	1420
6	1.75	250.00	251.75	1772
7	1.75	250.00	251.75	1661
8	1.75	250.00	251.75	1746
9	2.00	250.00	252.00	1590
10	2.90	250.00	252.90	1764
11	2.60	250.00	252.60	2197
12	2.50	250.00	252.50	1782
13	2.50	250.00	252.50	1944
14	-	-	-	-
15	-	-	-	-
16	2.60	250.00	252.60	1421
17	2.60	250.00	252.60	1595
18	2.20	250.00	252.20	1768
19	2.20	250.00	252.20	1668
20	3.00	250.00	253.00	1537
21	3.00	250.00	253.00	1702
Mean	2.22	250.00	252.22	1708

- = Data not available.

TABLE IV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Test Conditions and Concentrations - T-III

Exposure Day	Air Flow (L/Minute) Generator	Additional	Total	Concentration (ppm)
1	2.50	250.00	252.50	1360
2	2.40	250.00	252.40	1852
3	3.00	250.00	253.00	1778
4	3.00	250.00	253.00	1233
5	4.00	250.00	254.00	1601
6	4.00	250.00	254.00	1455
7	4.50	250.00	254.50	1801
8	3.70	250.00	253.70	1797
9	3.50	250.00	253.50	2043
10	4.10	250.00	254.10	1953
11	4.10	250.00	254.10	1827
12	4.10	250.00	254.10	1725
13	3.90	250.00	253.90	2647
14	-	-	-	-
15	-	-	-	-
16	3.90	250.00	253.90	1663
17	3.80	250.00	253.80	1686
18	4.50	250.00	254.50	2052
19	6.00	250.00	256.00	1875
20	6.00	250.00	256.00	1743
21	6.00	250.00	256.00	1716
Mean	4.11	250.00	254.11	1777

- = Data not available.

TABLE VI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Test Conditions and Concentrations - T-V

Exposure Day	Air Flow (L/Minute) Generator	Additional	Total	Concentration (ppm)
1	1.50	250.00	251.50	2383
2	1.50	250.00	251.50	1428
3	2.10	250.00	252.50	1628
4	3.00	250.00	253.00	1903
5	2.50	250.00	252.50	2282
6	2.50	250.00	252.50	1667
7	3.00	250.00	253.00	1771
8	2.10	250.00	252.10	1741
9	2.10	250.00	252.10	1828
10	2.10	250.00	252.10	1520
11	2.10	250.00	252.10	1613
12	2.10	250.00	253.10	1997
13	2.50	250.00	252.50	1829
14	-	-	-	-
15	-	-	-	-
16	2.25	250.00	252.25	1481
17	2.25	250.00	252.25	2325
18	2.25	250.00	252.25	1797
19	3.50	250.00	253.50	1851
20	3.50	250.00	253.50	1900
21	3.50	250.00	253.50	2295
Mean	2.33	250.00	252.33	1853

- = Data not available.

TABLE V

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Test Conditions and Concentrations - T-IV

Exposure Day	Air Flow (L/Minute) Generator	Additional	Total	Concentration (ppm)
1	1.25	250.00	251.25	1855
2	1.50	250.00	251.50	2580
3	1.75	250.00	251.75	1342
4	1.80	250.00	251.80	1923
5	1.80	250.00	251.80	1658
6	1.70	250.00	251.70	1713
7	2.50	250.00	252.50	1480
8	1.90	250.00	251.90	1982
9	1.90	250.00	251.90	2038
10	1.90	250.00	251.90	1783
11	1.90	250.00	251.90	1698
12	1.90	250.00	251.90	1778
13	1.90	250.00	251.90	1935
14	-	-	-	-
15	-	-	-	-
16	2.10	250.00	252.10	1910
17	2.10	250.00	252.10	1691
18	3.50	250.00	253.50	1900
19	3.50	250.00	253.50	1801
20	3.50	250.00	253.50	2084
21	3.50	250.00	253.50	1946
Mean	1.95	250.00	251.95	1847

- = Data not available.

TABLE VII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Test Conditions and Concentrations - T-VI

Exposure Day	Air Flow (L/Minute) Generator	Additional	Total	Concentration (ppm)
1	2.50	250.00	252.50	1713
2	3.00	250.00	253.00	1741
3	6.00	250.00	256.00	1388
4	6.00	250.00	256.00	1801
5	5.50	250.00	255.50	1866
6	5.90	250.00	255.90	1837
7	6.75	250.00	256.75	1622
8	6.00	250.00	256.00	1692
9	6.00	250.00	256.00	1850
10	6.00	250.00	256.00	1999
11	5.70	250.00	255.70	1658
12	5.70	250.00	255.70	1776
13	5.00	250.00	255.00	1756
14	-	-	-	-
15	-	-	-	-
16	6.00	250.00	256.00	1732
17	6.50	250.00	256.50	1627
18	6.50	250.00	256.50	1791
19	6.50	250.00	256.50	1744
20	7.75	250.00	257.75	1721
21	7.75	250.00	257.75	1814
Mean	5.81	250.00	255.81	1741

- = Data not available.

D. Observations

1. Mortality and Reactions

Checks for mortality and abnormal behavioral reactions were made daily during the 30-day investigational period.

2. Body Weights

Each animal was weighed prior to beginning the exposures and weekly thereafter during the study. The data were recorded and used as an index to growth.

3. Hematologic Studies

Hematologic studies, including determination of hemoglobin concentration, erythrocyte count, hematocrit value, both total and differential leukocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were conducted on all dogs, 10 male and 10 female rats and all rabbits in the untreated control, T-III and T-VI groups before initial exposure and on day 30 of the testing period.

4. Clinical Chemistry Studies

Clinical chemistry studies were conducted on the same animals on which the hematologic studies were performed. The parameters measured included blood urea nitrogen concentration (BUN), serum alkaline phosphatase activity (SAP), serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT) and fasting blood glucose.

5. Urinalyses

Urinalyses, including determinations of albumin, glucose, pH and microscopic elements, were conducted on the same animals used

for hematologic studies. Urine samples were collected during the 24-hour period preceding collection of blood samples.

6. Pathologic Studies

Arrangements were made to subject any animal which might die during the test to a gross necropsy examination. Also, in those cases in which postmortem changes were not advanced, sections of representative tissues and organs were taken for histopathologic study.

The following methods of sacrifice were used:

Dogs - All animals were sacrificed by exsanguination immediately following induction of anesthesia with an intravenous injection of a mixture of Ketaset* and Totaltox**.

Rats and Rabbits - All animals were rendered unconscious by exposure to carbon dioxide in a closed chamber and immediately exsanguinated.

At the time of sacrifice, the animals were subjected to a complete gross necropsy examination. The weights of the liver, kidneys, gonads, heart and brain of each animal were taken and recorded. Statistical analyses viz. an Analysis of Variance followed by Student's "t"-distribution tests, were conducted on the absolute organ weights and on the organ to body weight and organ to brain weight ratios.

A complete set of organs and other tissues was removed from each animal and preserved in 10 percent formalin solution (pH 7.0). These included adrenal gland, aorta (thoracic), bone, bone marrow, brain (cerebrum, cerebellum and pons), bronchi, caecum, colon, esophagus, eye, gall bladder

* Tradename of Ketamine Hydrochloride, Bristol Laboratories, Syracuse, N.Y.
** Tradename of a mixture of Pentobarbital, Propanol, Propylene Glycol, Holmes Serum Company, Chicago, Ill.

(dogs and rabbits only), gonads, heart, kidneys, liver, lungs, lymph nodes (cervical, peribronchial and mesenteric), muscle, optic nerve, pancreas, parathyroid gland, peripheral nerve (sciatic), pituitary gland, prostate, salivary gland (submaxillary), small intestine (duodenum, jejunum and ileum), spinal cord, spleen, sternum, stomach (cardia, fundus, pylorus), trachea, thyroid gland, urinary bladder and uterus.

Microscopic examinations of the above tissues and organs of untreated control, T-III and T-VI were conducted. In the event of significant pathologic findings microscopic examinations were conducted on additional test animals.

IV. Results

A. Mortality and Reactions

There were 3 deaths during the investigation. Two rats, 1 male untreated control and 1 male T-VI, died during the final blood sampling. A male T-VI rabbit died on day 21 of the investigational period.

The only untoward reaction noted among all test animals was hypoactivity. Ruffed fur was observed among all test rats and rabbits.

B. Body Weight Data

There were no significant weight gain differences between test and untreated control animals. A summary of body weight gain data is presented in Table VIII. The individual body weight data are presented in Tables IX through XIV.

TABLE VIII

TEST MATERIALS: Six Stabilised Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Summary of Body Weight Gain Data

	Body Weight Change Species:					
	Albino Rats (grams)		Albino Rabbits (kilograms)		Beagle Dogs (kilograms)	
Group	Males	Females	Males	Females	Males	Females
Untreated Control	118	38	0.64	0.59	0.83	0.76
T-I	95	40	0.44	0.15	0.95	0.05
T-II	108	50	0.55	0.29	0.46	0.96
T-III	92	46	0.42	0.27	0.08	0.35
T-IV	105	43	0.31	0.37	1.02	0.45
T-V	92	34	0.46	0.24	0.98	0.35
T-VI	94	45	0.20	0.51	0.88	0.68

TABLE IX

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Male Rats

Group	Animal Number	Individual Body Weights (grams) Week Number: 0	1	2	3	4	30-Day Weight Change (grams)
Untreated Control	1	225	262	276	288	293	68
	2	240	307	329	351	369	129
	3	252	312	329	343	369	117
	4	262	334	363	396	417	155
	5	248	314	343	360	382	134
	6	223	277	279	309	327	104
	7	214	276	277	311	335	121
	8	232	304	316	344	-	-
	9	256	302	317	341	359	103
	10	251	320	317	347	372	121
	11	230	308	333	365	381	151
	12	268	344	375	401	414	146
	13	272	313	317	342	357	85
	14	241	307	308	360	381	140
	15	257	326	338	374	395	138
	16	235	272	263	315	327	92
	17	257	292	297	352	366	109
	18	257	313	307	366	374	117
	19	234	267	272	319	335	101
	20	219	258	266	310	322	103
	Mean	243	300	312	345	361	118
T-1	21	227	297	312	354	356	129
	22	234	269	287	314	313	79
	23	237	278	293	318	320	83
	24	237	284	298	333	336	99
	25	263	304	318	351	359	96
	26	264	302	331	365	365	101
	27	222	255	275	294	300	78
	28	235	263	290	314	318	83
	29	228	263	284	310	318	90
	30	253	304	322	338	361	108
	Mean	240	282	301	331	364	95

TABLE IX continued

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Male Rats

Group	Animal Number	Individual Body Weights (grams) Week Number: 0	1	2	3	4	30-Day Weight Change (grams)
T-II	31	246	289	299	316	336	90
	32	240	273	288	308	323	83
	33	257	301	312	336	380	123
	34	266	303	305	409	364	98
	35	278	325	345	448	394	116
	36	247	303	308	408	346	99
	37	234	286	297	394	334	100
	38	224	269	286	390	336	112
	39	243	287	305	413	363	120
	40	248	335	361	486	403	155
	Mean	250	297	310	388	358	108
T-III	41	236	290	317	316	312	76
	42	250	312	336	348	367	117
	43	231	286	301	317	329	98
	44	266	313	337	352	374	108
	45	218	272	288	319	340	122
	46	232	280	303	329	342	110
	47	220	241	260	290	301	81
	48	251	280	293	310	320	69
	49	242	270	288	294	310	68
	50	261	289	310	319	332	71
	Mean	241	283	303	319	333	92
T-IV	51	238	268	295	316	334	96
	52	248	279	299	319	340	92
	53	243	288	313	332	366	123
	54	251	295	312	335	372	121
	55	253	274	308	330	354	101
	56	277	311	334	351	372	95
	57	255	302	315	340	358	103
	58	249	291	317	329	354	105
	59	259	309	342	361	395	136
	60	206	244	260	277	283	77
	Mean	248	286	310	329	353	105

TABLE IX continued

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Male Rats

Group	Animal Number	Individual Body Weights (grams) Week Number: 0	1	2	3	4	30-Day Weight Change (grams)
T-V	61	247	268	262	291	301	54
	62	257	302	314	348	365	108
	63	228	300	309	337	368	140
	64	282	299	323	363	394	112
	65	273	278	288	326	356	83
	66	281	325	344	372	396	115
	67	227	241	258	286	298	71
	68	255	282	298	323	333	78
	69	247	274	276	293	300	53
	70	248	282	307	333	350	102
	Mean	254	284	298	327	346	92
T-VI	71	248	298	324	327	-	-
	72	252	300	329	332	361	109
	73	237	271	284	298	309	72
	74	261	315	349	367	393	132
	75	248	282	307	309	330	82
	76	250	295	309	322	331	81
	77	240	279	296	307	317	67
	78	240	280	302	318	327	77
	79	244	299	320	344	365	121
	80	242	294	297	316	330	88
	Mean	247	290	310	325	340	94

TABLE X

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Female Rats

Group	Animal Number	Individual Body Weights (grams) Week Number: 0	1	2	3	4	30-Day Weight Change (grams)
Untreated Control	81	174	200	208	209	227	53
	82	196	232	239	249	254	58
	83	192	210	211	225	232	40
	84	213	230	238	247	249	36
	85	211	238	241	253	251	40
	86	211	233	243	249	258	47
	87	210	230	229	246	241	31
	88	213	246	241	256	264	51
	89	199	223	230	230	236	37
	90	202	220	226	238	240	38
	91	194	217	227	234	236	42
	92	204	227	221	232	232	28
	93	200	218	218	224	226	26
	94	205	230	226	232	237	32
	95	206	239	244	263	262	56
	96	188	212	215	214	220	32
	97	217	230	232	239	243	26
	98	203	223	226	234	237	34
	99	199	218	218	225	230	31
	100	202	244	234	231	228	26
	Mean	202	226	228	236	240	38
T-1	101	185	207	215	232	230	45
	102	189	218	223	226	226	37
	103	198	233	231	229	248	50
	104	192	216	226	244	247	55
	105	183	206	209	217	212	29
	106	192	219	224	230	230	38
	107	207	225	240	245	243	36
	108	209	218	236	246	246	37
	109	182	205	207	219	218	36
	110	184	209	197	219	222	38
	Mean	192	214	221	232	232	40

TABLE X continued

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Female Rats

Group	Animal Number	Individual Body Weights (grams) Week Number: 0	1	2	3	4	30-Day Weight Change (grams)
T-II	111	187	200	209	212	224	37
	112	191	212	227	240	242	51
	113	183	207	218	219	230	47
	114	193	225	241	258	274	81
	115	199	202	223	225	237	38
	116	203	235	240	248	247	44
	117	194	217	224	236	244	50
	118	186	198	213	215	230	44
	119	191	212	214	224	228	37
	120	181	216	229	245	256	75
	Mean	191	211	224	232	241	50
T-III	121	179	209	207	222	230	51
	122	191	221	229	235	243	52
	123	180	203	213	223	230	50
	124	172	194	203	209	216	44
	125	193	214	216	221	228	35
	126	200	217	222	226	230	30
	127	207	234	245	252	258	51
	128	210	212	232	231	258	48
	129	177	209	208	225	231	54
	130	188	218	213	233	238	50
	Mean	190	213	219	230	236	46
T-IV	131	209	229	243	252	245	36
	132	179	199	205	220	224	45
	133	216	243	245	256	262	46
	134	181	206	209	216	225	44
	135	198	222	219	238	257	59
	136	210	222	231	240	246	36
	137	204	217	223	242	245	41
	138	200	197	208	245	245	45
	139	210	232	254	252	269	59
	140	210	222	230	217	226	16
	Mean	202	219	227	238	244	43

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TABLE X continued

TEST MATERIALS: Six Stabilised Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Female Rats

Group	Animal Number	Individual Body Weights (grams) Week Number: 0	1	2	3	4	30-Day Weight Change (grams)
T-V	141	191	213	219	237	223	32
	142	208	221	227	241	232	24
	143	180	184	196	207	205	25
	144	196	217	218	233	238	42
	145	205	235	234	249	258	53
	146	201	204	217	232	231	30
	147	195	203	202	218	214	19
	148	196	220	221	221	225	29
	149	199	223	228	226	233	34
	150	202	230	232	243	253	51
	Mean	197	215	219	230	231	34
T-VI	151	189	217	218	223	235	46
	152	202	225	243	249	262	60
	153	179	194	206	215	227	48
	154	208	218	237	247	257	49
	155	209	225	237	237	254	45
	156	197	211	225	231	247	50
	157	193	201	202	215	218	25
	158	198	211	217	222	234	36
	159	201	224	223	237	247	46
	160	228	242	261	261	273	44
	Mean	200	217	227	233	245	45

TABLE XI

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Male Rabbits

Group	Animal Number	Individual Body Weights (kilograms) Week Number: 0	1	2	3	4	30-Day Weight Change (kilograms)
Untreated Control	1	1.67	1.70	1.86	2.23	2.43	0.76
	2	1.55	1.63	1.63	1.86	2.03	0.48
	3	1.68	1.89	1.98	2.40	2.60	0.92
	4	1.84	2.09	2.20	2.53	2.33	0.49
	5	1.67	1.74	1.84	1.69	2.10	0.43
	Mean	1.66	1.81	1.90	2.14	2.30	0.64
T-I	11	1.66	1.75	1.87	1.93	2.01	0.35
	12	1.58	1.72	1.87	1.98	2.09	0.51
	13	1.57	1.64	1.79	2.00	2.04	0.47
	Mean	1.60	1.70	1.84	1.96	2.05	0.44
T-II	17	1.43	1.81	2.09	2.17	2.33	0.90
	18	1.56	1.52	1.55	1.77	1.91	0.35
	19	1.55	1.58	1.86	1.63	1.95	0.40
	Mean	1.51	1.64	1.73	1.92	2.06	0.55
T-III	23	1.65	1.72	1.68	2.00	2.05	0.40
	24	1.39	1.50	1.65	1.84	1.95	0.56
	25	1.84	1.63	1.76	2.10	2.14	0.30
	Mean	1.63	1.68	1.70	1.98	2.05	0.42
T-IV	29	1.77	1.73	2.04	2.09	2.15	0.38
	30	1.65	1.55	1.83	1.77	1.56	-0.09
	31	1.54	1.31	1.80	1.96	2.19	0.65
	Mean	1.65	1.53	1.89	1.94	1.97	0.31
T-V	35	1.46	1.40	1.52	1.66	1.75	0.29
	36	1.39	1.50	1.50	1.67	1.77	0.38
	37	1.46	1.60	1.71	1.96	2.16	0.70
	Mean	1.44	1.50	1.58	1.76	1.89	0.46

TABLE XI continued

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Male Rabbits

Group	Animal Number	Individual Body Weights (kilograms) Week Number: 0	1	2	3	4	30-Day Weight Change (kilograms)
T-VI	41	1.62	1.78	1.75	1.91	1.93	0.31
	42	1.70	1.70	1.83	1.55	-	-
	43	1.87	1.90	1.90	1.89	1.96	0.09
	Mean	1.74	1.82	1.82	1.90	1.94	0.20

- = Animal died during the investigation.

TABLE XII

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Female Rabbits

Group	Animal Number	Individual Body Weights (kilograms) Week Number: 0	1	2	3	4	30-Day Weight Change (kilograms)
Untreated Control	6	1.81	1.98	2.37	2.56	2.67	0.86
	7	1.65	1.65	1.93	2.10	2.11	0.46
	8	1.44	1.32	1.60	1.98	2.10	0.66
	9	1.47	1.62	1.80	1.98	2.03	0.56
	10	1.61	1.84	1.68	2.00	2.04	0.43
	Mean	1.60	1.62	1.88	2.11	2.19	0.59
T-I	14	1.91	2.02	2.17	2.00	2.16	0.25
	15	1.58	1.53	1.70	1.80	1.65	0.07
	16	1.67	1.69	1.90	1.65	1.80	0.13
	Mean	1.72	1.75	1.92	1.82	1.87	0.15
T-II	20	1.55	1.46	1.83	1.66	1.83	0.28
	21	1.62	1.49	1.64	1.77	1.83	0.21
	22	1.52	1.40	1.67	1.73	1.90	0.38
	Mean	1.56	1.45	1.61	1.72	1.85	0.29
T-III	26	1.57	1.36	1.66	1.80	1.81	0.24
	27	1.60	1.61	1.62	1.79	1.86	0.26
	28	1.72	1.72	1.80	1.91	2.03	0.31
	Mean	1.63	1.56	1.69	1.83	1.90	0.27
T-IV	32	1.98	1.85	1.90	2.10	2.15	0.57
	33	1.82	1.74	1.92	2.00	2.13	0.31
	34	1.71	1.74	1.83	1.90	1.93	0.22
	Mean	1.70	1.78	1.88	2.00	2.07	0.37
T-V	38	1.44	1.37	1.72	1.77	1.71	0.27
	39	1.42	1.25	1.35	1.53	1.35	-0.07
	40	1.62	1.56	1.91	1.96	2.15	0.53
	Mean	1.49	1.38	1.66	1.75	1.74	0.24

TABLE XII continued

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Female Rabbits

Group	Animal Number	Individual Body Weights (kilograms) Week Number: 0	1	2	3	4	30-Day Weight Change (kilograms)
T-VI	44	1.47	1.33	1.60	1.84	1.96	0.49
	45	1.90	1.35	1.70	2.04	2.20	0.70
	46	1.50	1.46	1.61	1.73	1.83	0.33
	Mean	1.49	1.38	1.64	1.87	2.00	0.51

TABLE XIII

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Male Dogs

Group	Animal Number	Individual Body Weights (kilograms) Week Number: 0	1	2	3	4	30-Day Weight Change (kilograms)
Untreated Control	1	7.60	7.40	8.06	8.42	7.88	0.28
	2	8.50	9.06	9.55	9.20	9.73	1.23
	3	7.00	7.56	7.90	7.69	8.11	1.11
	4	7.10	7.10	7.75	7.75	7.80	0.70
	Mean	7.55	7.78	8.32	8.26	8.38	0.83
T-I	9	9.60	8.80	9.50	9.70	10.40	0.80
	10	9.60	10.10	11.00	10.50	10.70	1.10
	Mean	9.60	9.45	10.25	10.10	10.55	0.95
T-II	13	9.10	9.35	8.90	9.00	9.77	0.67
	14	9.30	9.40	9.60	9.50	9.55	0.25
	Mean	9.20	9.38	9.25	9.25	9.66	0.46
T-III	17	11.20	11.00	12.20	12.10	11.85	0.65
	18	9.15	8.90	8.80	8.64	8.75	-0.40
	Mean	10.18	9.95	10.50	10.37	10.30	0.08
T-IV	21	10.50	11.30	11.05	12.05	11.80	1.30
	22	9.30	9.72	9.95	9.75	10.05	0.75
	Mean	9.90	10.51	10.50	10.90	10.92	1.02
T-V	25	13.50	14.00	14.75	14.85	15.00	1.50
	26	11.00	11.40	11.90	11.80	11.45	0.45
	Mean	12.25	12.70	13.32	13.32	13.22	0.98
T-VI	29	9.50	10.10	9.75	10.35	10.35	0.85
	30	6.60	7.20	6.80	7.50	7.50	0.90
	Mean	8.05	8.65	8.28	8.92	8.92	0.88

TABLE XIV

TEST MATERIALS: Six Stabilized Methylchloroform Formulations

30-Day Subacute Inhalation Toxicity Study
Albino Rats, Albino Rabbits and Beagle Dogs

Individual Body Weight and Weight Change Data - Female Dogs

Group	Animal Number	Individual Body Weights (kilograms) Week Number: 0	1	2	3	4	30-Day Weight Change (kilograms)
Untreated Control	5	6.50	6.48	6.50	6.43	6.36	-0.14
	6	7.05	7.25	7.65	7.03	7.05	0.00
	7	6.75	6.90	7.10	7.65	7.75	1.00
	8	7.35	8.90	8.60	8.60	8.55	1.20
	Mean	6.91	7.38	7.46	7.43	7.43	0.76
T-I	11	7.50	7.50	8.00	8.75	7.25	-0.25
	12	8.20	8.30	9.20	8.75	8.35	0.15
	Mean	7.85	7.90	8.60	8.75	7.80	-0.05
T-II	15	6.40	6.35	7.40	7.45	7.70	1.30
	16	7.10	7.20	7.80	7.30	7.71	0.61
	Mean	6.75	6.78	7.60	7.33	7.71	0.96
T-III	19	6.00	6.44	6.50	6.25	6.00	0.00
	20	8.60	9.30	8.90	8.90	9.30	0.70
	Mean	7.30	7.87	7.70	7.58	7.65	0.35
T-IV	23	10.20	10.30	9.85	10.06	10.25	0.05
	24	6.90	7.20	7.10	8.40	7.75	0.85
	Mean	8.55	8.75	8.48	9.23	9.00	0.45
T-V	27	6.35	6.80	7.05	7.00	6.65	0.30
	28	8.50	9.40	9.75	8.90	8.90	0.40
	Mean	7.42	7.95	8.40	7.95	7.77	0.35
T-VI	31	5.70	6.70	6.34	6.30	6.30	0.60
	32	7.60	8.75	8.55	8.35	8.35	0.75
	Mean	6.65	7.72	7.44	7.32	7.32	0.68

C. Hematologic Studies

The results of hematologic studies done on blood from male and female rats, rabbits and dogs from the control group and from treatment groups T-III and T-VI are presented in Tables XV through XXXII.

Mean values for total leukocyte and erythrocyte counts, differential leukocyte counts, hemoglobin concentration and hematocrit of treated animals were normal when compared to similar values from control animals after 30 days of treatment. Erythrocyte indices (MCV, MCH and MCHC) of treated animals were also normal when compared to those of control animals after 30 days of treatment.

TABLE XV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rats

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
Untreated Control	1	7.3	8.7	9.18	8.81	16.9	16.3	56	53
	2	8.7	9.8	7.27	8.00	14.8	16.7	48	51
	3	8.6	9.9	7.58	8.26	14.9	16.9	49	51
	4	7.7	12.8	8.04	7.46	15.8	15.4	50	50
	5	.8	11.9	7.53	7.62	15.1	16.0	48	49
	6	7.0	7.7	7.20	7.95	14.4	17.0	48	53
	7	9.4	11.7	7.27	8.41	15.4	16.8	50	54
	8	9.9	12.3	7.20	8.48	14.3	17.0	47	52
	9	12.5	11.8	7.85	8.01	14.8	17.0	47	51
	10	7.6	15.0	6.94	7.74	14.2	16.8	48	52
	Mean	8.8	11.2	7.52	8.07	15.1	16.6	49	52
T-III	41	8.9	7.8	8.03	8.48	14.8	16.3	47	51
	42	6.9	7.5	8.17	8.85	15.6	16.8	50	50
	43	7.9	11.1	7.93	7.80	15.4	16.7	49	50
	44	5.9	11.8	8.11	7.78	15.7	15.4	50	48
	45	6.9	9.2	7.58	8.50	15.0	17.1	49	55
	46	10.8	10.4	7.85	8.43	15.1	16.7	50	53
	47	7.5	5.6	7.88	8.24	15.7	16.7	50	50
	48	5.4	6.3	8.15	8.68	15.8	16.5	49	50
	49	7.5	9.9	7.60	8.11	14.6	16.3	49	51
	50	6.1	8.5	8.14	8.52	14.6	16.8	47	52
	Mean	7.4	8.8	7.94	8.24	15.2	16.5	49	51

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TABLE XV continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rats

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
T-VI	71	10.8	13.8	8.11	7.77	14.8	15.8	49	49
	72	7.9	15.2	7.83	7.94	14.2	15.9	48	50
	73	6.4	13.3	8.44	7.48	16.6	15.3	52	49
	74	12.7	14.1	8.38	7.95	15.5	16.0	51	50
	75	7.8	8.2	8.42	8.75	15.2	16.6	48	53
	76	13.8	9.4	8.83	7.87	15.4	16.1	50	52
	77	7.8	5.6	8.11	8.72	15.4	17.3	50	53
	78	7.9	7.2	8.24	8.86	15.1	16.3	48	50
	79	11.2	15.4	7.51	8.57	13.6	15.8	45	49
	80	9.3	13.3	7.52	8.14	14.4	16.4	47	49
	Mean	9.2	11.6	8.06	8.20	15.0	16.1	49	50

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TABLE XVI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rats

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
Untreated Control	81	6.7	6.7	8.74	8.00	14.7	15.6	47	49
	82	12.2	5.9	7.51	8.05	13.4	15.5	44	47
	83	7.6	11.7	8.67	9.30	15.0	16.6	46	50
	84	6.6	8.3	7.82	8.31	14.3	16.7	45	50
	85	6.8	6.1	7.62	8.32	13.6	16.5	44	48
	86	9.8	6.4	7.49	8.03	14.0	15.5	45	49
	87	13.1	9.7	7.30	8.03	14.2	16.8	44	49
	88	10.5	5.8	7.31	-	14.2	16.6	45	50
	89	5.5	5.0	7.90	8.41	14.4	16.0	46	49
	90	7.2	8.1	7.15	8.07	13.8	15.9	44	48
	Mean	8.6	7.4	7.72	8.28	14.2	16.2	45	49
T-III	121	6.6	7.7	7.62	7.93	14.9	16.3	48	49
	122	6.4	8.9	7.26	7.70	13.8	15.4	44	49
	123	5.8	5.9	8.04	8.66	14.4	15.9	46	48
	124	3.6	7.1	8.67	8.99	15.3	15.7	49	49
	125	6.2	6.8	7.70	7.93	14.3	15.1	45	46
	126	5.8	5.6	8.02	8.58	14.6	15.5	46	49
	127	7.8	10.3	7.51	7.93	13.7	15.0	44	46
	128	11.1	11.5	7.85	8.60	13.9	16.0	44	49
	129	10.7	8.2	7.71	8.13	13.8	15.2	43	48
	130	8.8	7.3	8.88	8.67	14.4	16.0	46	49
	Mean	7.1	7.9	7.84	8.31	14.2	15.6	46	48

- Data not available

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TABLE XVI continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rats

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
T-VI	151	5.1	10.8	7.71	8.41	13.5	16.2	43	49
	152	6.9	10.5	7.62	8.38	14.4	16.0	45	48
	153	8.5	5.9	7.78	8.55	14.4	16.8	46	50
	154	6.8	2.4	7.84	8.38	14.3	16.2	46	49
	155	7.8	5.1	7.48	9.03	13.4	17.1	42	51
	156	5.4	16.2	7.52	4.72	13.8	9.8	44	34
	157	9.4	12.8	8.24	8.32	14.1	15.1	45	48
	158	7.8	4.3	7.99	8.21	14.5	16.1	46	48
	159	7.6	7.4	7.68	8.36	14.4	17.8	46	49
	160	9.1	9.8	7.68	8.37	14.6	16.8	46	49
	Mean	7.4	8.4	7.74	8.07	14.1	15.7	45	48

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TABLE XVII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rabbits

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
Untreated Control	1	9.4	12.6	6.31	6.83	12.7	13.7	39	40
	2	11.4	13.7	6.34	6.82	12.5	13.9	41	43
	3	8.2	11.5	6.51	6.79	12.8	14.0	41	43
	4	13.7	9.6	5.43	5.39	10.6	11.8	35	38
	5	18.8	12.3	6.08	5.66	11.6	12.0	37	37
	Mean	12.3	11.9	6.13	5.98	12.0	13.1	38	40
T-III	23	8.2	8.0	6.09	5.53	12.8	12.6	41	36
	24	9.7	6.8	6.59	6.01	12.8	12.0	41	37
	25	6.3	14.7	4.87	5.34	10.1	12.8	33	37
	Mean	8.1	9.8	5.85	5.63	11.9	12.2	38	37
T-VI	41	12.5	8.7	6.66	6.17	13.4	12.4	42	37
	42	9.1	9.3	5.65	5.83	11.6	12.7	37	38
	43	12.2	7.6	5.97	5.73	11.0	13.4	36	40
	Mean	11.3	8.5	6.09	5.91	12.0	12.8	38	38

TAB XVIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rabbits

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
Untreated Control	6	9.4	10.9	6.16	5.66	12.2	11.8	38	38
	7	8.7	8.2	5.94	5.85	11.9	12.4	38	39
	8	8.1	7.0	6.53	5.02	13.5	10.8	42	34
	9	5.4	8.1	6.48	6.21	13.8	14.0	45	44
	10	7.7	8.7	6.84	6.13	13.9	12.9	43	38
	Mean	7.9	8.6	6.39	5.77	13.0	12.4	41	39
T-III	26	5.7	7.0	6.41	5.90	14.0	13.5	44	41
	27	7.3	11.7	6.82	5.85	13.0	12.8	40	40
	28	9.2	6.2	6.15	5.91	13.3	13.1	42	40
	Mean	7.4	8.3	6.19	5.89	13.4	13.1	42	40
T-VI	44	7.2	7.3	6.23	5.72	14.0	13.5	44	41
	45	13.6	7.3	6.36	5.41	13.9	12.6	43	37
	46	8.7	6.6	5.93	6.40	12.6	13.1	41	42
	Mean	9.8	7.1	6.17	5.84	13.5	13.1	43	40

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TABLE XIX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Dogs

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
Untreated Control	1	12.8	11.3	5.90	5.70	14.0	13.0	41	40
	2	19.4	21.0	5.42	6.75	12.6	15.4	40	46
	3	26.8	11.0	5.42	6.21	12.0	14.3	38	42
	4	9.5	10.8	5.67	6.26	11.3	13.5	36	41
	Mean	15.6	13.5	5.45	6.23	12.5	14.1	38	42
T-III	17	12.4	12.2	6.69	6.03	16.5	14.1	48	41
	18	19.0	13.8	6.47	6.70	14.4	14.0	44	41
	Mean	15.7	13.0	6.58	6.37	15.4	14.1	46	41
T-VI	29	13.3	14.6	6.10	6.67	14.7	15.6	44	46
	30	15.0	21.0	5.82	6.30	13.3	14.3	40	41
	Mean	14.2	17.8	5.96	6.48	14.0	14.9	42	43

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TABLE XX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Dogs

Group	Animal Number	Total Leukocyte Count (thousands/mm^3) Test Day: 0	30	Erythrocyte Count (millions/mm^3) Test Day: 0	30	Hemoglobin Concentration (g/100 ml) Test Day: 0	30	Hematocrit Value (%) Test Day: 0	30
Untreated Control	5	11.6	7.1	5.21	7.08	12.6	16.2	38	47
	6	17.4	12.4	6.07	6.69	14.8	15.9	43	46
	7	32.3	18.6	5.80	6.40	13.2	13.9	40	41
	8	15.7	13.1	5.78	6.61	12.9	14.4	39	43
	Mean	19.2	12.8	5.74	6.70	13.2	15.1	40	44
T-III	19	14.2	18.3	6.47	6.76	16.4	15.6	46	44
	20	11.8	12.9	7.33	6.72	17.0	15.6	52	44
	Mean	13.0	15.6	6.90	6.74	16.7	15.6	49	44
T-VI	31	11.7	8.6	6.31	6.55	14.1	14.5	42	42
	32	12.4	18.0	6.21	7.10	15.3	16.0	45	46
	Mean	12.0	13.3	6.26	6.82	14.7	15.2	44	44

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TABLE XXI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rats

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred) Neutrophils Test Day: 0	30	Lymphocytes Test Day: 0	30	Monocytes Test Day: 0	30	Eosinophils Test Day: 0	30	Basophils Test Day: 0	30
Untreated Control	1	6	16	94	80	0	1	0	2	0	0
	2	4	10	96	89	0	1	0	0	0	0
	3	4	8	96	90	0	1	0	1	0	0
	4	2	18	96	80	1	2	1	0	0	0
	5	1	19	96	80	2	0	1	1	0	0
	6	7	6	92	94	1	0	0	0	0	0
	7	4	7	96	91	0	2	0	0	0	0
	8	3	3	96	95	1	1	0	1	0	0
	9	5	8	93	89	2	3	0	0	0	0
	10	11	11	87	87	1	0	1	2	0	0
	Mean	4.7	10.6	94.2	87.5	0.8	1.1	0.3	0.7	0.0	0.0
T-III	41	2	15	97	85	1	0	0	0	0	0
	42	6	16	92	83	1	1	1	0	0	0
	43	2	9	97	91	0	0	1	0	0	0
	44	3	7	97	91	0	0	0	2	0	0
	45	11	8	87	88	2	3	0	1	0	0
	46	5	11	95	88	0	0	0	1	0	0
	47	4	15	94	83	2	2	0	0	0	0
	48	4	12	96	86	0	1	0	1	0	0
	49	3	12	97	85	0	0	0	0	0	0
	50	1	-	99	-	0	-	0	-	0	-
	Mean	4.1	11.5	95.1	87.0	0.6	0.8	0.2	0.6	0.0	0.0

- = Data not available.

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TABLE XXI continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rats

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred)									
		Neutrophils Test Day:		Lymphocytes Test Day:		Monocytes Test Day:		Eosinophils Test Day:		Basophils Test Day:	
		0	30	0	30	0	30	0	30	0	30
T-VI	71	1	5	99	95	0	0	0	0	0	0
	72	3	11	96	88	1	0	0	1	0	0
	73	2	10	98	89	0	1	0	0	0	0
	74	14	10	85	86	1	1	0	2	0	0
	75	7	6	93	92	0	2	0	0	0	0
	76	5	19	95	81	0	0	0	0	0	0
	77	7	8	92	92	1	0	0	0	0	0
	78	16	7	84	92	0	0	0	1	0	0
	79	1	22	99	77	0	1	0	0	0	0
	80	8	8	92	91	0	1	0	0	0	0
	Mean	6.4	10.6	93.3	88.3	0.3	0.5	0.0	0.4	0.0	0.0

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TABLE XXII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rats

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred) Neutrophils Test Day: 0	30	Lymphocytes Test Day: 0	30	Monocytes Test Day: 0	30	Eosinophils Test Day: 0	30	Basophils Test Day: 0	30
Untreated Control	81	3	14	96	85	1	1	0	0	0	0
	82	13	12	85	86	0	0	2	2	0	0
	83	7	6	91	94	0	0	2	0	0	0
	84	6	13	94	85	0	1	0	1	0	0
	85	7	10	91	89	2	0	0	1	0	0
	86	14	10	85	89	1	1	0	0	0	0
	87	14	14	85	84	1	0	0	2	0	0
	88	2	15	97	54	0	1	1	0	0	0
	89	13	15	82	82	1	2	4	1	0	0
	90	7	7	91	93	2	0	0	0	0	0
	Mean	8.6	14.6	89.7	34.1	0.8	0.6	0.9	0.7	0.0	0.0
T-III	121	3	4	96	96	1	0	0	0	0	0
	122	7	15	91	82	0	0	2	3	0	0
	123	3	13	97	84	0	1	0	2	0	0
	124	17	9	82	90	0	1	1	0	0	0
	125	6	10	92	90	0	0	2	0	0	0
	126	5	16	95	83	0	1	0	0	0	0
	127	8	2	91	96	1	1	0	1	0	0
	128	6	8	92	91	2	1	0	0	0	0
	129	6	12	94	89	0	0	1	0	0	0
	130	8	11	91	89	0	0	1	0	0	0
	Mean	6.9	10.0	92.1	88.9	0.4	0.5	0.6	0.6	0.0	0.0

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TABLE XXIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rabbits

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred) Neutrophils Test Day: 0	30	Lymphocytes Test Day: 0	30	Monocytes Test Day: 0	30	Eosinophils Test Day: 0	30	Basophils Test Day: 0	30
Untreated Control	1	29	51	70	48	0	0	1	1	0	0
	2	2	44	98	53	0	3	0	0	0	0
	3	52	31	48	65	0	0	0	3	0	1
	4	27	61	72	39	1	0	0	0	0	0
	5	29	71	71	28	0	0	0	1	0	0
	Mean	27.8	51.6	71.8	46.6	0.2	0.6	0.2	1.0	0.0	0.2
T-III	23	60	67	36	30	0	2	2	0	2	1
	24	39	83	56	17	1	0	2	0	2	0
	25	35	53	60	47	1	0	2	0	2	0
	Mean	44.7	67.7	50.7	31.3	0.7	0.7	2.0	0.0	2.0	0.3
T-VI	41	58	65	40	34	0	0	0	1	2	0
	42	18	48	79	59	1	0	1	0	1	1
	43	53	64	44	33	1	0	1	2	0	1
	Mean	43.3	56.3	54.3	42.0	0.7	0.0	0.7	1.0	1.0	0.7

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TABLE XXIV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rabbits

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred)									
		Neutrophils Test Day:		Lymphocytes Test Day:		Monocytes Test Day:		Eosinophils Test Day:		Basophils Test Day:	
		0	30	0	30	0	30	0	30	0	30
Untreated Control	6	55	80	40	20	4	0	1	0	0	0
	7	47	77	49	22	3	0	1	1	0	0
	8	53	47	46	53	0	0	1	0	0	0
	9	33	65	65	34	0	0	2	1	0	0
	10	55	57	43	41	1	1	0	0	0	1
	Mean	48.8	65.2	48.6	34.0	1.6	0.2	1.0	0.4	0.0	0.2
T-III	26	43	69	57	31	0	0	0	0	0	0
	27	15	55	85	43	0	2	0	0	0	0
	28	46	55	54	44	0	1	0	0	0	0
	Mean	34.7	59.7	65.3	39.3	0.0	1.0	0.0	0.0	0.0	0.0
T-VI	44	68	84	28	16	2	0	0	0	2	0
	45	58	52	36	48	2	0	4	0	0	0
	46	59	66	40	34	1	0	0	0	0	0
	Mean	61.7	67.3	34.7	32.7	1.7	0.0	1.3	0.0	0.7	0.0

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TABLE XXV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Dogs

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred)									
		Neutrophils Test Day:		Lymphocytes Test Day:		Monocytes Test Day:		Eosinophils Test Day:		Basophils Test Day:	
		0	30	0	30	0	30	0	30	0	30
Untreated Control	1	49	45	38	36	8	4	5	15	0	0
	2	67	67	21	22	8	7	4	4	0	0
	3	67	45	23	47	4	5	5	3	0	0
	4	55	48	33	38	5	2	5	12	0	0
	Mean	59.5	51.2	28.8	35.8	6.2	4.5	4.8	8.5	0.0	0.0
T-III	17	51	61	39	34	9	4	0	0	0	0
	18	65	67	29	17	4	11	2	5	0	0
	Mean	58.0	64.0	34.0	25.5	6.0	7.5	1.0	2.5	0.0	0.0
T-VI	29	67	67	28	24	10	5	1	2	0	0
	30	80	79	11	18	8	1	0	0	0	0
	Mean	73.5	73.0	15.5	21.0	9.0	3.0	0.5	1.0	0.0	0.0

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TABLE XXVI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Dogs

Group	Animal Number	Differential Leukocyte Count (number of cells per hundred) Neutrophils Test Day: 0	30	Lymphocytes Test Day: 0	30	Monocytes Test Day: 0	30	Eosinophils Test Day: 0	30	Basophils Test Day: 0	30
Untreated Control	5	66	52	26	42	5	3	2	3	0	0
	6	69	62	19	31	11	5	1	2	0	0
	7	80	61	18	34	10	4	0	1	0	0
	8	76	65	18	38	6	2	0	3	0	0
	Mean	72.8	60.0	18.2	34.2	8.0	3.5	0.8	2.2	0.0	0.0
T-III	19	67	86	23	15	9	5	0	0	0	0
	20	54	65	42	27	4	7	0	1	0	0
	Mean	60.5	72.5	32.5	21.0	6.5	6.0	0.0	0.5	0.0	0.0
T-VI	31	76	67	19	28	5	5	0	0	0	0
	32	49	74	42	19	5	4	4	3	0	0
	Mean	62.5	70.5	30.5	23.5	5.0	4.5	2.0	1.5	0.0	0.0

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TABLE XXVII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rats

Group	Animal Number	Erythrocyte Indices: MCV (μ^3) Test Day: 0	MCV (μ^3) Test Day: 30	MCH (μμg) Test Day: 0	MCH (μμg) Test Day: 30	MCHC (%) Test Day: 0	MCHC (%) Test Day: 30
Untreated Control	1	61.0	59.6	18.4	18.6	30.2	31.1
	2	66.0	64.3	20.4	20.9	30.8	32.5
	3	64.6	61.7	19.6	20.5	30.4	33.1
	4	62.2	67.0	19.6	20.6	31.6	30.8
	5	63.7	65.6	20.0	21.0	31.4	32.3
	6	65.7	64.6	20.0	21.4	30.8	32.1
	7	68.8	64.2	21.2	20.0	30.8	31.1
	8	65.3	61.9	19.9	20.2	30.4	32.7
	9	66.7	64.3	21.0	21.3	31.5	33.1
	10	69.2	67.2	20.5	21.8	29.6	32.4
	Mean	65.4	64.2	20.1	20.6	30.7	32.1
T-III	41	58.5	59.5	18.4	19.3	31.5	32.4
	42	61.2	62.2	19.1	19.9	31.2	32.0
	43	61.8	64.1	19.4	21.4	31.4	33.4
	44	61.6	61.1	19.4	19.7	31.4	32.3
	45	64.6	64.7	19.8	20.1	30.6	31.1
	46	63.7	62.2	19.2	19.8	30.2	31.8
	47	63.4	62.5	19.9	20.9	31.4	33.5
	48	60.1	57.6	18.4	19.0	30.6	33.0
	49	64.5	62.3	19.2	20.0	29.8	32.2
	50	57.7	61.0	17.9	19.8	31.1	32.4
	Mean	61.7	61.7	19.1	20.0	30.9	32.4

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TABLE XXVII continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rats

Group	Animal Number	Erythrocyte Indices MCV (μ^3) Test Day: 0	30	MCH (pg) Test Day: 0	30	MCHC (%) Test Day: 0	30
T-VI	71	60.4	63.7	18.2	20.3	30.2	31.9
	72	61.3	62.9	16.1	20.0	29.6	31.8
	73	61.6	65.5	19.7	20.7	31.9	31.5
	74	60.8	62.9	18.5	20.1	30.4	32.0
	75	57.0	60.6	18.0	19.0	31.7	31.3
	76	62.3	66.1	19.2	20.5	30.8	31.1
	77	61.6	60.2	19.0	19.8	30.8	32.9
	78	58.2	56.4	18.3	18.4	31.4	32.6
	79	59.9	56.6	15.1	17.5	30.2	30.8
	80	62.5	60.8	19.1	20.2	30.6	33.2
	Mean	60.6	61.6	18.6	19.6	30.8	31.9

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TABLE XXVIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rats

Group	Animal Number	Erythrocyte Indices: MCV (μ^3) Test Day: 0	MCV (μ^3) Test Day: 30	MCH ($\mu\mu g$) Test Day: 0	MCH ($\mu\mu g$) Test Day: 30	MCHC (%) Test Day: 0	MCHC (%) Test Day: 30
Untreated Control	81	53.8	61.3	16.8	19.4	31.3	31.7
	82	58.6	58.4	17.8	19.2	30.4	32.9
	83	53.0	53.8	17.3	17.9	32.6	33.3
	84	57.1	60.2	18.1	20.1	31.8	33.4
	85	59.3	57.1	18.3	19.8	30.9	34.7
	86	60.1	61.0	18.7	19.3	31.1	31.6
	87	62.8	61.6	20.0	21.0	32.3	34.0
	88	60.9	66.4	19.2	22.0	31.6	33.2
	89	58.2	57.7	18.2	19.0	31.3	33.0
	990	61.5	59.5	19.3	19.6	31.4	33.0
	Mean	58.4	59.7	18.4	19.7	31.5	33.1
T-III	121	63.8	61.2	19.6	20.5	31.0	33.5
	122	60.6	63.0	19.0	20.8	31.4	31.8
	123	57.5	55.4	18.0	18.4	31.3	33.1
	124	56.5	53.9	17.6	17.5	31.2	32.4
	125	58.4	58.6	18.6	19.0	31.8	32.5
	126	57.4	56.5	18.2	18.1	31.7	32.0
	127	58.6	58.6	18.2	18.9	31.1	32.2
	128	56.0	56.4	17.7	18.6	31.6	33.0
	129	55.8	59.8	16.9	18.7	30.2	31.7
	130	56.9	57.1	17.8	18.5	31.3	32.3
	Mean	58.1	58.0	18.2	18.8	31.3	32.4

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TABLE XXVIII continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rats

Group	Animal Number	Erythrocyte Indices MCV (μ^3) Test Day: 0	30	MCH (pg) Test Day: 0	30	MCHC (%) Test Day: 0	30
T-VI	151	55.8	58.9	17.5	19.3	31.4	32.7
	152	59.0	56.7	18.9	19.1	32.0	33.7
	153	59.1	58.5	18.5	19.7	31.3	33.7
	154	58.7	58.5	18.2	19.3	31.1	33.1
	155	56.1	57.0	17.9	18.9	31.9	33.2
	156	58.5	72.0	18.4	20.8	31.4	28.8
	157	56.4	57.7	17.1	18.1	31.3	31.5
	158	57.6	57.9	18.1	19.6	31.5	33.9
	159	60.5	59.2	18.9	20.3	31.3	34.2
	160	60.5	59.1	19.2	20.1	31.7	33.9
	Mean	58.0	59.5	18.3	19.5	31.5	32.9

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TABLE XXIX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Rabbits

Group	Animal Number	Erythrocyte Indices MCV (μ^3) Test Day: 0	MCV (μ^3) Test Day: 30	MCH ($\mu\mu g$) Test Day: 0	MCH ($\mu\mu g$) Test Day: 30	MCHC (%) Test Day: 0	MCHC (%) Test Day: 30
Untreated Control	1	61.8	66.3	20.1	22.7	32.6	34.3
	2	63.9	71.4	19.7	23.1	30.9	32.3
	3	62.2	63.3	19.7	20.6	31.6	32.6
	4	63.5	70.5	19.5	21.9	30.7	31.1
	5	60.9	65.4	19.1	21.2	31.4	32.4
	Mean	62.5	67.4	19.6	21.9	31.4	32.5
T-III	23	68.2	65.1	21.0	22.8	30.8	35.0
	24	61.5	61.6	19.4	20.0	31.6	32.4
	25	67.8	69.3	20.8	22.5	30.8	32.4
	Mean	65.8	65.3	20.4	21.7	31.1	33.3
T-VI	41	63.1	60.0	20.1	20.1	31.9	33.5
	42	65.5	65.2	20.5	21.8	31.4	33.4
	43	60.3	69.8	18.4	23.4	30.6	33.5
	Mean	63.0	65.0	19.7	21.8	31.3	33.5

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TABLE XXX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Rabbits

Group	Animal Number	Erythrocyte Indices MCV (μ^3) Test Day: 0	30	MCH (μμg) Test Day: 0	30	MCHC (%) Test Day: 0	30
Untreated Control	6	61.7	67.1	19.8	20.8	32.1	30.9
	7	64.0	66.7	19.9	21.2	31.2	31.8
	8	64.3	67.7	20.7	21.5	32.1	31.8
	9	69.4	69.9	21.2	22.5	30.6	31.8
	10	62.1	62.0	20.2	21.1	32.6	33.9
	Mean	64.3	66.9	20.4	21.4	31.7	32.0
T-III	26	68.6	69.5	21.8	22.9	31.7	32.9
	27	66.4	68.4	21.6	21.9	32.5	32.0
	28	68.3	67.7	21.6	22.1	31.7	32.6
	Mean	67.8	68.5	21.7	22.3	32.0	32.5
T-VI	44	71.4	71.7	22.4	23.5	31.3	32.8
	45	67.6	68.4	21.8	23.3	32.2	34.1
	46	69.1	65.6	21.2	20.5	30.7	31.3
	Mean	69.4	68.6	21.8	22.5	31.4	32.7

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TABLE XXXI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Male Dogs

Group	Animal Number	Erythrocyte Indices: MCV (μ^3) Test Day: 0	MCV (μ^3) Test Day: 30	MCH (µµg) Test Day: 0	MCH (µµg) Test Day: 30	MCHC (%) Test Day: 0	MCHC (%) Test Day: 30
Untreated Control	1	69.5	69.3	23.7	22.9	34.1	33.0
	2	73.8	68.1	23.2	22.9	31.5	33.6
	3	72.1	67.6	22.1	23.8	31.6	34.0
	4	71.8	65.3	22.3	21.6	31.4	32.5
	Mean	71.1	67.9	22.8	22.6	32.2	33.3
T-III	17	71.7	68.0	24.7	23.5	34.3	34.5
	18	68.0	60.4	22.3	20.9	32.7	34.6
	Mean	69.8	64.2	23.5	22.2	33.5	34.5
T-VI	29	72.1	69.0	24.1	23.5	33.4	34.0
	30	68.7	64.3	22.8	22.6	33.2	35.2
	Mean	70.4	66.6	23.4	23.0	33.3	34.6

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TABLE XXXII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Hematologic Data - Female Dogs

Group	Animal Number	Erythrocyte Indices MCV (μ^3) Test Day: 0	MCV (μ^3) Test Day: 30	MCH (µµg) Test Day: 0	MCH (µµg) Test Day: 30	MCHC (%) Test Day: 0	MCHC (%) Test Day: 30
Untreated Control	5	72.9	66.4	24.2	22.9	33.2	34.5
	6	70.8	69.5	23.1	23.8	32.5	34.2
	7	67.8	64.8	22.4	21.8	33.0	34.0
	8	67.5	65.1	22.3	21.8	33.1	33.5
	Mean	69.8	66.2	23.0	22.6	33.0	34.0
T-III	19	71.1	65.1	25.3	23.1	35.6	35.5
	20	70.9	66.2	23.2	23.2	32.7	35.1
	Mean	71.0	65.7	24.2	23.1	34.2	35.3
T-VI	31	66.6	64.1	22.3	22.1	33.6	34.4
	32	72.5	64.8	24.6	22.5	34.0	34.8
	Mean	69.6	64.5	23.4	22.3	33.8	34.6

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D. Clinical Chemistry Studies

The results of clinical chemistry done on blood from male and female rats, rabbits and dogs from the control group and from treatment groups T-III and T-IV are presented in Tables XXXIII through XXXVIII.

Mean values for fasting blood glucose, BUN, SAP, SGPT and SGOT of treated animals were normal when compared to similar values of control animals after 30 days of treatment.

TABLE XXXIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Male Rats

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	30	BUN (mg/100 ml) Test Day: 0	30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	30	SGPT (International Units) Test Day: 0	30	SGOT (International Units) Test Day: 0	30
Untreated Control	1	115	123	8	14	17.4	12.4	47	52	64	97
	2	139	115	8	15	15.6	8.5	44	47	78	69
	3	129	106	4	14	13.0	8.7	26	38	70	79
	4	123	107	6	13	16.5	9.4	34	37	74	91
	5	133	103	9	15	27.4	12.2	37	41	56	76
	6	123	101	7	13	12.1	8.5	25	33	76	47
	7	139	106	6	14	12.7	8.5	20	24	73	54
	8	127	115	8	12	13.3	8.3	34	33	66	71
	9	137	120	8	20	13.8	9.4	26	37	66	73
	10	130	124	5	12	13.3	9.5	19	29	85	67
	Mean	129	112	7	14	15.6	9.5	31	37	71	72
T-III	41	122	84	5	13	12.1	8.5	20	22	67	66
	42	119	84	7	14	11.8	8.3	25	32	71	53
	43	118	91	7	16	11.6	8.4	31	29	67	61
	44	122	94	6	14	14.5	10.6	21	30	67	59
	45	127	107	7	19	12.5	10.5	28	40	51	64
	46	128	93	6	9	22.2	12.6	35	44	-	75
	47	125	90	8	12	9.2	6.6	34	45	72	77
	48	122	98	9	9	14.6	8.2	72	37	66	76
	49	125	105	9	12	15.6	9.9	41	41	59	78
	50	131	106	6	11	9.5	6.2	33	46	69	85
	Mean	124	94	7	13	13.4	9.0	33	37	65	69

- = Data not available.

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TABLE XXXIII continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Male Rats

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	Glucose (mg/100 ml) Test Day: 30	BUN (mg/100 ml) Test Day: 0	BUN (mg/100 ml) Test Day: 30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	SAP (Bessey-Lowry-Brock Units) Test Day: 30	SGPT (International Units) Test Day: 0	SGPT (International Units) Test Day: 30	SGOT (International Units) Test Day: 0	SGOT (International Units) Test Day: 30
T-VI	71	124	90	5	10	17.4	10.5	35	42	63	85
	72	123	116	8	11	23.1	10.6	32	37	66	72
	73	126	78	12	10	10.4	5.8	127	37	48	74
	74	126	104	10	12	13.9	9.7	45	40	58	73
	75	133	109	6	10	11.7	7.1	40	51	43	82
	76	128	115	10	13	13.2	10.6	32	67	50	111
	77	123	103	8	12	10.3	8.3	30	42	47	68
	78	126	93	4	12	10.3	7.0	39	46	67	91
	79	126	90	8	10	23.4	11.2	44	37	63	83
	80	127	116	6	13	10.4	7.2	31	46	62	72
	Mean	125	101	8	11	14.4	8.8	46	45	56	81

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TABLE XXXIV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Female Rats

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	30	BUN (mg/100 ml) Test Day: 0	30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	30	SGPT (International Units) Test Day: 0	30	SGOT (International Units) Test Day: 0	30
Untreated Control	81	129	106	13	17	5.3	4.6	31	33	82	73
	82	91	103	8	16	11.1	7.7	30	37	75	72
	83	90	110	8	19	6.9	5.8	32	44	93	79
	84	133	117	8	16	5.3	3.9	30	26	97	90
	85	112	120	11	17	7.6	4.9	27	28	87	53
	86	123	115	8	17	7.8	4.5	30	31	72	53
	87	108	123	7	14	5.6	3.1	38	33	69	54
	88	117	122	8	13	9.1	6.2	29	26	78	77
	89	120	109	9	16	6.4	4.2	22	23	103	57
	90	115	96	6	16	10.6	6.8	27	40	111	86
	Mean	112	112	9	16	7.6	5.2	30	32	87	69
T-III	121	109	100	12	16	5.2	3.7	36	42	113	65
	122	108	106	8	18	6.6	5.2	71	33	165	93
	123	105	106	9	19	5.5	5.3	35	44	79	72
	124	128	104	9	16	5.0	4.1	36	36	141	67
	125	118	109	10	18	9.0	5.7	30	27	90	73
	126	111	98	11	17	10.5	7.3	26	19	103	48
	127	121	106	11	16	9.6	7.2	39	34	89	44
	128	99	96	10	15	5.3	3.4	31	30	83	42
	129	116	99	9	16	5.3	3.8	30	24	84	39
	130	102	96	8	16	8.5	5.4	26	46	105	46
	Mean	110	102	10	17	7.0	5.1	36	34	105	59

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TABLE XXXIV continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Female Rats

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	30	BUN (mg/100 ml) Test Day: 0	30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	30	SGPT (International Units) Test Day: 0	30	SGOT (International Units) Test Day: 0	30
T-VI	152	92	98	7	16	6.1	3.6	22	22	91	47
	152	104	101	8	19	6.0	3.2	25	27	105	77
	153	101	115	8	14	6.6	5.0	24	29	95	60
	154	107	102	8	17	4.6	2.4	31	26	137	56
	155	100	122	6	18	9.2	5.2	24	32	85	70
	156	109	103	9	17	5.6	3.3	29	41	83	61
	157	98	128	10	13	9.1	6.2	37	46	95	67
	158	95	112	8	13	7.4	5.6	41	41	88	59
	159	99	106	9	15	9.3	5.4	26	34	95	59
	160	125	93	7	15	7.6	5.8	30	37	94	61
	Mean	113	108	8	16	7.2	5.0	29	34	97	62

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TABLE XXXV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Male Rabbits

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	30	BUN (mg/100 ml) Test Day: 0	30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	30	SGPT (International Units) Test Day: 0	30	SGOT (International Units) Test Day: 0	30
Untreated Control	1	155	114	15	29	7.0	9.0	51	43	23	28
	2	133	116	14	36	9.9	7.0	91	51	31	42
	3	134	109	10	20	7.9	8.2	53	42	26	33
	4	129	106	8	17	5.4	10.1	61	60	19	30
	5	114	117	16	19	3.5	13.1	54	52	22	29
	Mean	133	112	13	22	6.7	9.5	62	50	24	32
T-III	23	165	131	17	23	8.4	9.7	76	77	29	45
	24	132	128	19	14	6.7	10.1	49	48	15	41
	25	125	119	22	24	6.8	7.0	112	83	30	59
	Mean	141	126	19	20	7.3	9.3	79	69	25	48
T-VI	41	165	122	19	21	5.9	6.1	92	82	41	60
	42	153	113	15	21	9.9	8.8	96	62	41	43
	43	114	110	17	24	4.5	9.0	51	58	25	57
	Mean	144	117	17	22	6.8	7.7	80	67	36	53

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TABLE XXXVI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Female Rabbits

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	30	BUN (mg/100 ml) Test Day: 0	30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	30	SGPT (International Units) Test Day: 0	30	SGOT (International Units) Test Day: 0	30
Untreated Control	6	135	130	12	12	9.1	10.7	55	52	23	49
	7	141	113	12	16	7.1	9.5	65	45	31	42
	8	117	107	27	12	6.6	6.7	89	61	28	38
	9	147	125	19	18	15.4	17.9	56	63	35	51
	10	121	113	19	24	10.2	8.1	66	57	36	28
	Mean	132	118	18	16	9.7	10.6	66	56	31	42
T-III	26	128	116	24	17	11.4	8.5	61	76	52	65
	27	127	109	22	22	8.8	8.4	64	50	51	32
	28	136	122	19	20	10.2	9.8	93	65	46	39
	Mean	130	116	22	20	10.1	8.9	73	64	50	45
T-VI	44	119	135	22	11	5.1	8.0	53	50	45	29
	45	133	128	25	13	9.2	11.0	68	50	58	40
	46	149	150	26	22	7.1	19.2	46	67	46	39
	Mean	134	135	24	15	7.1	15.7	56	56	50	36

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TABLE XXXVII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Male Dogs

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	30	BUN (mg/100 ml) Test Day: 0	30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	30	SGPT (International Units) Test Day: 0	30	SGOT (International Units) Test Day: 0	30
Untreated Control	1	93	86	16	17	3.0	2.4	34	23	22	14
	2	110	95	9	12	11.1	10.0	35	20	20	23
	3	109	98	10	10	4.2	4.3	33	29	18	22
	4	111	104	13	14	4.2	4.6	33	33	17	25
	Mean	106	96	12	13	5.6	5.3	34	26	19	21
T-III	17	101	111	15	17	4.2	4.0	39	24	24	21
	18	96	93	14	14	4.7	4.4	40	18	20	14
	Mean	103	104	14	16	4.4	4.2	40	21	22	18
T-VI	29	107	108	12	13	4.5	4.4	43	33	24	19
	30	89	90	12	15	4.7	5.3	31	25	28	30
	Mean	98	99	12	14	4.6	4.8	37	29	26	24

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TABLE XXXVIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Clinical Chemistry Data - Female Dogs

Group	Animal Number	Glucose (mg/100 ml) Test Day: 0	Glucose (mg/100 ml) Test Day: 30	BUN (mg/100 ml) Test Day: 0	BUN (mg/100 ml) Test Day: 30	SAP (Bessey-Lowry-Brock Units) Test Day: 0	SAP (Bessey-Lowry-Brock Units) Test Day: 30	SGPT (International Units) Test Day: 0	SGPT (International Units) Test Day: 30	SGOT (International Units) Test Day: 0	SGOT (International Units) Test Day: 30
Untreated Control	5	109	99	12	17	1.4	1.6	32	34	24	24
	6	110	88	9	15	3.6	4.2	43	39	21	29
	7	100	98	10	16	6.1	6.2	39	34	23	34
	8	105	112	12	14	2.9	2.7	47	37	23	30
	Mean	106	97	11	16	3.8	3.5	40	36	23	29
T-III	19	104	91	16	16	4.3	5.5	35	18	28	28
	20	109	99	14	15	4.2	5.3	43	23	30	27
	Mean	106	95	15	16	4.2	5.4	39	20	29	24
T-VI	31	99	90	13	15	4.6	5.8	52	36	19	25
	32	90	104	20	17	3.9	4.6	40	30	23	23
	Mean	94	97	16	16	4.2	5.2	46	33	21	24

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E. Urinalyses

The results of examination of specimens of urine collected from male and female rats, rabbits and dogs in the control group and in treatment groups T-III and T-VI are presented in Tables XXXIX through L.

Mean values for urine content of blood, ketones, glucose and protein, for pH and for microscopic constituents of urinary sediments of treated animals were normal when compared to similar values of control animals after 30 days of treatment.

0 = normal
T = trace amounts
+ = slight
++ = moderate
+++ = marked in severity
++++ = extreme in severity

TABLE XXXIX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Rats

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
Untreated Control	1	0	0	0	0	0	0	0	T	7.0	6.5
	2	0	0	0	0	0	0	+	+	7.0	6.5
	3	0	0	0	0	0	0	0	T	7.0	7.5
	4	0	0	0	0	0	0	0	T	7.0	6.5
	5	0	0	0	0	0	0	+	T	7.0	7.0
	6	0	0	0	0	0	0	0	T	7.5	8.0
	7	0	0	0	0	0	0	+	T	7.5	7.0
	8	0	0	0	0	0	0	0	+	7.0	7.0
	9	0	0	0	0	0	0	0	T	8.5	7.0
	10	0	0	0	0	0	0	0	T	7.0	7.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.6	7.2	7.0
T-III	41	0	0	0	0	0	0	0	0	7.5	6.0
	42	0	0	0	0	0	0	0	T	7.0	6.5
	43	0	0	0	0	0	0	T	+	7.0	7.0
	44	0	0	0	0	0	0	0	0	7.0	6.5
	45	0	0	0	0	0	0	0	T	7.5	6.5
	46	0	0	0	0	0	0	0	T	7.5	6.5
	47	0	0	0	0	0	0	0	T	8.0	7.0
	48	0	0	0	0	0	0	T	T	7.0	7.0
	49	0	0	0	0	0	0	0	+	8.0	6.5
	50	0	0	0	0	0	0	0	T	7.5	7.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.5	7.4	6.6

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TABLE XXXIX continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Rats

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
T-VI	71	0	0	0	0	0	0	0	T	7.5	7.0
	72	0	0	0	0	0	0	0	T	7.5	6.0
	73	0	0	0	0	0	0	0	T	7.0	7.0
	74	0	0	0	0	0	0	T	T	7.5	6.5
	75	0	0	0	0	0	0	0	T	6.5	6.0
	76	0	0	0	0	0	0	+	++	6.5	7.0
	77	0	0	0	0	0	0	0	0	7.5	7.0
	78	0	0	0	0	0	0	0	+	7.5	7.0
	79	0	0	0	0	0	0	0	T	7.0	7.0
	80	0	0	0	0	0	0	0	+	8.0	6.5
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.7	7.2	6.7

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TABLE XL

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Female Rats

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
Untreated Control	81	0	0	0	0	0	0	T	0	6.5	6.0
	82	0	+	0	0	0	0	0	+	6.5	6.5
	83	0	0	0	0	0	0	0	0	7.0	7.0
	84	0	0	0	0	0	0	T	0	7.0	7.0
	85	0	+	0	0	0	0	T	0	7.0	8.0
	86	0	0	0	0	0	0	T	0	7.0	6.5
	87	0	0	0	0	0	0	T	0	7.0	6.5
	88	0	++	0	0	0	0	0	0	8.0	7.0
	89	0	0	0	0	0	0	0	0	9.0	6.0
	90	0	0	0	0	0	0	0	0	6.0	6.0
	Mean	0.0	0.4	0.0	0.0	0.0	0.0	0.5	0.2	7.1	6.7
T-III	121	0	0	0	0	0	0	0	0	7.0	6.0
	122	0	0	0	0	0	0	0	0	7.0	6.5
	123	0	0	0	0	0	0	+	0	6.0	6.5
	124	0	0	0	0	0	0	0	0	8.5	6.0
	125	0	0	0	0	0	0	0	A	7.0	6.5
	126	0	0	0	0	0	0	0	0	7.0	6.5
	127	0	0	0	0	0	0	0	0	8.5	6.0
	128	0	0	0	0	0	0	0	0	7.5	6.0
	129	0	0	0	0	0	0	0	0	7.0	6.0
	130	0	0	0	0	++	0	1	0	7.5	6.0
	Mean	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	7.3	6.2

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TABLE XL continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Female Rats

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
I-VI	151	0	0	0	0	0	0	0	0	7.0	6.0
	152	0	0	0	0	0	0	T	0	7.0	6.5
	153	0	0	0	0	0	0	0	0	7.0	6.0
	154	0	0	0	0	0	0	0	0	7.5	6.0
	155	0	0	0	0	0	0	0	T	6.5	6.5
	156	0	0	0	0	0	0	T	0	9.0	6.5
	157	0	0	0	0	0	0	0	0	7.0	6.0
	158	0	0	0	0	0	0	0	T	6.5	6.0
	159	0	0	0	0	0	0	0	0	7.0	6.0
	160	0	0	0	0	0	0	0	0	7.0	6.5
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	7.2	6.2

TABLE XLI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Rabbits

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
Untreated Control	1	T	++	0	0	0	0	0	T	8.5	9.0
	2	T	0	0	0	0	0	0	T	9.0	9.0
	3	-	++	-	0	-	0	-	0	-	8.0
	4	0	T	0	0	0	0	0	0	8.0	7.0
	5	T	T	0	0	0	0	0	0	8.0	7.0
	Mean	0.8	1.2	0.0	0.0	0.0	0.0	0.0	0.4	8.4	8.0
T-III	23	0	+++	0	0	0	0	0	+++	7.0	7.5
	24	0	0	0	0	0	0	0	0	8.5	6.0
	25	++	T	0	0	0	0	0	0	6.0	7.0
	Mean	0.7	1.2	0.0	0.0	0.0	0.0	0.0	1.0	7.2	6.8
T-VI	41	T	T	0	0	0	0	0	0	7.0	6.0
	42	0	T	0	0	0	0	0	0	6.0	7.0
	43	0	T	0	0	0	0	0	+	8.5	9.0
	Mean	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.3	7.2	7.3

- = Data not available.

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TABLE XLII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Female Rabbits

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
Untreated Control	6	0	0	0	0	0	0	0	0	6.5	6.0
	7	0	0	0	0	0	0	0	0	7.0	6.0
	8	+	++	0	0	0	0	0	0	7.0	7.0
	9	0	++	0	0	0	0	0	0	9.0	7.5
	10	0	++	0	0	0	0	0	0	8.0	6.5
	Mean	0.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0	7.5	6.6
T-III	26	++	T	0	0	0	0	+	0	8.0	9.0
	27	0	++	0	0	0	0	0	T	9.0	7.0
	28	0	0	0	0	0	0	0	0	6.0	8.0
	Mean	0.7	0.8	0.0	0.0	0.0	0.0	0.2	0.2	7.7	8.0
T-VI	44	+	0	0	0	0	0	0	0	7.5	7.0
	45	+	0	0	0	0	0	0	0	6.5	6.0
	46	0	0	0	0	0	0	0	0	8.0	6.5
	Mean	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.3	6.5

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TABLE XLIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Dogs

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
Untreated Control	1	0	0	0	0	0	0	+	++	6.0	9.0
	2	0	0	0	0	0	0	0	0	7.0	7.0
	3	0	0	0	0	0	0	++	+	6.0	6.5
	4	0	0	0	0	0	0	0	0	7.0	8.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.6	6.5	7.6
T-III	17	0	0	0	0	0	0	++	+	7.0	6.5
	18	0	0	0	0	0	0	+	+	7.0	6.5
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.0	7.0	6.5
T-VI	29	0	0	0	0	0	0	0	+	6.0	7.0
	30	0	0	0	0	0	0	0	0	8.0	8.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	7.5	7.5

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TABLE XLIV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalysis Data - Female Dogs

Group	Animal Number	Blood Test Day: 0	Blood Test Day: 30	Ketones Test Day: 0	Ketones Test Day: 30	Glucose Test Day: 0	Glucose Test Day: 30	Proteins Test Day: 0	Proteins Test Day: 30	pH Test Day: 0	pH Test Day: 30
Untreated Control	5	0	0	0	0	0	0	0	++++	7.0	9.0
	6	0	0	0	0	0	0	0	0	7.0	8.0
	7	0	0	0	0	0	0	0	0	5.0	6.5
	8	0	0	0	0	0	0	0	++	7.0	9.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	6.5	8.1
T-III	19	0	0	0	0	0	0	0	++	7.5	9.0
	20	0	0	0	0	0	0	0	T	7.5	8.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	7.5	8.5
T-VI	31	0	0	0	0	0	0	0	+++	6.0	9.0
	32	0	0	0	0	0	0	0	T	8.0	6.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	7.0	7.5

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TABLE XLV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Rats

Group	Animal Number	Microscopic Examination Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
Untreated Control	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	+
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	7	0	0	0	0	0	0
	8	0	0	0	0	0	0
	9	0	0	0	0	0	0
	10	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.1
T-III	41	0	0	0	0	0	0
	42	0	0	0	0	0	0
	43	0	0	0	0	0	0
	44	0	0	0	0	0	0
	45	0	0	0	0	0	0
	46	0	0	0	0	0	0
	47	0	0	0	0	0	0
	48	0	0	0	0	0	0
	49	0	0	0	0	0	0
	50	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0

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TABLE XLV continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Rats

Group	Animal Number	Microscopic Examination Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
T-VI	71	0	0	0	0	0	0
	72	0	0	0	0	0	0
	73	0	0	0	0	0	0
	74	0	0	0	0	0	0
	75	0	0	0	0	0	0
	76	0	0	0	0	0	0
	77	0	0	0	0	0	0
	78	0	0	0	0	0	0
	79	0	0	0	0	0	0
	80	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0

TABLE XLVI continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Female Rats

Group	Animal Number	Microscopic Examination: Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
T-VI	151	0	0	0	0	+	0
	152	0	0	0	0	+	0
	153	0	0	0	0	0	0
	154	0	0	0	0	0	0
	155	0	0	0	0	0	0
	156	0	0	0	0	0	0
	157	0	0	0	0	+	0
	158	0	0	0	0	++	0
	159	1	0	0	0	0	0
	160	0	0	0	0	0	0
	Mean	0.1	0.0	0.0	0.0	0.4	0.0

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TABLE XLVII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Rabbits

Group	Animal Number	Microscopic Examination: Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
Untreated Control	1	6	0	0	0	0	0
	2	1	0	0	0	0	0
	3	–	0	–	0	–	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	Mean	0.3	0.0	0.0	0.0	0.0	0.0
T-III	23	0	0	0	0	0	0
	24	0	0	0	0	0	0
	25	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0
T-VI	41	0	0	0	0	0	0
	42	0	0	0	0	0	0
	43	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0

– = Data not available.

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TABLE XLVIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Female Rabbits

Group	Animal Number	Microscopic Examination: Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
Untreated Control	6	0	0	0	0	0	0
	7	0	0	0	0	0	0
	8	0	0	0	0	0	0
	9	0	0	0	0	+	0
	10	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.2	0.0
T-III	26	0	0	0	0	+++	0
	27	0	0	0	0	0	0
	28	0	0	0	0	0	++
	Mean	0.0	0.0	0.0	0.0	0.6	0.4
T-VI	44	0	0	0	0	0	0
	45	0	0	0	0	0	0
	46	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0

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TABLE XLIX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Male Dogs

Group	Animal Number	Microscopic Examination: Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
Untreated Control	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0
T-III	17	6	0	0	0	0	0
	18	0	0	0	0	+	0
	Mean	3.0	0.0	2.0	0.0	0.5	0.0
T-VI	29	0	0	0	0	0	0
	30	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0

TABLE I

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Urinalyses Data - Female Dogs

Group	Animal Number	Microscopic Examination Leukocytes (aver./hpf-400X) Test Day: 0	30	Erythrocytes (aver./hpf-400X) Test Day: 0	30	Crystals Test Day: 0	30
Untreated Control	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	7	0	0	0	0	0	0
	8	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0
T-III	19	0	0	0	0	0	0
	20	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0
T-VI	31	0	0	0	0	0	+
	32	0	0	0	0	0	0
	Mean	0.0	0.0	0.0	0.0	0.0	0.5

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F. Organ Weight Data

Organ weight data consisting of absolute organ weights as well as organ to body weight and organ to brain weight ratios are presented in Tables LI through LXV.

No consistent or dose-related test material effects were noted among the test animals when compared to the controls.

TABLE LI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rats

Organ: Liver

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	9.237	6.526	2.5541	2.7165	4.6484	3.3597
T-I	9.841	7.261	2.9489*	3.1292	5.1216	3.8012
T-II	9.009	6.418	2.5130	2.6658	4.3954	3.3726
T-III	8.251	6.260	2.4829	2.6514	4.2307	3.2389
T-IV	8.900	6.895	2.5474	2.5457	4.4587	3.6099
T-V	9.256	6.725	2.6868	2.9111	4.6763	3.5852
T-VI	9.116	6.633	2.7996	2.7849	4.4531	3.5129

* Statistically significant difference from the untreated control group at the 95 percent level of confidence.

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TABLE LII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rats

Organ: Both Kidneys

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	2.608	1.721	0.7218	0.7166	1.3106	0.8844
T-I	2.639	1.767	0.7875	0.7616	1.3721	0.9249
T-II	2.595	1.710	0.7251	0.7104	1.2671	0.8981
T-III	2.570	1.644	0.7721	0.6976	1.3137	0.8613
T-IV	2.690	1.747	0.7647	0.6482	1.3347	0.9139
T-V	2.697	1.670	0.7784	0.7248	1.3567	0.8907
T-VI	2.659	1.684	0.7921	0.6882	1.2983	0.8918

No statistically significant treatment related effects found.

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TABLE LIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rats

Organ: Both Gonads

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	3.285	0.085	0.9179	0.0356	1.5578	0.0439
T-I	2.957	0.078	0.8881	0.0302	1.5385	0.0367
T-II	3.673	0.075	0.8628	0.0310	1.5013	0.0393
T-III	3.057	0.069	0.9245	0.0293	1.5617	0.0365
T-IV	3.042	0.078	0.8697	0.0284	1.5089	0.0408
T-V	3.064	0.082	0.8884	0.0354	1.5432	0.0435
T-VI	3.122	0.075	0.9286	0.0308	1.5232	0.0399

No statistically significant treatment related effects found.

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TABLE LIV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rats

Organ: Heart

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	1.220	0.894	0.3376	0.3720	0.6138	0.4596
T-I	1.134	0.853	0.3400	0.3677	0.5912	0.4468
T-II	1.189	0.892	0.3326	0.3707	0.5798	0.4685
T-III	1.150	0.890	0.3467	0.3776	0.5885	0.4665
T-IV	1.214	0.914	0.3457	0.3809	0.6032	0.4779
T-V	1.197	0.856	0.3462	0.3699	0.6016	0.4565
T-VI	1.210	0.907	0.3592	0.3692	0.5911	0.4794

No statistically significant treatment related effects found.

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TABLE LV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rats

Organ: Brain

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	1.988	1.945	0.5526	0.8116	1.0000	1.0000
T-I	1.925	1.989	0.5772	0.8237	1.0000	1.0000
T-II	2.052	1.999	0.5756	0.7935	1.0000	1.0000
T-III	1.956	1.913	0.5907	0.8113	1.0000	1.0000
T-IV	2.016	1.911	0.5749	0.7043	1.0000	1.0000
T-V	1.984	1.875	0.5768	0.8128	1.0000	1.0000
T-VI	2.052	1.894	0.6129	0.7756	1.0000	1.0000

No statistically significant treatment related effects found.

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TABLE LVI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rabbits

Organ: Liver

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	72.346	91.294	31.3439	42.4482	8.2661	11.0543
T-I	60.887	63.570	29.6886	33.8368	7.2644	7.6306
T-II	58.233	51.503	28.5021	27.7667	7.1171	6.3603
T-III	56.818	50.423	27.4696	26.5744	6.8181	6.2282
T-IV	83.517	96.797	41.1083	46.3633	10.2018	11.8823
T-V	103.667	72.403	54.8435	40.6760	12.7593	9.0971
T-VI	81.270	91.037	37.3859	45.0578	9.4719	10.9669

No statistically significant treatment related effects found.

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TABLE LVII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rabbits

Organ: Both Kidneys

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	14.224	13.194	6.2127	6.0439	1.6294	1.6258
T-I	13.583	13.237	6.6387	7.1623	1.6091	1.5838
T-II	13.847	12.913	6.7786	6.9643	1.6737	1.5933
T-III	12.970	11.260	6.3360	5.9368	1.5751	1.3907
T-IV	13.683	14.253	7.0619	6.8847	1.6483	1.7472
T-V	14.373	12.733	7.6816	7.4046	1.7639	1.6287
T-VI	12.985	13.417	5.9390	6.7479	1.5138	1.6004

No statistically significant treatment related effects found.

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TABLE LVIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rabbits

Organ: Both Gonads

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	2.300	0.131	1.0458	0.0599	0.2724	0.0158
T-I	1.967	0.113	0.9699	0.0638	0.2281	0.0137
T-II	0.957	0.144	0.4729	0.0781	0.1164	0.0179
T-III	1.360	0.117	0.6669	0.0610	0.1656	0.0144
T-IV	1.560	0.099	0.7918	0.0475	0.1906	0.0120
T-V	1.353	0.112	0.7442	0.0668	0.1647	0.0142
T-VI	2.605	0.113	1.1999	0.0564	0.3037	0.0134

No statistically significant treatment related effects found.

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TABLE LIX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rabbits

Organ: Heart

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	5.424	4.972	2.3642	2.3064	0.6215	0.6065
T-I	4.643	4.860	2.2680	2.1680	0.5519	0.4890
T-II	4.617	4.017	2.2322	2.1661	0.5577	0.4965
T-III	4.487	4.050	2.1945	2.1317	0.5436	0.5005
T-IV	4.567	4.887	2.3535	2.3588	0.5505	0.5999
T-V	4.283	4.117	2.2191	2.3731	0.5168	0.5245
T-VI	5.005	4.547	2.3361	2.2882	0.5927	0.5460

No statistically significant treatment related effects found.

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TABLE LX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Rabbits

Organ: Brain

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	8.747	8.120	3.8399	3.7578	1.0000	1.0000
T-I	8.464	8.278	4.1385	4.4251	1.0000	1.0000
T-II	8.269	8.086	4.0342	4.3614	1.0000	1.0000
T-III	8.270	8.094	4.0497	4.2780	1.0000	1.0000
T-IV	8.358	8.145	4.3898	3.9432	1.0000	1.0000
T-V	8.144	7.865	4.3466	4.6988	1.0003	1.0000
T-VI	8.585	8.369	3.9933	4.2139	1.0000	1.0000

No statistically significant treatment related effects found.

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TABLE LXI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Dogs

Organ: Liver

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	275.248	275.245	32.8011	37.1905	3.5293	3.8876
T-I	319.500	226.430	30.2866	29.2453	4.3703	3.0122
T-II	266.575	264.120	27.5780	34.2849	3.5954	3.5241
T-III	357.800	262.445	35.2133	33.8902	4.7340	3.8058
T-IV	307.565	267.440	28.1937	29.8305	3.6621	3.7032
T-V	[illegible].695	268.020	28.3374	34.9341	4.7695	3.4047
T-VI	278.170	245.435	31.0207	33.9291	3.7450	3.5457

No statistically significant treatment related effects found.

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TABLE LXII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Dogs

Organ: Both Kidneys

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	45.520	41.350	5.4355	5.5655	0.5829	0.5858
T-I	51.605	36.375	4.8351	4.6657	0.7116	0.4833
T-II	42.760	36.310	4.4297	4.7129	0.5741	0.4823
T-III	50.295	33.015	5.0435	4.4037	0.6646	0.4830
T-IV	46.190	37.350	4.2449	4.1666	0.5492	0.5172
T-V	54.250	38.975	4.1371	5.0871	0.6849	0.4954
T-VI	41.685	34.150	4.7308	4.7523	0.5569	0.4931

No statistically significant treatment related effects found.

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TABLE LXIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Dogs

Organ: Both Gonads

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	7.850	0.544	0.9460	0.0746	0.0999	0.0078
T-I	12.805	0.639	1.2195	0.0830	0.1702	0.0085
T-II	14.215	0.678	1.4724	0.0880	0.1999	0.0069
T-III	12.415	0.799	1.1903	0.1045	0.1646	0.0116
T-IV	16.735	0.676	1.5415	0.0741	0.1987	0.0094
T-V	13.380	0.523	1.0540	0.0667	0.1687	0.0066
T-VI	15.125	0.616	1.7878	0.0827	0.2089	0.0089

No statistically significant treatment related effects found.

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TABLE LXIV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Dogs

Organ: Heart

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	67.633	62.832	8.0799	8.3884	0.8671	0.8847
T-I	76.315	66.385	7.2333	8.5285	1.0445	0.8814
T-II	72.090	61.790	7.4618	8.0198	0.9693	0.8184
T-III	72.185	59.210	7.1454	7.8520	0.9547	0.8649
T-IV	79.165	71.775	7.3267	8.0423	0.9375	0.9537
T-V	96.140*	73.155	7.3601	9.5257	1.2143	0.9289
T-VI	71.800	71.485	8.1016	9.7763	0.9617	1.0335

* Statistically significant difference from the untreated control group at the 95 percent level of confidence.

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TABLE LXV

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Mean Organ Weight and Ratio Data - Dogs

Organ: Brain

Group	Absolute Organ Weight (g)		Organ/Body Weight Ratio (g/1,000 g)		Organ/Brain Weight Ratio (g/g)	
	Male	Female	Male	Female	Male	Female
Untreated Control	78.265	70.773	9.4152	9.5976	1.0000	1.0000
T-I	73.785	75.215	6.9961	9.6827	1.0000	1.0000
T-II	74.440	75.750	7.7183	9.8312	1.0000	1.0000
T-III	75.640	68.000	7.5261	9.2100	1.0000	1.0000
T-IV	84.395	72.275	7.8038	8.1961	1.0000	1.0000
T-V	79.135	78.780	6.8794	10.2511	1.0000	1.0000
T-VI	75.695	69.265	8.8042	9.6590	1.0000	1.0000

No statistically significant treatment related effects found.

Industrial BIO-TEST Laboratories, Inc.

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C. Pathology

The results of histopathologic evaluation of tissues from male and female rats, rabbits and dogs from the control groups and from treatment groups T-III and T-VI are presented in Tables LXVI through LXXI.

No histopathologic alterations attributable to the effects of the test material were observed in any of the treated animals examined. Histopathologic alterations observed in treated animals were similar to those found in control animals of the same species.

The grading system used is as follows:

0.5 = trace
1.0 = minimal in severity
2.0 = mild in severity
3.0 = moderate in severity
4.0 = marked in severity
5.0 = extreme in severity
p = present

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Veterinary Pathologists

TABLE LXVI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Male Rats

Organ	Findings	Group: Untreated Control Number Examined: 20 Incidence	Average Grade	T-III 10 Incidence	Average Grade	T-VI 10 Incidence	Average Grade
Trachea	Chronic tracheitis, focal	1	1.0	0	-	1	1.0
Lung	Focal lymphocytic infiltrates, peribronchial	10	0.8	6	0.7	8	0.8
	Focal lymphocytic infiltrates, peribronchiolar	9	0.5	8	0.5	8	0.6
	Focal lymphocytic infiltrates, perivascular	6	0.9	7	0.7	10	0.9
	Focal subacute alveolitis	18	1.0	9	1.1	10	0.9
	Focal hemorrhage	1	0.5	0	-	2	1.0
	Congestion	2	1.5	0	-	0	-
	Edema	0	-	1	0.5	0	-
	Focal accumulation of macrophages in alveoli	1	1.0	0	-	0	-
Liver	Focal lymphocytic infiltrates, central distribution	0	-	0	-	1	1.0
Colon	Parasite, probably S. obvelata, in in lumen (nematodiasis)	3	P	1	P	2	P

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TABLE LXVI continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Male Rats

		Group: Untreated Control		T-III		T-VI	
	Number Examined:	20		10		10	
Organ	Findings	Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Lymph nodes	Acute serous lymphadenitis	1	0.5	0	-	0	-
Kidneys	Microconcretions in tubules deep in cortex, unilateral	1	0.5	1	0.5	0	-
	Hyperplasia, pelvic epithelium, unilateral	1	1.0	0	-	1	0.5
	Focal fibrosis of papilla, unilateral	1	0.5	0	-	0	-
	Focal mineralization in papilla, unilateral	1	0.5	0	-	0	-

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TABLE LXVII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Female Rats

Organ	Findings	Group: Untreated Control Number Examined: 20 Incidence	Average Grade	T-III 10 Incidence	Average Grade	T-VI 10 Incidence	Average Grade
Trachea	Chronic tracheitis, diffuse	3	0.6	0	-	0	-
Lung	Focal lymphocytic infiltrates, peribronchial	13	0.8	7	0.7	3	1.0
	Focal lymphocytic infiltrates, peribronchiolar	12	0.6	7	0.7	5	0.5
	Focal lymphocytic infiltrates, perivascular	10	0.7	4	0.8	7	0.9
	Focal subacute alveolitis	18	1.3	8	0.6	10	0.8
	Focal hemorrhage	0	-	1	0.5	2	0.7
	Edema	1	0.5	0	-	0	-
	Focal accumulation of macrophages in alveoli	0	-	0	-	1	0.5
	Focal acute bronchopneumonia	0	-	0	-	1	0.5
Liver	Bile duct proliferation	1	0.5	0	-	0	-
Kidneys	Microconcretions in tubules deep in cortex, unilateral	1	0.5	0	-	0	-
	Microconcretions in tubules deep in cortex, bilateral	14	1.1	4	1.0	7	0.8
Uterus	Hydrometra, unilateral	1	1.0	1	1.9	1	0.5
	Hydrometra, bilateral	6	1.3	1	1.0	2	1.2

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TABLE LXVIII

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Male Rabbits

		Group:	Untreated Control		T-III		T-VI	
		Number Examined:	5		3		3	
Organ	Findings		Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Heart	Congestion		0	-	0	-	1	2.5
Lung	Focal lymphocytic infiltrates, peribronchial		4	0.7	3	1.0	2	1.0
	Focal lymphocytic infiltrates, peribronchiolar		3	0.5	3	0.6	2	1.0
	Focal lymphocytic infiltrates, perivascular		3	1.0	3	0.6	0	-
	Focal subacute alveolitis		5	1.0	3	0.6	2	0.5
	Congestion		0	-	0	-	1	3.0
	Edema		0	-	0	-	1	3.0
	Acute bronchopneumonia		0	-	0	-	1	1.0
	Inhaled bone fragment		1	P	0	-	0	-
Liver	Focal lymphocytic infiltrates, portal distribution		2	0.7	2	1.7	0	-
	Focal cytoplasmic vacuolation of hepatocytes, glycogen, central distribution		1	1.0	0	-	0	-
	Congestion		0	-	0	-	1	2.0

TABLE LXVIII continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Male Rabbits

		Group:	Untreated Control		T-III		T-VI	
		Number Examined:	5		3		3	
Organ	Findings		Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Pancreas	Focal subacute pancreatitis		0	-	0	-	1	1.0
Lymph nodes	Acute serous lymphadenitis		0	-	1	1.0	0	-
Kidneys	Focal interstitial lymphocytic infiltrates, bilateral		2	0.5	1	1.5	0	-
	Congestion		0	-	0	-	1	2.0
Gonads	Juvenile, bilateral		5	P	1	P	1	P
	Atrophy, diffuse with fibrosis		0	-	1	1.0	0	-
Brain	Focal granulomatous meningo-encephalitis (probably encephalito-zoonosis), multiple		1	1.0	0	-	0	-

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TABLE LXIX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Female Rabbits

		Group:	Untreated Control		T-III		T-VI	
		Number Examined:	5		3		3	
Organ	Findings		Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Heart	Congestion		0	-	0	-	1	1.0
Lung	Focal lymphocytic infiltrates, peribronchial		5	0.8	3	0.7	3	1.0
	Focal lymphocytic infiltrates, peribronchiolar		4	0.7	3	0.6	3	0.8
	Focal lymphocytic infiltrates, perivascular		2	1.0	2	0.5	1	1.0
	Focal subacute alveolitis		5	0.9	3	0.6	3	0.8
	Focal hemorrhage		0	-	0	-	1	1.0
	Focal accumulation of hemosiderin-laden macrophages in alveoli		1	1.5	0	-	0	-
Liver	Focal lymphocytic infiltrates, portal distribution		1	1.0	2	1.5	0	-
	Focal lymphocytic infiltrates, random distribution		1	1.5	0	-	0	-
	Focal lymphocytic infiltrates, diffuse		0	-	1	1.0	0	-

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TABLE LXIX continued

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Female Rabbits

		Group:	Untreated Control		T-III		T-VI	
		Number Examined:	5		3		3	
Organ	Findings		Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Lymph nodes	Acute serous lymphadenitis		0	-	0	-	1	0.5
Kidneys	Focal interstitial lymphocytic infiltrates, bilateral		1	1.0	1	1.5	0	-
	Hyaline casts in tubules, unilateral		0	-	0	-	1	1.0
	Infarct, unilateral		1	1.0	0	-	0	-
	Encephalitozoonosis		1	1.0	0	-	0	-
Brain	Focal granulomatous meningo-encephalitis (encephalitozoonosis), multiple		1	1.0	1	1.0	0	-

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TABLE LXX

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Male Dogs

	Group:	Untreated Control		T-III		T-VI	
	Number Examined:	4		2		2	
Organ	Findings	Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Lung	Focal lymphocytic infiltrates, perivascular	1	0.5	0	-	0	-
	Focal subacute alveolitis	3	1.6	1	2.0	2	1.0
	Focal acute bronchopneumonia	0	-	1	2.0	0	-
	Focal accumulation of macrophages in alveoli	0	-	0	-	1	0.5
	Focal fibrosis	0	-	1	1.0	0	-
	Focal thrombosis	1	0.5	0	-	0	-
	Focal granulomatous pneumonia with acicular anisotropic material in granulomas	1	2.0	0	-	0	-
Liver	Focal congestion	0	-	0	-	1	0.5
Gonads	Atrophy, seminiferous epithelium with hyperplasia of Leydig cells	1	1.5	0	-	0	-

TABLE LXXI

TEST MATERIALS: Six Stabilized Methyl Chloroform Formulations

30-Day Subacute Inhalation Toxicity Study - Albino Rats, Albino Rabbits and Beagle Dogs

Histopathologic Changes

Final Sacrifice

Sex and Species: Female Dogs

		Group:	Untreated Control		T-III		T-VI	
		Number Examined:	4		2		2	
Organ	Findings		Incidence	Average Grade	Incidence	Average Grade	Incidence	Average Grade
Lung	Focal lymphocytic infiltrates, peribronchiolar		1	0.5	0	-	0	-
	Focal subacute alveolitis		4	1.5	2	1.2	2	1.2
	Congestion		0	-	1	0.5	0	-
Stomach	Focal subacute arteritis		0	-	1	1.0	0	-
Kidneys	Focal interstitial lymphocytic infiltrates, unilateral		1	1.0	0	-	0	-
	Microconcretions in tubules deep in cortex, bilateral		1	0.5	0	-	0	-
	Microconcretions in papilla, bilateral		0	-	0	-	1	0.5
	Focal mineralization of papilla, bilateral		1	0.5	0	-	0	-
Gonads	Focal mineralization of atretic follicle, unilateral		1	0.5	0	-	0	-

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Mr. L. W. Rampy
Toxicology Research Laboratory
Health and Environmental Research
1803 Building
Dow Chemical U.S.A.
Midland, Michigan 48640

Our Ref.: IBT No. 663-06334
Your Ref.: P.O. No. MM-917244

The following study has been scheduled as indicated below:

Test Material(s):
Six Methylchloroform Formulations:
1. XN-6058.1-L 1x inhibitor
2. XN-6058.1-L 2.5x inhibitor
3. XN-6058.1-L 5x inhibitor
4. XN-6080 1x inhibitor
5. XN-6080 2.5x inhibitor
6. XN-6080 5x inhibitor

Type of Test(s):

30-Day Subacute Inhalation Toxicity Study -
Beagle Dogs, Albino Rats, Albino Rabbits

Starting Date: 10/20/75

Termination Date: 11/23/75

Mailing Date: 1/13/76

Project Coordinator: Dr. John W. Goode - Decatur Research Laboratories

The above dates are approximations, and you will be
tified of any delays. If there are any questions, please contact the project coordinator above.
hank you.

Industrial BIO-TEST Laboratories, Inc.
Project Center

OFFICE OF TOXIC SUBSTANCES

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ACUTE PERCUTANEOUS ABSORPTION POTENTIAL OF CHLOROTHENE* VG SOLVENT

Chemical Name (300 per name)	CAS No. (10)
CHLOROTHENE VG	71-55-6

A.K. 5/24/83

D.D. Lockwood 5/8/78

R & D REPORT

878210695 D-268

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LABORATORY REPORT CODE

DATE ISSUED: April 7, 1978

LAB. NO. | PROBLEM NO.

DEPARTMENT: Toxicology Research Laboratory

TITLE: ACUTE PERCUTANEOUS ABSORPTION POTENTIAL OF CHLOROTHENE* VG SOLVENT

PAGES IN FULL REPORT

AUTHOR(S): P. A. Keeler, D. D. Lockwood

AUTHOR(S) SIGNATURE(S): 4-10-78 PAK, D.D. Lockwood 4/7/78

REVIEWER'S SIGNATURE: K. J. Olson 4/11/78

K. J. Olson

This report is: ☐ INTERIM ☒ FINAL and mainly: ☒ NEW ☐ REVIEW

DATA REFERENCES (book and page):

None

(Refer also to earlier related reports and publications.)

PATENT STATUS: ☐ disclosure submitted ☐ case filed ☐ no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

A clear colorless liquid sample of CHLOROTHENE*VG solvent, was submitted to the Toxicology Research Laboratory for evaluation of acute percutaneous absorption potential.

Two male and two female albino rabbits per dose level were exposed for 24 hours to 2520 or 5000 mg/kg of the undiluted test material. During the exposure period the material is confined to the skin by applying the dose under a plastic sleeve secured around the animal's trunk with tape. All of the animals survived. Slight to moderate redness and swelling were revealed when the sleeves were removed following the 24 hr. exposure period. Three animals from the high dose group also exhibited slight necrosis (skin burns). About 4 to 7 days after treatment, all of the animals exhibited tough leathery skin on the treated areas. Eventually this skin peeled off and left scars. All animals on the low dose level and the females on the high dose level regained pre-treatment weight in 3 weeks. The males from the high dose group did not regain pre-treatment weight in 6 weeks, although they appeared healthy and alert. These males lost about 200 grams during treatment, and remained at 200-400 grams under pre-treatment weight throughout the 6-week post-exposure observation period. Gross pathological examination, revealed no observable injury to internal organs.

CHLOROTHENE VG is known to be absorbed through skin, but is low in toxicity by this route of exposure.

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MUTAGENIC EVALUATION OF

COMPOUND DA

Chemical Name (300 per name)	25	CAS No. (10)	26
COMPOUND DA		9999999-4	
1,1,1- TRICHLOROETHANE		71-55-6	

AK 5/31/83

1A

D-269

878210696

LBI PROJECT # 2505

MUTAGENIC EVALUATION OF COMPOUND D6

SUBMITTED TO

DOW CHEMICAL COMPANY
TOXICOLOGY RESEARCH LABORATORY
1701 BUILDING
MIDLAND, MICHIGAN 48640

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND

JANUARY 20, 1975

BIONETICS

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EVALUATION SUMMARY

Compound D6 appeared to demonstrate mutagenic activity for indicator strain TA-1535 at very high dose levels in suspension tests with liver microsome fractions. The tests were repeated and mutagenic activity was still detected. No activity was observed in corresponding non-activation suspension tests with TA-1535 or in direct or activation plate test assays. No mutagenic or recombinogenic activity was demonstrated in any of the other indicator strains. This compound should be considered a presumptive mutagen based on these test results.

DATE: January 20, 1975

SPONSOR: Dow Chemical Company, Toxicology Research Laboratory

SUBJECT: Mutagenic Evaluation of Compound D6

I. OBJECTIVE

The objective of this study was to assess the genetic activity of the test material in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

II. MATERIALS

A. Test Material

Approximately 100 ml. of a clear liquid designated D6

B. Indicator Organisms

The indicator organisms used for all tests are described below:

- *Saccharomyces cerevisiae*, strain D4: a / a ade 2-2 try 5-12 / ade 2-1, try 5-27
- *Salmonella typhimurium*, strains:

TA-1538:	*his*G, *uvr*B, *rfa*	Missense Mutant
TA-1537:	*his*C, *uvr*B, *rfa*	Frameshift Mutant
TA-1538:	*his*D, *uvr*B, *rfa*	Frameshift Mutant

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml	
1. TPN (sodium salt)	6	uM
2. Isocitric acid	49	uM
3. Tris buffer, pH 7.4	28	uM
4. $MgCl_2$	1.7	uM
5. Isocitric dehydrogenase	1.0	Unit
6. Tissue homogenate or cell fraction	72	mg

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

D. Tissue Homogenates and Supernatant

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males. When induced tissues are used, the animals are pretreated with a mixture of polychlorinated biphenyls.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity[b]
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS

a Concentrations given in the Results Section
b BPS = base-pair substitution; FS = frameshift
c Previously shown to be non-mutagenic (see Appendix)

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

In the non-activation procedure, approximately 10^9 cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1 x 10^9 cells/ml and 5 x 10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.6 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

D. Preperation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.

IV. RESULTS SECTION

SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND: Received December 4, 1974

   Approximately 100 ml. of a clear liquid designated D6

2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY:

   This chemical was not soluble at the treatment concentrations employed. All tests were conducted in an aqueous environment.

3. OTHER COMMENTS:

   Because of the low solubility of this compound in the aqueous testing environment, the toxicity from test to test was quite variable depending upon the ability to effectively disperse the compound in the testing medium.

TOXICITY AND DOSAGE DETERMINATIONS

COMPOUND D6 TEST DATE: 12-15-74

Range of concentrations of the test compound used to determine the 50% survival level

Dose Number	% Concentration
1	10.0
2	1.0
3	0.1
4	0.01
5	0.001

Concentrations of the test chemical required for mutagenicity tests

Dose	% Concentration	
	Bacteria	Yeast
1/4 50% Survival	2.5	2.25
1/2 50% Survival	5.0	4.50
50% Survival	>5.0	>5.00 (No toxicity)
Plate Test	5.0	--

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SUMMARY OF TEST RESULTS

COMPOUND D6

A. Suspension Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Reversion Frequencies (x 10^{-8}) TA-1535	TA-1537	TA-1538	Saccharomyces D6 Conversion Frequencies (x 10^{-5}) Ade+	Trp+
NA-PC	-	-	54.08	164.54	101.81	52.37	38.65
NA-NC	-	-	3.00	16.34	7.78	5.70	20.56
NA-H	-	-	3.48	2.90	11.07	7.84	13.56
NA-L	-	-	0.88	8.82	9.00	6.76	20.62
A-NC (-C)	-	-	0.98	2.00	9.70	3.16	14.58
A-NC (+C)	-	-	2.90	4.85	6.76	4.36	21.24
A-PC	R	Li	27.32	7.40	41.01	6.54	19.75
A-PC	R	Lu	1.37	1.79	10.06	5.41	14.15
A-PC	R	T	1.49	7.06	10.06	4.08	19.77
A-H	R	Li	44.29	1.33*	7.13	5.77	20.56
A-L	R		7.11	1.67*	4.97	5.03	11.55
A-H	R	Lu	2.38	1.03	10.22	4.26	17.26
A-L	R		2.86	1.61	5.81	0.00	0.00
A-H	R	T	2.80	1.59	10.95	5.50	19.61
A-L	R		2.68	4.29	6.94	0.00	0.00

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

* Data from repeat tests

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COMPOUND D6

3. Plate Tests

Test[a]	Activation: Species[b]	Activation: Organ[c]	Salmonella Responses: TA-1535	TA-1537	TA-1538
NA-PC	-	-	+	+	+
NA-NC	-	-	-	-	-
NA-N	-	-	-	-	-
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	N	Li	+	+	+
A-PC	N	Lu	-	-	-
A-PC	N	T	-	-	-
A-N	N	Li	-	-	-
A-N	N	Lu	-	-	-
A-N	N	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
N = high dose
L = low dose

[b] N = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

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B. Plate Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Responses TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	R	Li	-	-	-
A-PC	R	Lu	+	+	+
A-PC	R	T	-	-	-
A-H	R	Li	-	-	-
A-H	R	Lu	-	-	-
A-H	R	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

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COMPOUND DS

B. Plate Tests

Test[a]	Activation Species[b]	Activation Organ[c]	Salmonella Responses TA-1535	TA-1537	TA-1538
NA-PC	-	-			
NA-NC	-	-			
NA-H	-	-			
A-NC (-C)	-	-	-	-	-
A-NC (+C)	-	-	-	-	-
A-PC	Mo	Li	-	-	-
A-PC	Mo	Lu	+	+	+
A-PC	Mo	T	-	-	-
A-H	Mo	Li	-	-	-
A-H	Mo	Lu	-	-	-
A-H	Mo	T	-	-	-

[a] NA = non activation
NC = negative control
PC = positive control
A = activation
H = high dose
L = low dose

[b] M = mouse
Mo = monkey
R = rat

[c] Li = liver
Lu = lung
T = testes

(-C) = solvent control
(+C) = chemical control

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V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound 06 was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate Tests

At a concentration of 5.0%, this chemical was not mutagenic for any of the bacterial indicator organisms in the direct or activation plate tests.

2. Non-activation Suspension Tests

These tests were all negative.

3. Activation Suspension Tests (Rat Tissues)

Original tests with this chemical produced increases in tests with liver tissue, using strains TA-1535 and TA-1537. Repeat tests with both strains were conducted at multiple dose levels. The results with TA-1537 were negative. The results with strain TA-1535 were again positive but only at very high dose levels (5.0%), which exhibited extreme toxicity. Results of this type are difficult to interpret.

B. Saccharomyces cerevisiae

1. Non-activation Suspension Tests

These tests were all negative.

2. Activation Suspension Tests (Rat Tissues)

These tests were all negative.

Conclusions

The mutagenicity of this chemical for indicator strain TA-1535 must be considered presumptive for the following reasons:

A. The positive response is only evident at high dose levels which generally result in low population survivals (high toxicity). Thus one cannot exclude some type of selection.

B. The data from activation plate tests does not indicate any activity. The activation plate tests were repeated and were negative in those tests as well.

David Brusick

David Brusick, Ph.D.
Director
Department of Genetics

Appendix

PLATE TEST RESULTS

COMPOUND D6

DATE 12-16-74

Test	Species	Tissue	TA-1538 1	TA-1538 2	TA-1537 1	TA-1537 2	TA-1535 1	TA-1535 2
1. Non-activation								
Solvent Control	---	---	6	3	11	8	13	10
Positive Control[a]	---	---	>1000	>1000	>500	>500	>100	>100
D6 5.0%	---	---	0	4	8	10	12	9
2. Activation								
Negative Control	---	---	4	6	9	14	11	7
Solvent Control	---	---	6	6	10	8	15	12
Reaction Mixture Control	---	---						
Positive Control[b]	Mouse	Liver	>500	>500	>100	>100	>500	>500
Positive Control		Lung	8	5	11	9	14	16
Positive Control		Testes	3	2	8	10	9	9
Positive Control	Rat	Liver	>500	>500	>100	>100	>500	>500
Positive Control		Lung	6	6	11	12	13	17
Positive Control		Testes	7	3	11	8	8	16
Positive Control	Monkey	Liver	>500	>500	>100	>100	>500	>500
Positive Control		Lung	9	3	19	8	14	14
Positive Control		Testes	2	10	8	10	13	7
D6 5.0%	Mouse	Liver	2	4	6	8	15	10
D6 5.0%		Lung	2	0	9	9	9	11
D6 5.0%		Testes	1	1	9	10	17	12
D6 5.0%	Rat	Liver	6	4	10	9	12	14
D6 5.0%		Lung	3	3	8	6	8	16
D6 5.0%		Testes	4	4	8	11	17	23
D6 5.0%	Monkey	Liver	1	1	8	9	9	13
D6 5.0%		Lung	0	6	12	8	17	17
D6 5.0%		Testes	2	3	10	11	-	15

[a]

TA-1535	EMS	10 µl/plate	
TA-1537	9AA	20 µg/plate	
TA-1538	NF	100 µg/plate	

[b]

TA-1535	DMNA	25 µm/plate
TA-1537	AAF	100 µg/plate
TA-1538	AAF	100 µg/plate

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Non Activation: Solvent Control NAP = Non Activation: Positive Control NA1 = Non Activation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control A-C = Activation: Solvent Control ACP = Activation: Positive Control ACT = Activation: Test Compound LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2 PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = X 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = X 10^0). For strain D4, MUT 1 represents the number of ADE^+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY^+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE^+ value.
FREQ 2	Only used for strain D4 and, represents the TRY^+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethyl Methanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (*Macaca mulatta*)

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT DON

EXPERIMENT 436001 DETECTOR TA1535 SPECIES PROJECT 02506

DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SALINE	0600	0018	3.00	0
	NAP		EMS 0.05 %	0612	0331	54.08	0
D06	NA1		0005-0 PCT.	0606	0021	3.48	0
D06	NA2		0025-1 PCT.	0569	0005	0.88	0

120000

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT ODN
EXPERIMENT 436002 DETECTOR TA1537 SPECIES PROJECT 07506
DATE - 01/20/75

COMPOUND	TEST	SRG ID	CONCENTRATION	PRPM1 EP+6	PRIT1 EP+0	FREQ1 EP-8	CONTAM
	MAN		SALINE	0361	0059	16.34	0
	ENP		ON 0.01 MG/ML	0141	0232	164.54	0
006	NA1		0005-0 PCT.	0792	0023	2.90	0
006	NA2		0025-1 PCT.	0408	0036	8.82	0

220000

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 000
EXPERIMENT 436401 DETECTOR TA1S 3 SPECIES PROJECT 02506
DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAS		DMSO	0527	0041	7.78	0
	NAP		MF 1.25 MG/ML	0332	0338	101.81	0
D06	NA1		0005-0 PCT.	0542	0060	11.07	0
D06	NA2		0025-1 PCT.	0511	0046	9.00	2

000023

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 00N
EXPERIMENT 436101 DETECTOR 000004 SPECIES PROJECT 02506
DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	[illegible]		SALINE	0895	0051	0184	5.70	20.56	1
	MA9		EMS 1.0 %	0892	0420	0310	52.37	38.65	0
006	MA1		0045-1 PCT.	0804	0063	0109	7.84	13.56	0
006	MA2		0225-2 PCT.	0873	0059	0100	6.76	20.62	0

420000

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 80M

EXPERIMENT 435101 DETECTOR TA1535 SPECIES SPROAM PROJECT 02506 DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ! EP-6	CONTAM
	A+C		DMM 25 UM/ML	1041	0026	2.50	2
	A-C		SALINE	1431	0014	0.98	0
	ACP	LI	DMM 25 UM/ML	1380	0377	27.32	2
	ACP	LU	DMM 25 UM/ML	1331	0018	1.37	2
	ACP	TE	DMM 25 UM/ML	1480	0022	1.49	2
D06	ACT	LI1	0005-0 PCT.	0727	0322	44.29	2
D06	ACT	LI2	0025-1 PCT.	0239	0017	7.11	2
D06	ACT	LU1	0005-0 PCT.	0378	0009	2.38	2
D06	ACT	LU2	0025-1 PCT.	0874	0025	2.86	0
D06	ACT	TE1	0005-0 PCT.	1037	0029	2.80	2
D06	ACT	TE2	0025-1 PCT.	1233	0033	2.68	2

000025

REPEAT DOSE RESPONSE TEST FOR D6 USING
RAT LIVER MICROSOME FRACTIONS AND INDICATOR TA-1535

COMPOUND	TEST	ORG ID	CONCENTRATION	FREQ. X 10^{-8}	PERCENT SURVIVAL
	A-C	-	DMSO	3.49	100
D6	ACP	LI	0.313%	1.39	93
D6	ACP	LI	0.625%	1.46	100
D6	ACP	LI	1.25%	2.67	100
D6	ACP	LI	2.5%	6.42	37
D6	ACP	LI	5.0%	142.86	1

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 004 PROJECT 02506
EXPERIMENT 435701 DETECTOR TA1537 SPECIES SPRDAW DATE - 01/20/75

COMPOUND	TEST	DOS ID	CONCENTRATION	POPU EP=0	MUTI EP=0	FREQ1 EP=0	CONTAM
	A+C		AAF 0.31 MG/ML	1155	0056	4.85	3
	A-C		DMSO	1351	0027	2.00	2
	ACP	LI	AAF 0.31 MG/ML	1460	0108	7.40	2
	ACP	LU	AAF 0.31 MG/ML	1397	0025	1.79	2
	ACP	TE	AAF 0.31 MG/ML	1119	0079	7.06	0
D06	ACT	LI1	0005-0 PCT.	0000	0000	******	1
D06	ACT	LI2	0025-1 PCT.	0000	0000	******	3
D06	ACT	LU1	0005-0 PCT.	0000	0000	******	0
D06	ACT	LU2	0025-1 PCT.	0000	0000	******	2
D06	ACT	TE1	0005-0 PCT.	0000	0000	******	2
D06	ACT	TE2	0025-1 PCT.	0000	0000	******	2

000027

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT DOW
EXPERIMENT 436505 DETECTOR TA1537 SPECIES SPRDAW PROJECT 02506 DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-0	CONTAM
	A-C		DMSO	0958	0015	1.57	0
D06	ACT	LI1	0005-0 PCT.	0480	0097	20.21	0
D06	ACT	LI2	0025-1 PCT.	0402	0113	28.11	0
D06	ACT	LU1	0005-0 PCT.	0487	0005	1.03	0
D06	ACT	LU2	0025-1 PCT.	0622	0010	1.61	0
D06	ACT	TE1	0005-0 PCT.	0629	0010	1.59	0
D06	ACT	TE2	0025-1 PCT.	0466	0020	4.29	0

8202028

REPEAT DOSE RESPONSE TEST FOR D6 USING
RAT LIVER MICROSOME FRACTIONS AND INDICATOR TA-1537

COMPOUND	TEST	ORG ID	CONCENTRATION	FREQ. X 10^{-8}
	A-C	-	DMSO	0.68
D6	ACP	LI	0.625%	1.34
D6	ACP	LI	2.5%	1.33
D6	ACP	LI	5.0%	1.67

BIONETICS

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT DOH

EXPERIMENT 435201 DETECTOR TA1538 SPECIES SPRDAW PROJECT 02506 DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AAF 0.31 MG/ML	1657	0112	6.76	0
	A-C		DMSO	1381	0134	9.70	0
	ACP	LI	AAF 0.31 MG/ML	0951	0390	41.01	?
	ACP	LU	AAF 0.31 MG/ML	0954	0096	10.06	2
	ACP	TE	AAF 0.31 MG/ML	1084	0109	10.06	?
D06	ACT	LI1	0005-0 PCT.	1403	0100	7.13	2
D06	ACT	LI2	0025-1 PCT.	0785	0039	4.97	0
D06	ACT	LU1	0005-0 PCT.	1047	0107	10.22	?
D06	ACT	LU2	0025-1 PCT.	0344	0020	5.81	0
D06	ACT	TE1	0005-0 PCT.	1114	0122	10.95	?
D06	ACT	TE2	0025-1 PCT.	0749	0052	6.94	?

000030

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT DOW
EXPERIMENT 43540I DETECTOR 000004 SPECIES SPROAM PROJECT 02506
DATE - 01/20/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 150 UM/ML	0871	0038	0185	4.36	21.24	1
	A-C		SALINE	1077	0034	0157	3.16	14.58	0
	ACP	LI	DMN 150 UM/ML	087.	0057	0172	6.54	19.75	0
	ACP	LU	DMN 150 UM/ML	0961	0052	0156	5.41	14.15	0
	ACP	TE	DMN 150 UM/ML	0784	0032	0155	4.08	19.77	0
D06	ACT	LI1	0045-1 PCT.	0710	0041	0146	5.77	20.56	4
D06	ACT	LI2	0225-2 PCT.	0735	0037	0085	5.03	11.56	0
D06	ACT	LU1	0045-1 PCT.	0728	0031	0130	4.26	17.86	0
D0L	ACT	LU2	0225-2 PCT.	0008	0000	0000	0.00	0.00	0
D06	ACT	TE1	0045-1 PCT.	0673	0037	0132	5.50	19.61	0
D06	ACT	TE2	0225-2 PCT.	0011	0000	0000	0.00	0.00	0

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Chemical Name (300 per name)	CAS No. (10)
CHLOROTHENE NU	71-55-6

A.K 5/27/83 1A

878210697

Biochemical Research Laboratory

The Dow Chemical Company

Midland, Michigan

RECEIVED
OCT 20 1967

A COMPARATIVE STUDY OF THE EFFECT OF INHALED WOLY FUR SPRAY AND ITS INGREDIENTS ON LABORATORY RATS

File:

Chg:

Sub. by: A. W. Schaffer
Rept. by: T. R. Torkelson

Signed T R Torkelson Date April 2, 1968

Checked Bent K. J. Lange Date April 5, 1968

PROBLEM

Alleged human illness and discomfort were reported in Europe following use of Woly Fur Spray for treating shoes and clothing. An earlier study (wire from A. W. Schaffer to M. J. Blankenship, February 23, 1967) had shown that the spray was markedly injurious to the lungs of rats and presented a considerable hazard. The spray contains several ingredients including Chlorothene NU.* Hence, the possible role of Chlorothene NU in the toxicity of the mixture needed to be evaluated.

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RESULTS

1. Under the conditions of this study, Woly Fur Spray was again shown to be markedly injurious to the lungs of laboratory rats. Omission of either Chlorothene NU or one other ingredient, a fluorocarbon, markedly reduced the injury.

2. Chlorothene NU should not be used in Woly Fur Spray.

3. This study illustrates that synergistic effects of Chlorothene NU in aerosol formulations are possible. Hence, these must be considered and all complete formulations containing this solvent or Aerothene TT* should be evaluated by the producer.

METHOD

Exposures

The procedure used in this study was a rather severe test recommended on pages 58 and 59 of SAFETY OF CHEMICALS IN FOOD, DRUGS & COSMETICS.[1]

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Five male rats weighing 250-350 grams each were exposed under static conditions to an aerosol of the test material. With the exception of Sample #10 exposures were of four hours or one hour duration. (Animals exposed to Sample #10 received seven and three hour exposures instead of four and one hour exposures.) Repeated 30-second releases from the test can were made into the test chamber at 30-minute intervals. During the first 14.5 minutes after the 30-second release, no air was moved through the chamber. During the next 15 minutes the chamber was flushed with room air. The procedure was then repeated until the exposure period was complete. The animals exposed four and one hour were exposed simultaneously by adding the second group one hour before the end of the exposure.

The animals were observed for anesthesia, nasal discharge and other effects during the exposure period. Following the last exposure the animals were returned to their cages and frequently observed for latent effects. Animals that died during exposure or immediately thereafter were necropsied as soon as possible with particular attention given to gross changes in the lungs and respiratory tract. Where indicated occasional other rats were sacrificed for

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necropsy, all rats that survived two weeks after exposure were necropsied and gross observations made.

Test Material

Propellant 12 was present in all formulations. The complete formulation for Woly Fur Spray is confidential. It contains 47.3% Chlorothene NU and five other ingredients in addition to Freon 12. Four of these ingredients are hydrophobic materials and the fifth is a perfume at a concentration of 0.04%. Two samples of the complete formulation were studied. The original sample was received in February 1967; the second samples was received December 7, 1967. Samples 2-6 omitted one of the five ingredients. Sample 7 used propellant 11 as a solvent rather than Chlorothene NU. Sample 8 used a petroleum solvent instead of Chlorothene NU. Samples 10, 11 and 12 contained only the fluorocarbon and either Chlorothene NU (sample 10), propellant 11 (sample 11), or hexane (sample 12). The composition of the samples is listed in Tables 1 and 2.

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RESULTS

Results of four hour exposure are given in Table 1 and the results of one hour exposure in Table 2. It is obvious that Chlorothene NU had its expected anesthetic action. Anesthesia was not observed with Freon 11, with the petroleum solvent, or with hexane. Little other injury can be attributed to the solvents alone, but the following observations indicate that a complex interrelationship of activities exists between Chlorothene NU and the fluorocarbon.

1. The response of animals exposed for four hours (ten applications of spray) to the original sample and sample #1 were similar and very severe. The animals were quickly anesthetized and did not readily recover from their anesthesia. There were many deaths during or immediately after exposure. Necropsy of the dead animals revealed extensive edema and hemorrhage in the lungs. Delayed deaths which occurred throughout the 11 days following exposure appeared to be the result of pulmonary damage and respiratory failure. The livers

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and kidneys also showed some damage in these animals, but the effect on these organs may have been secondary to the severe pulmonary injury.

2. The response of animals exposed to Samples 2, 3, 4, and 6 were similar to those observed in the original sample and sample #1, except that the degree of anesthesia seemed to have been less. However, there were fewer delayed deaths during the first post-exposure week and practically none in the second post-exposure week. The body weights of the animals dropped considerably following exposure and did not return to the initial weights by the end of the two week observation period. Pulmonary injury was far less serious than with sample 1 and was not marked by severe hemorrhage and edema.

3. The response of animals to samples 5, 9 and 10 were similar and differed from those described for the original sample and samples 1, 2, 3, 4, and 6. The animals were moderately anesthetized, but recovered rapidly from their anesthesia. Furthermore, these samples did not cause any deaths and there was either

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no or a very slight loss of body weight. By the end of the 14-day observation period, the body weights in general exceeded the initial weight of the animals and pathology in the lungs and other organs was not remarkable.

4. The animals exposed to sample 7, 8, 11, and 12 did not become anesthetized. Delayed deaths occurred in animals exposed to sample 7 but not to samples 8, 11 and 12. There was a loss of body weight immediately after exposures, but survivors regained and exceeded their initial body weight by the end of the observation period. Pathological changes in the lungs were minimal.

5. Based upon these experimental results, the observed deaths and severe injury must have been due to a joint action which was by far the most severe when both the fluorocarbon and Chlorothene NU were present in the complete formulation. When either one or the other was omitted, samples 5 and 9 the severe response in the lungs did not occur. Neither did it occur when only Chlorothene NU and the fluorocarbon were present, sample 10.

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As seen in Table 2, similar reactions occurred in animals exposed for a shorter duration to the test material (two applications in one hour). The primary differences were an absence of immediate deaths and a more rapid regaining of lost body weight.

We should make every effort to get Chlorothene NU out of the Woly Fur Spray formulation. The formulation prepared in petroleum solvent appears to present the least hazard but I suggest the Manufacturer have the toxicity of any formulation evaluated extensively.

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REFERENCES

1. Association of Food & Drug Officials of the United States, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Edited and Published by The Editorial Committee of the Association of Food and Drug Officials of the United States, 2nd printing, 1967, p. 38-59.

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RESULTS OF TEN EXPOSURES OF MALE RATS TO WOLY FUR SPRAY AND MODIFIED FORMULATIONS

Test Material Pressurized with Propellant 12	Average Conc. At Each Appl. mg/l	Mortality		Observation During Exposure		Condition of Lungs at Necropsy		
		No. Rats Died/ Exposed	No. Days Between Exposure & Death	Anes-thesia	Respira-tory Distress/ Nasal Discharge	Post-Exposure Or Next Day	During 1st Week	During 2nd Week
0. Original Spray Received 2-67	121	5/5	0,4,7, 9,11	Yes	Yes	Hemorrhage Edema	Congested	Congested
1. Same as original. Received 12/67	141	5/5	0,0,1, 3,10	Yes	Yes	Hemorrhage	—	—
2. Ingredient #1 omitted	96	5/5	1,3,3, 7,10	Yes	Yes	—	—	—
3. Ingredient #2 omitted	144	3/5	2,2,2	Yes	Yes	Hemorrhage Edema	—	Pneumonia
4. Ingredient #3 omitted	139	3/5	0,2,6	Yes	Yes	—	—	Congested
5. Fluorocarbon omitted	105	0/5	—	Slight	No	—	—	NVL
6. Perfume omitted	106	3/5	2,2,6	Yes	Yes	Hemorrhage Congested	—	Pneumonia
7. Substitution of Propellant 11 for Calorothene MU	116	3/5	3,3,7	No: Hyper-active	Yes	—	Congested	Grey in Color

CCCO11

RESULTS OF TEN EXPOSURES OF MALE RATS TO MOLY FUR SPRAY AND MODIFIED FORMULATIONS

Test Material Pressurized with Propellant 12	Average Conc. At Each Appl. mg/l	Mortality		Observation During Exposure		Condition of Lungs at Necropsy		
		No. Rats Died/ Exposed	No. Days Between Exposure & Death	Anesthesia	Respiratory Distress/ Nasal Discharge	Post-Exposure Or Next Day	During 1st Week	During 2nd Week
8. Substitution of Propellant 11 for Chlorothene NU	99	0/5	—	No	No	—	—	NVL*
9. Chlorothene NU	98	0/5	—	Slight	No	—	—	NVL
10. 1% Fluorocarbon* in Chlorothene NU	104	0/3	—	Yes	No	Questionable; old lesions not related to exposure	—	NVL
11. 1% Fluorocarbon for Propellant 11	1[illegible]	0/4	—	Slight	No	NVL	—	NVL
12. 1% Fluorocarbon in hexane	94	0/4	—	No	No	NVL	—	NVL

*Seven hours of exposure; 14 exposures.

*NVL = No Visible Lesions.

000012

RESULTS OF FOUR EXPOSURES OF MALE RATS TO WOLY FUR SPRAY AND MODIFIED FORMULATIONS

Test Material Pressurized with Propellant 12	Average Conc. At Each Appl. mg/l	Mortality		Observation During Exposure		Condition of Lungs at Necropsy		
		No. Rats Died/ Exposed	No. Days Between Exposure & Death	Anes-thesia	Respira-tory Distress/ Nasal Discharge	Post-Exposure Or Next Day	During 1st Week	During 2nd Week
0. Original spray received 2/67	121	3/5	1,1,1	Yes	Yes	—	—	Fibrotic pneumonia adhesions
1. Same as original received 12/67	141	5/5	3,3,3, 4,12	Yes	Yes	Congested Hemorrhage	—	Congested
2. Ingredient #1 omitted	96	2/5	3,3	Yes	No	—	—	NVL
3. Ingredient #2 omitted	144	4/5	1,2,2,8	No	Yes	—	Congested	Congested
4. Ingredient #3 omitted	139	3/5	1,3,10	Yes	Yes	—	Congested	Fibrotic pneumonia
5. Fluorocarbon omitted	105	0/5	—	No	No	—	—	NVL
6. Perfume omitted	106	3/5	—	Yes	Yes	—	Congested	NVL
7. Substitution of Propellant 11 for Chlorothene NU	116	0/5	—	No	No	—	—	Question-able pneumonia

000013

RESULTS OF FOUR EXPOSURES OF MALE RATS TO WCLY FOR SPRAY AND MODIFIED FORMULATIONS

Test Material Pressurized with Propellant 12	Average Conc. At Exact Appl. mg/l	Mortality		Observation During Exposure		Condition of Lungs at Necropsy		
		No. Rats Died/ Exposed	No. Days Between Exposure & Death	Anesthesia	Respiratory Distress/ Nasal Discharge	Post-Exposure Or Next Day	1st Week	2nd Week
8. Substitution of Petroleum solvent for Chlorothene NU	99	0/5	—	No	No	—	—	NVL*
9. Chlorothene NU	96	0/5	—	No	Slight	—	—	Chronic pneumonia
10. 1% Fluorocarbon in Chlorothene NU*	104	0/4	—	Slight	No	NVL	—	NVL
11. 1% Fluorocarbon in Propellant 11	139	0/4	—	No	No	NVL	—	NVL
12. 1% Fluorocarbon in hexane	94	0/4	—	No	No	NVL	—	NVL

*Three hours of exposure; 6 exposures.

*NVL = No Visible Lesions.

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OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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EFFECTS OF REPEATED INHALATION OF AEROSOLS OF CHLOROTHENE NU, CHLOROTHENE NU PLUS FC 310 RESIN, XYLOL, AND XYLOL PLUS FC 310 RESIN

Chemical Name (300 per name) 25	CAS No. (10) 24
CHLOROTHENE NU	71-55-6

A.K. 5/27/82

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EFFECTS OF REPEATED INHALATION OF AEROSOLS OF CHLOROTHENE® NU, CHLOROTHENE® NU PLUS FC 310 RESIN, XYLOL, AND XYLOL PLUS FC 310 RESIN

RELEASED

By

Leong, B. K. J., P. B. Conran, R. A. DeGesero
and T. R. Torkelson

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PROBLEM

Clinical cases of respiratory distress and liver intoxication among workers exposed to aerosols escaping from solvent-fluorocarbon resin spray coating operations were reported from several fabric and shoe factories in Europe.[1,2] The solvent most often employed was CHLOROTHENE NU from Dow and the fluorocarbon resin was identified as FC 310 resin from 3M company of St. Paul, Minnesota.

Two earlier investigations on Woly Fur Spray and a series of modified formulations with or without FC 310 and CHLOROTHENE NU indicated that, in general, the presence of both materials in an aerosol produced narcosis and pulmonary injury in the experimental animals.[3,4]

The present problems are:

I. To determine whether the injurious effects reported in European factories were indeed caused by FC 310, CHLOROTHENE NU, or both materials.

II. To determine the dose-response relationship between the toxic ingredient(s) and the mortality of the animals.

III. To determine the type of pathological changes caused by the material(s) in the experimental animals.

IV. To investigate possible mechanisms of toxic effects on the experimental animals.

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CONCLUSIONS

I. Cause of Injurious Effects

(1) Exposure of rats to CHLOROTHENE NU aerosols up to the concentration of 6.6 mg/l caused only slight narcosis. This aerosol concentration was equivalent to 1200 ppm of the vapors of CHLOROTHENE NU.

(2) Exposure to an equivalent aerosol generated from CHLOROTHENE NU containing 1% w/w of FC 310 resin resulted in narcosis, pulmonary injury and death.

(3) Exposure to an aerosol of FC 310 resin which was generated from a solution of 1% w/w of resin in xylol* resulted also in pulmonary injury and death. (The xylol vaporised readily leaving behind the resin particulates in the chamber atmosphere).

(4) Exposure to xylol vapor at an equivalent concentration (1200 ppm) did not cause any adverse effect other than very slight narcosis.

Since exposures of animals to CHLOROTHENE NU aerosol and xylol vapors did not produce any serious adverse effect, the pulmonary injury and deaths which occurred in the combination exposure in (2) and (3) were therefore considered to be caused primarily by the FC 310 resin.

*Xylol is a mixture of ortho, meta and para xylene $C_6H_4(CH_3)_2$ dimethylbenzenes with the meta isomer (1,3 dimethylbenzene) as the chief constituent.

II. Dose-response Relationship

(1) There was a definite dose-response relationship between FC 310 and the mortality of animals. However, there was no such relationship for CHLOROTHENE NU and xylol under the present experimental conditions.

(2) The critical (Ct), which will be defined as the magnitude of exposure, expressed in terms of the product of concentration and time, at which dramatic alteration of trends of dose-mortality response occurred, was obtained by graphical interpolations and were found to be approximately 0.6 to 0.7 day• mg/l for FC 310 resins.

(3) No Threshold Limit Value, and no-effect level for FC 310 resin toxicity was obtained under the experimental conditions employed. However, the data suggested that the value was below 0.06 day•mg/l.

III. Pathological Changes

(1) Gross pathological examination revealed acute pulmonary edema and hemorrhage in the animals which succumbed after exposures to CHLOROTHENE NU or xylol plus FC 310 resins. However, no compound-related lesion was observed in the survivors of these same treatments.

(2) Histopathological examination of the lungs of the animals, which succumbed after inhalations of

aerosols containing the FC 310 resins, revealed exudative pneumonia. The pathology was characterized by edema, congestion, hemorrhage and the presence of macrophages and polymorphonuclear inflammatory cells in the alveoli. In the survivors of the FC 310 treated animals, the CHLOROTHENE NU aerosols and the xylol vapor exposed animals, the histological changes were comparable to those of the control animals, and were attributed to chronic murine pneumonia.

(3) No noteworthy histopathological condition was observed in other tissues in any group of animals. Particulate matter, which were morphologically and tinctorially similar to FC 310 resin particles, was detected in the cytoplasm of occasional macrophages in the lungs of rats exposed to the resin.

(4) Hemograms for animals in various treatment groups were comparable to those of the control animals.

(5) Clinical evaluation of liver function, using serum glutamic pyruvic transaminase (SGPT) levels, revealed no significant difference between the various compound exposed and the control animals.

IV. <u>Possible Mechanisms of Toxic Effects</u>

(1) Several egg-white injury, "sensitization", tests (which causes libration of endogenous histamine) were attempted. In the resin treated animals, the tests

resulted in an isolated case of anaphylactic shock, suggesting the possibility of increases susceptibility following inhalation of FC 310 resin.

(2) Animals which were pretreated with injection of egg-white were rendered more susceptible to the action of inhaled FC 310 resin.

(3) Respiratory failure as the result of mechanical blockage of the respiratory tract could be discarded as a cause of death because no sizable quantity of material was observed in the respiratory passages.

(4) Alteration of the surface active properties of the lung tissue by this hydrophobic resin warrants further investigation.

METHODS

A. Experimental Design

The general experimental design consisted of three sets of experiments shown in the following table.

Experiments	Animal Groups	Exposures
I.	1. Control	Filtered room air
	2. CHLOROTHENE NU	CHLOROTHENE NU aerosol
	3. CHLOROTHENE NU plus FC 310	CHLOROTHENE NU plus FC 310 (1% w/w) aerosol
II.	1. Control	Filtered room air
	2. Xylol	Xylol vapor and fine aerosol
	3. Xylol plus FC 310	Xylol vapor plus FC 310
III.	Egg-white injury "Sensitization" tests	Intraperitoneal injection of fresh egg-white in a portion of the animals from experiment II.

In Experiment I, 90 male rats of Sherman strain, weighing approximately 100 grams each were statistically randomized into three equal groups. The treatment for each group was also assigned at random. Prior to the exposure, base lines of animal body weight and hematological parameters including routine cell counts, hemoglobin concentration, hematocrit and SGPT levels were determined. During the exposures, the body weights were followed three times weekly and the hematological studies were conducted after 1, 3, 20 and 60 days of exposures. The duration of exposure was six hours per day and five days per week. Animal behavior and health were observed carefully throughout the studies. Animals which succumbed during the experiment

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were necropsied for gross and microscopic examination. Similar examinations were performed on animals which were sacrificed at the termination of the scheduled 65-day period.

The surviving animals from each group were starved overnight prior to necropsy. The animals were anesthetized with one and one-half (1.5) cc of SOMNOPENTYL® and the tracheas were ligated immediately caudal to the larynx. The rats were then killed by decapitation. All major organs with the exception of the brain were examined grossly. The lungs, livers, kidneys, and testes were weighed prior to sectioning for determination of organ to body weight ratios. Sections of trachea, lungs, liver, kidneys, adrenals, stomach, terminal colon, urinary bladder, and gonads were saved, fixed in neutral buffered formalin, and processed in the usual manner for histopathology examination (H & E stain).

The exposure schedule, i.e., the number of days during which the animals were exposed to a given concentration of CHLOROTHENE NU and CHLOROTHENE NU plus FC 310 are presented in the following table. It can be seen that the exposure concentration was increased in five steps. Because some of the solvent was inhaled as vapor and some as aerosol, the concentrations are given in mg/l with the equivalent concentration in ppm by volume.

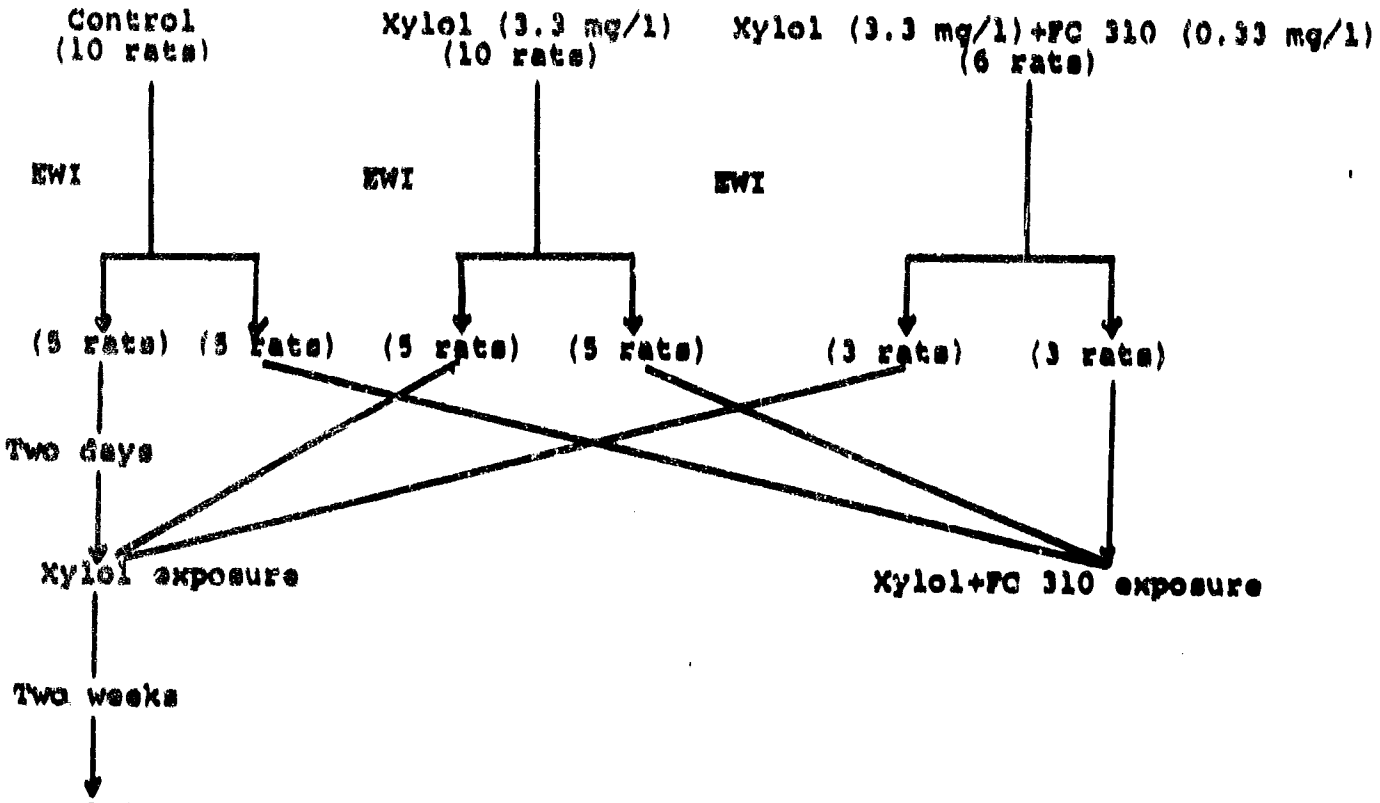
	Group 1	Group 2		Group 3	
Exposure Duration (Days)	Filtered room air	CHLOROTHENE NU Chamber mg/l ≈ ppm		CHLOROTHENE NU plus FC 310 resin CHLOROTHENE NU mg/l	FC 310 mg/l
25	0	0.6	100	0.6	0.006
5	0	1.1	200	1.1	0.011
10	0	1.7	300	1.7	0.017
20	0	3.3	600	3.3	0.033
2.5	0	6.6	1200	6.6	0.066

In Experiment II the experimental procedures were essentially similar to those carried out in Experiment I. The exceptions were: (1) older animals having age and weight corresponding to animals in Experiment I after eight weeks of exposures were used; (2) the exposure concentrations administered throughout the six week studies were 3.30 mg/l (≈690 ppm) for xylol and 0.033 mg/l for FC 310.

For both Experiments I and II, the relationship between mortality and the cumulative (Ct) was followed daily.

In Experiment III, the egg-white injury "sensitization" tests,[5,6,7,8,9] the experimental animals were obtained from Experiment II at the termination of the exposure experiments. Ten animals from the control and xylol groups together with six of the ten survivors from the xylol plus FC 310 group were used. Each animal was injected intraperitoneally with 1 ml of fresh egg-white and observed for acute anaphylactic response.

Animals which did not show anaphylaxis were kept for two days and then regrouped for another exposure to xylol vapor or xylol plus FC 310 aerosols as shown below.



EWI = Egg-white injury

B. Exposure and Analytical Techniques

Exposure Chamber. All exposures were conducted in stainless steel and glass Rochester type chambers of 1000 liter volume. In each chamber a constant airflow was maintained by means of a rotary pump connected to the exhaust pipe at the bottom of the chamber. The rates of airflow ranged from 100-250 liters per minute depending on the required chamber concentrations. The air was filtered through Cambridge® Absolute filters prior to entering the chambers.

Aerosol Generation. Each type of aerosol was generated at a calculated rate by means of a positive pressure spray nozzle. Each solvent or 1% FC 310 solution was metered into the spray nozzle with a precision syringe feeder. The aerosols were ejected from a spray nozzle located at the geometric center of the chamber. The aerosols circulated upward toward the air inlet at the top of the chamber, where further dispersion by the incoming filtered room air took place. The diluted aerosols then descended, passing the animals, to the exhaust. Uniformity of aerosol distribution in the chamber was verified by particle size analyses of air samples taken at various locations within the chamber.

Chamber Concentration Analyses. The nominal concentration of each material was calculated from the ratio of the rate of compound delivery into the spray nozzle to the rate of total airflow (volume of air ejected through the spray nozzle plus the volume of makeup air drawn through the chamber via the

air inlet). The analytical concentrations of CHLOROTHENE NU were obtained by using a calibrated Davis halide meter,[10] and those of the FC 310 were determined gravimetrically using a Millipore filter sampling technique. In the xylol plus FC 310 studies, only the FC 310 concentration was analytically determined. All analyses were carried out daily.

Particle Size Analyses. Aerosol particle size analyses were obtained daily by using a Royco Particle Analyser[11] (a light-scattering measuring instrument) and spot-checking with a microscopic counting technique. All calculations of arithmetic mean diameter, and geometric mean diameter from the size distribution data were computerized.

RESULTS

Experiment I: Air Exposed Control, CHLOROTHENE NU, CHLOROTHENE NU + FC 310 (1% w/w)

Physical Parameters

Chamber Concentrations. The analytical concentrations of CHLOROTHENE NU in the CHLOROTHENE NU chamber and the CHLOROTHENE NU + FC 310 chamber which was spot-checked routinely with a Davis halide meter were found to be within ±10% of the nominal concentrations.

Aerosol Particle Size Analyses. Particle size analyses of the chamber atmosphere with a Royco Particle Analyser at various experimental time points showed the following arithmetic mean diameters in microns.

CHLOROTHENE NU		Chambers		
mg/l	ppm	CHLOROTHENE NU μ	CHLOROTHENE NU + FC310 μ	Control (FR) μ
0.6	100	0.8 (23)	1.00 (23)	0.2 (32)
1.1	200	0.7 (6)	1.4 (6)	
1.7	300	0.7 (3)	2.1 (4)	
3.3	600	-	2.1 (1)	
6.6	1200	2.1 (2)	2.2 (2)	

( ) = Number of analyses
(FR) = Filtered room air

The data indicated that (1) at low concentrations the mean particle size of FC310 aerosols was larger than the CHLOROTHENE NU aerosols; (2) at high concentrations (6.6 mg/l or ≈1200 ppm), the mean particle diameters for both materials were of the same magnitude; (3) submicron particles of 0.2 μ and smaller were present in the chambers despite absolute filters in the inlet air stream.

Biological Parameters

Appearance, Behavior and Growth of Animals. During exposures, both the CHLOROTHENE NU and the CHLOROTHENE NU plus FC310 groups of animals became lethargic and crouched motionlessly together. However, all animals remained responsive to light and noise throughout the exposure period. Upon removal from the chamber at the termination of each exposure, the animals rapidly exhibited vivacity comparable to the control animals. The

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control and CHLOROTHENE NU animals were normal in appearance after each exposure session throughout the 12-week studies. However, in the CHLOROTHENE NU plus FC 310 animals, many deaths occurred. (The terminal events of death will be further described). Because the deaths occurred rapidly with little loss of body weight, the mean body weights of the survivors were not affected appreciably and thus no difference in growth between the control and the treated groups is apparent in Figure 1 and Table I.

Hematology and Clinical. Examination of red, white and differential cell counts, together with hematocrit, hemoglobin concentration and SGPT levels at various time points did not show any significant differences between the control and the treated groups (Tables II and III).

Mortality. There were no deaths among the control animals and the animals exposed successively to increasing concentrations of CHLOROTHENE NU aerosols. The CHLOROTHENE NU plus FC 310 animals, however, exhibited the following events (Figure 1, Table IV). There was no adverse effect detected until approximately the 10th exposure day when a few animals acquired respiratory rales. The body weight of these animals remained normal for several days and then suddenly decreased 10-20 grams within a day. The affected animals then died of acute respiratory failure the following day. Death was preceded by several hours of shallow and rapid respiration and nasal discharge. Three animals died but the remaining animals appeared normal until the chamber concentration was raised.

The same death and survival pattern was observed in each subsequent elevation of chamber concentrations up to 0.033 mg/l of FC 310 in 600 ppm of CHLOROTHENE NU.

During the above 10-week period, a total of seven animals succumbed. When the chamber concentration was raised to 0.066 mg/l of FC 310 in 1200 ppm of CHLOROTHENE NU (cumulative Ct of FC 310 ≈0.74 day*-mg/l) most aniamls exhibited respiratory distress and the mortality rate suddenly increased. In 2.5 days of exposure (cumulative Ct of FC 310 ≈0.806 day-mg/l) five animals succumbed and nine more died within one week although exposures were terminated. A total of 21 animals (70%) in this group succumbed, compared to no deaths in the other two groups.

Organ to Body Weight Ratios. The terminal body weights, organ weights and the calculated organ to body weight ratios are presented in Table V. All parameters were similar between the three groups of animals except the spleen weight to body weight ratio of the CHLOROTHENE NU plus FC 310 group was significantly lower than the control value.

Gross necropsy. Examination of the animals which succumbed after exposures to aerosols of CHLOROTHENE NU containing FC 310 resins, revealed acute pulmonary edema, congestion and hemorrhage. The lungs were characterized by being plum-red and moist. Occasionally there were multi-focal, irregular pink foci on normal lung parenchyma noted on the surface. On cut sections white to yellow froth exuded from the major bronchi, while

*Day = 1 Day is equivalent to 6 exposure hours

serosanguinous fluid exuded from the parenchyma. Moderate, passive congestion of the liver, kidney and myocardium was noted in many, but not all of the animals that died spontaneously.

Comparable lesions were not observed in the air-exposed controls or the CHLOROTHENE NU controls. Other lesions, including chronic murine pneumonia, (CMP) were observed in all groups but were interpreted as being spontaneous, and not compound related. None of the surviving animals from any group exhibited pulmonary lesions other than those attributable to chronic murine pneumonia (CMP).

Histopathology. Microscopically, there was congestion, multi-focal areas of acute hemorrhage and diffuse pulmonary edema in animals which succumbed. The bronchi and bronchioles were cuffed with lymphocytes which were attributed to the early stages of CMP. Occasionally, polymorphonuclear inflammatory cells were noted in the bronchial walls and in two instances, an acute desquamative bronchitis was noted. The acute bronchial changes were interpreted as being compound related.

The alveoli were, for the most part, filled with proteinaceous fluid and there was occasional dilation of respiratory bronchioles. Varying numbers of alveolar macrophages were noted intimately associated with the alveolar wall and in the alveolar lumena. Often-times vast numbers of these macrophages, while being contiguous with the alveolar wall, appeared to coalesce and undergo degeneration, filling

the alveoli with necrotic debris. Polymorphonuclear inflammatory cells were occasionally noted co-existing with the macrophages, emphasizing the acuteness of the response.

Using paraffin imbedded tissues and staining them with Oil Red O, 2-3 micron, red birefringent crystals in the cytoplasm of occasional macrophages were observed. These crystals were morphologically compatible with, and possessed similar tinctorial properties to, FC 310 particles collected on glass slides placed on the chambers.

The remaining histological lesions observed in other organs were interpreted as being spontaneous in nature and not compound related.

Experiment II: Control, xylol and xylol plus FC 310 (1% w/w)

Physical Parameters

Chamber Concentrations. The nominal concentrations of xylol in the chamber were verified daily by measuring the volume of the liquid delivered by a precision syringe feeder.

Aerosol Particle Size Analyser. The results of particle size analyses with the Royco Particle Analyser and microscopic technique are as follows.

Xylol Conc.		Chambers					
		Xylol		Xylol + FC 310		Control	
mg/l	ppm	A.M.	G.M.	A.M.	G.M.	A.M.	
3.3	690	0.6 (20)	0.5	2.7 (20) [3.4*]	2.0 [2.5*]	0.2 (32)	

( ) = Number of analyses
A.M. = Arithematic Mean
G.M. = Geometric Mean
[ ] = Microscopic
* = Average from two counting analyses checks

The data indicate that aerosol particle sizes of xylol were much smaller than aerosols generated from xylol plus FC 310.

Biological Parameters

Appearance, Behavior and Growth of Animals. The responses of the animals to xylol and xylol plus FC 310 exposures were similar to those observed among the CHLOROTHENE NU and CHLOROTHENE NU plus FC 310 exposures. Examination of the weekly mean body weight changes indicated no effect of treatment. (Figure 2). It must be pointed out that (1) the animals used in this experiment were of the same age and weight range of the animals in Experiment I after the 8th exposure week, so that the xylol plus FC 310 animals would receive the same magnitude of dose (Ct/kg) of FC 310 as the CHLOROTHENE NU plus FC 310 animals when started at a higher FC 310 concentration of 0.033 mg/l; (2) the xylol animal body weights were slightly lower than the other two groups because they were slightly younger animals. (The original xylol group was killed by operational accident and

had to be replaced).

Hematology and Clinical. As in the CHLOROTHENE NU and CHLOROTHENE NU plus FC 310 animals, there was no compound related effects observed (Table VII).

Mortality. There was no death in either the control or the xylol aerosols exposed animals. The xylol plus FC 310 animals, however, succumbed acutely in a pattern similar to that of the CHLOROTHENE NU plus FC 310 animals (Figure 2, Table VIII). When the xylol plus FC 310 animals had received a total of approximately 0.615 day·mg/l of FC 310 (compared to an approximate 0.74 day·mg/l of FC 310 for the CHLOROTHENE NU plus FC 310 animals), the mortality rate was increased sharply. A total of 20 deaths (66.6%) occurred by the termination of the 6-week exposures.

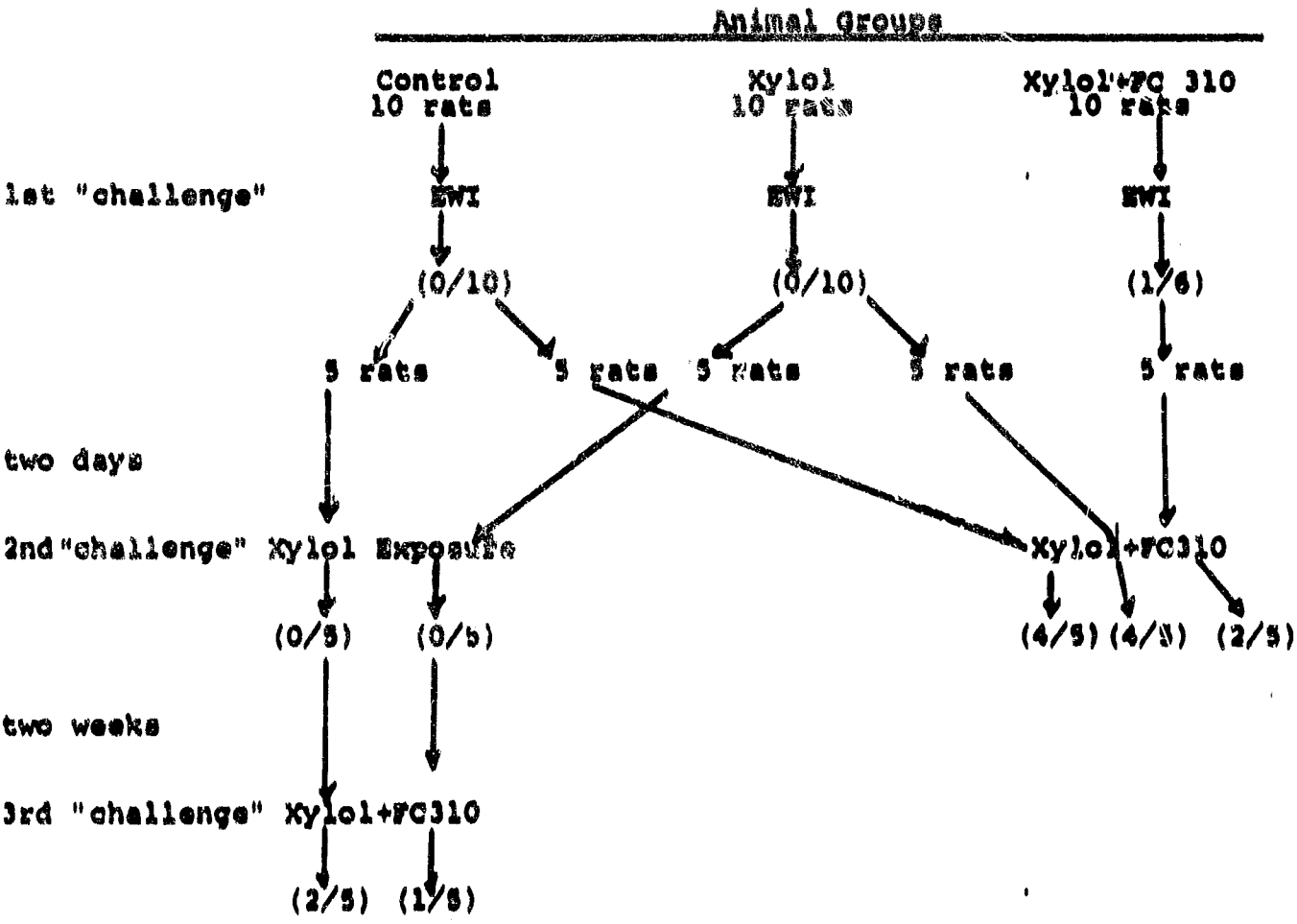
Gross necropsy. Necropsy of the animals, which succumbed of acute pulmonary distress, as indicated by pulmonary edema and hemorrhage, revealed lesions identical to those observed in CHLOROTHENE NU plus FC 310 animals. Examinations of the survivors of the xylol plus FC 310 and xylol animals which were sacrificed did not reveal any abnormal deviation from the control animals.

Histopathology. Microscopic examination of the tissues from animals which succumbed or were sacrificed revealed similar alterations as those seen in Experiment I.

Organ to Body Weight Ratios. All organ weight and their ratios to the tested body weights were comparable between the control and the treated animals (Table IX).

Experiment III: Egg-White Injury "Sensitization" Tests

The results of the various "sensitization" tests, using mortality as the criterion of response, can be summarized in the following diagram:



EWI = Egg-white injury

(1) = Number of deaths/number of animals per group

After a two-day period, the egg-white injected animals from the control and xylol groups together with the egg-white injection survivors from the xylol plus FC 310 group were re-exposed to xylol or xylol plus FC 310 at the same concentration as in Experiment II. All animals survived the xylol exposures whereas most animals succumbed to xylol plus FC 310 exposure. Thus, the animals which received egg-white injury appeared more susceptible to the toxic effect of FC 310.

DISCUSSION

Inhalation Toxicity

Vapors of CHLOROTHENE NU solvent (1,1,1-trichloroethane) have been shown to have a very low toxicity from repeated inhalation.[12,13,14,15] The present studies demonstrate that the inhalation of the solvent in the form of an aerosol is equally low in toxicity. In the 3-month experiment, rats which were exposed for 65 days to concentrations up to 1200 ppm (6.6 mg/l) exhibited no adverse effects with respect to growth, hematologic and clinical parameters. The only observable effect was light narcosis during exposures. A rapid onset and recovery of exposed animals from narcosis indicates that CHLOROTHENE NU solvent is rapidly absorbed and excreted from the lungs.[14] Anesthesia was less pronounced in animals exposed to xylol aerosols.

Repeated exposures of animals to CHLOROTHENE NU aerosols containing 1% w/w of FC 310 resin, however, resulted in high mortality (70%). Sickness and deaths were all-or-none responses. Survivors were totally unaffected whereas those animals that succumbed did so very rapidly with marked pulmonary injury. The lungs were the only organs significantly affected. All other lesions were considered unrelated to exposure or secondary to the severe acute pulmonary injury. The decreased spleen weight in the absence of histological changes is probably an artifact. From the dose-mortality data, a plot of

the logarithmic value of cumulative (Ct) of FC 310 versus the cumulative per cent mrotality showed a linear relationship up to a value of 0.74 day·mg/l. Continued exposure of animals over this "critical (Ct)" led to a sharp increase in mortality rate (Figure 3). This abrupt alteration of rate suggested that the causes of death of the experimental animals at (Ct) below and above the "critical" value might be different. A similar mortality rate was observed in the xylol plus FC 310 animals with the exception that the "critical (Ct)" was 0.62 days mg/l (Figure 4). The slightly smaller "critical (Ct)" for xylol might be attributed by the inherent higher inhalation toxicity of xylol. (The lethal concentrations for xylol, depending on the proportions of meta, ortho and para isomers, are reported to be between 7,000-11,300 ppm[16] whereas the lethal concentration for CHLOROTHENE NU (1,1,1-trichloroethane) is approximately 16,000 ppm (4 hour LC_{50})[17]). Xylol is also more of a respiratory irritant than CHLOROTHENE NU but is not as potent an anesthetic. In spite of the differences in solvent toxicities, the animals in the CHLOROTHENE NU plus FC 310 and the xylol plus FC 310 groups exhibited practically identical dose-mortality responses (Figure 5). These observations suggest that FC 310 aerosols were the main causative ingredient of pulmonary injury and death, while the toxicities of the two solvents appear to exert no significant effect.

Mechanisms of toxic effect

Chemical and physical irritations: Histopathological examination of the animals, which succumbed after exposures to FC 310 aerosols revealed non-specific, exudative pneumonia. Considering the chemical and physical properties of this fluorocarbon resin, the material may exert direct irritation on the pulmonary tissues. It may also be postulated that this highly hydrophobic resin may alter the surface properties of the alveolar walls and the permeability of capillary membranes leading to pulmonary edema.

"Sensitisation" reaction: The acuteness of the terminal event indicated by the sudden occurrence of respiratory distress, weight loss and death, suggests that a non-specific sensitivity reaction might be involved. Indeed, the egg-white injury "sensitisation" tests on six FC 310 treated animals resulted in one case of typical anaphylactic death. Subsequent tests showed that animals previously "sensitised" by egg-white injection appeared more susceptible to the toxic effects of FC 310. Although there exists the possibility of "cross-sensitisation" between egg-white and FC 310 resin, no conclusive evidence was established in the present experiment.

The reaction of the experimental animals following exposure of aerosols of solvent-resin showed remarkably close resemblance to that described in human cases. The main adverse effect was respiratory distress, and the onset of the reaction was sudden and the development fast.

Based upon these experimental data, it must be concluded that the FC 310 resin was a causative agent of pulmonary injury caused by the coating-spray products.
A no effect level was not determined by these studies since the lowest concentration studied, 0.006 mg/l, appeared to cause an increase in mortality after two weeks of exposure. Hence, it is not possible to recommend a level safe for human exposure. The level probably should be considerably below 0.006 mg/l to provide a margin of safety. It is also recommended that workers with predisposing pulmonary conditions or allergies should avoid exposures to the resin containing the compound.

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TABLE I

MEAN BODY WEIGHTS OF ANIMALS EXPOSED TO AIR, CHLOROTHENE® NU AEROSOLS AND CHLOROTHENE® NU + FC 310 AEROSOLS

Experimental Duration (week)	Exposure Duration (day)	Animal Groups		
		Control gm	Chlorothene® NU gm	Chlorothene® NU + FC 310 1% w/w gm
0	0	97 ± 15(30)	103 ± 13(30)	100 ± 18(30)
1	5	131 ± 18	139 ± 17	133 ± 22
2	10	172 ± 25	182 ± 22	175 ± 26
3	15	210 ± 28	217 ± 27	208 ± 30(29)
4	20	244 ± 29	245 ± 30	244 ± 25(27)
5	25	266 ± 28	268 ± 33	269 ± 26
6	30	282 ± 38	285 ± 34	285 ± 27(26)
7	35	308 ± 33	311 ± 37	308 ± 321(25)
8	40	326 ± 36	332 ± 40	328 ± 34
9	45	339 ± 37	346 ± 39	335 ± 37(23)
10	50	347 ± 35	358 ± 42	344 ± 35
11	55	344 ± 46	360 ± 46	351 ± 48(14)
12	60	368 ± 48	390 ± 48	348 ± 54(9)
13	65	374 ± 45	394 ± 49	354 ± 62
Necropsy (Starved)	66	354 ± 45	370 ± 47	335 ± 58

( ) = Number of Animals

TABLE II

HEMATOLOGICAL PARAMETERS OF RATS EXPOSED TO AIR, CHLOROTHENE® NU AND CHLOROTHENE® NU + PC 310 1% w/w AEROSOLS

Animal Groups		Experimental Duration week	Exposure Duration day	Cumulative (Ct) of PC 310 day-mg/l	HCT %	RBC X10^6	Hgb g/100 cc.	WBC X10^3	Neut. Seg	Neut. e/j	Lym	Mn	Bas	Eos
Control	(6)	0	0	—	43.5	6.06	12.0	13.9	24.8	0.5	68.8	4.5	1.0	0
	(3)		1	—	43.0	5.77	12.2	14.0	18.6	0	76.3	4.0	1.0	0
	(3)		2	—	41.3	5.51	11.7	15.0	17.0	0	77.0	5.6	0.3	0
	(6)	1	5	—	42.1	5.64	12.0	14.5	17.8	0	76.6	4.8	0.6	0
	(6)	2	10	—	44.3	6.10	12.9	13.4	23.0	0	72.8	4.0	0	0
	(6)	4	20	—	49.1	7.10	16.7	15.2	21.5	0	74.0	3.6	0.8	0
	(6)	13	53	—	55.8	9.00	17.8	19.8	18.8	0	77.4	2.2	1.2	0
Chlorothene® NU	(6)	0	0	—	43.5	5.81	12.5	14.8	20.8	0.3	71.1	4.3	0.6	0
	(3)		1	—	42.8	5.35	12.2	14.1	19.0	0	76.3	4.0	[illegible]	0
	(3)		2	—	43.0	5.83	12.5	14.5	23.6	0	69.3	6.9	0.[illegible]	0
	(6)	1	5	—	42.9	5.89	12.3	14.3	21.3	0	72.8	5.3	0.5	0
	(6)	2	10	—	47.2	6.20	13.5	7.6	26.4	0	70.2	3.0	0.4	0
	(6)	4	20	—	48.2	6.75	16.3	15.9	21.5	0	75.8	2.3	0.3	0
	(6)	13	53	—	53.0	8.62	17.0	16.6	24.8	0	72.6	2.0	1.8	0
Chlorothene® NU + PC 310 1% w/w	(6)	0	0	—	42.6	5.65	12.0	11.9	27.6	0	67.0	4.6	0	0
	(3)		1	0.0054	43.3	5.35	11.8	16.7	15.3	0	76.0	6.3	0.6	0
	(3)		2	0.0108	44.8	6.18	13.0	15.8	24.3	0	73.3	2.0	0.3	0
	(6)	1	5	0.027	44.1	5.77	12.3	16.0	20.1	0	74.3	5.0	0.5	0
	(6)	2	10	0.055	44.1	6.10	13.3	16.2	28.6	0	68.3	2.6	0.2	0
	(6)	4	20	0.11	49.3	6.68	15.9	16.3	26.2	0	71.5	1.7	0.5	0
	(6)	13	53	0.87	—	—	—	—	—	—	—	—	—	—

( ) = Number of animals examined

TABLE XXX

SGPT ACTIVITY (KARMEN UNITS) OF RATS EXPOSED TO VARIOUS EXPERIMENTAL SYSTEMS

Experimental Duration (week)	Exposure Duration (day)	Animal Groups		
		Control	Chlorothene® NU	Chlorothene® NU + FC 310 1% w/w
0	0	62.0*(8)	62.7*(8)	65.1*(8)
	1	39.4*(4)	53.0*(4)	45.8*(4)
	2	43.4*(4)	63.6*(4)	53.3*(4)
4	20	46.9 (8)	49.4 (8)	45.0 (8)
12	60	40.3 (8)	43.1 (8)	56.4 (3)
		Control	Xylol	Xylol + FC 310 1% w/w
0	0	71.1 (3)	42.8 (5)	53.4 (5)
	1	49.1 (5)	36.0 (5)	42.8 (5)
	2	39.8 (5)	31.5 (5)	40.4 (5)
2	10	36.8 (5)	32.9 (5)	30.8 (5)
4	20	46.7 (5)	36.4 (5)	39.7 (5)

* = Direct conversion of S.F. units to Karmen units.
( ) = Number of animals examined.

000032

TABLE IV

CUMULATIVE MORTALITY OF RATS EXPOSED TO AEROSOLS OF CHLOROTHENE® NU + PC 310 1% w/w

Exposure Conditions				Animal Groups		
Cumulative Exposure (days)	Nominal Concentrations Chlorothene® (ppm)	Nominal Concentrations PC 310 (mg/l)	Cumulative Ct (days-mg/l)	Cumulative % Mortality Control	Cumulative % Mortality Chlorothene®	Cumulative % Mortality PC310
11	0.545	0.005	0.059	—	—	3.33
15	0.545	0.005	0.081	—	—	6.67
16	0.545	0.005	0.092	—	—	10.00
26	1.089	0.011	0.146	—	—	13.33
34	1.635	0.017	0.250	—	—	16.67
43	3.270	0.033	0.446	—	—	23.33
50	6.540	0.066	0.675	—	—	30.00
51	6.540	0.066	0.740	—	—	33.33
52	6.540	0.066	0.806	—	—	40.00
53	6.540	0.066	0.871	—	—	53.33
*	—	—	—	—	—	70.50

*Post-exposure mortality

000033

TABLE V

MEAN TERMINAL BODY AND ORGAN WEIGHTS AND BODY TO ORGAN WEIGHT RATIOS OF RATS EXPOSED TO AIR, CHLOROTHENE[5] NU AEROSOL, OR CHLOROTHENE[6] NU + PC 310 AEROSOLS FOR 3 MONTHS

Species: Sherman Rats

Sex: Male

Specimens	Treatments					
	Control		Chlorothene[6] NU		Chlorothene[6] NU + PC 310 % w/w	
	Weight, g	g/100g	Weight, g	g/100g	Weight, g	g/100g
Terminal Body Weight	354 ± 45 (30)	—	360 ± 73 (39)	—	334 ± 58 (9)	—
Lung	3.28 ± 0.66	0.95	3.13 ± 0.62	0.86	3.10 ± 0.78	0.98
Heart	1.21 ± 0.15	0.33	1.29 ± 0.17	0.35	1.03 ± 0.09	0.31
Liver	7.85 ± 1.09	2.23	8.44 ± 1.24	2.28	7.58 ± 1.20	2.27
Kidney	2.17 ± 0.27	0.61	2.34 ± 0.36	0.62	1.97 ± 0.44	0.57
Spleen	0.62 ± 0.08	0.19	0.57 ± 0.09	0.15	0.39 ± 0.10	0.12*
Testes	3.41 ± 0.27	0.96	3.44 ± 0.28	0.94	3.80 ± 0.50	0.92

( ) = Number of animals examined

*P<0.01 for both control versus CHLOROTHENE NU + PC 310, and CHLOROTHENE NU versus CHLOROTHENE NU + PC 310

TABLE VI

MEAN BODY WEIGHTS OF ANIMALS EXPOSED TO AIR, XYLOL AEROSOL OR XYLOL + FC 310 AEROSOL

Experimental Duration (Week)	Exposure Duration (Day)	Animal Groups		
		Control gm	Xylol gm	Xylol + FC 310 (1% w/w) gm
0	0	358**± 44 (30)	304*± 35 (30)	354**± 36 (30)
1	5	372 ± 44	319 ± 35	372 ± 37 (25)
2	10	387 ± 44	332 ± 37	391 ± 36 (21)
3	15	397 ± 43	343 ± 38	396 ± 38 (18)
4	20	412 ± 46	356 ± 37	401 ± 37 (14)
5	25	416 ± 47	357 ± 39	402 ± 45 (11)
6	30	420 ± 54	361 ± 44	420 ± 38 (10)

*Replacement animals of younger age.
**Animals received four extra days of exposures.

TABLE VII

HEMATOLOGICAL PARAMETERS OF RATS EXPOSED TO AIR, XYLENE OR XYLENE + FC 310 1% W/W AEROSOL

Animal Groups	Experimental Duration Week	Exposure Duration Day	Cumulative (Ct) of day-mg/l	HCT %	RBC X10^6	Hgb g/100 cc.	MCV X10^3	Reticulocytes Exp	Reticulocytes b/j	Lym	Mon	Eos	Bas
Control	0	0	—	49.3	8.31	15.7	18.3	26	0	69.4	2	0.6	0
		1	—	48.9	7.93	15.3	21.9	18	0	78.8	1.8	1.8	0
		2	—	49.5	8.21	16.3	23.7	18	0	78.8	2.6	0.2	0
	4	20	—	47.0	7.93	15.0	21.6	28.2	0	66.8	5.0	2.6	0
Xylol	0	0	—	48.4	8.00	16.1	21.0	19.4	0	77.2	2.4	0.8	0
		1	—	43.0	7.15	14.1	17.0	27.6	0	70.0	1.8	0.6	0
		2	—	47.0	7.61	15.5	17.6	23.6	0	74.4	1.0	1.0	0
	4	20	—	49.4	8.02	16.1	19.5	25.8	0	71.6	1.8	0.8	0
Xylol + FC 310 (1% w/w)	0	0	—	48.6	9.06	15.5	19.3	22.6	0	73.6	1.4	0	0
		1	0.033	50.7	8.36	16.1	19.5	26.2	0	71.0	2.6	0	0
		2	0.066	49.2	8.36	16.8	19.5	26.8	0	71.6	1.6	2	0
	4	20	0.652	49.2	7.90	16.2	20.4	37.8	0	57.8	2.2	1	0

006036

TABLE VIII

CUMULATIVE MORTALITY OF RATS EXPOSED TO AIR OR AEROSOLS OF XYLOL, OR XYLOL + PC 310 1% w/w

Exposure Conditions				Animal Groups		
	Nominal Concentrations			Cumulative % Mortality		
Cumulative Exposure (days)	Xylol (mg/l)	Xylol + PC 310 (mg/l)	Cumulative Ct of PC 310 (mg-day/l)	Control	Xylol	PC310
5	3.30	0.033	0.119	—	—	10.00
7	3.30	0.033	0.182	—	—	13.33
8	3.30	0.033	0.218	—	—	16.67
10	3.30	0.033	0.290	—	—	20.00
14	3.30	0.033	0.435	—	—	30.00
18	3.30	0.033	0.579	—	—	33.33
19	3.30	0.033	0.615	—	—	36.67
20	3.30	0.033	0.652	—	—	40.00
21	3.30	0.033	0.687	—	—	46.67
23	3.30	0.033	0.759	—	—	53.33
25	3.30	0.033	0.838	—	—	56.67
26	3.30	0.033	0.568	—	—	60.00
27	3.30	0.033	0.903	—	—	63.33
*	—	—	—	—	—	66.66

*Post-exposure mortality

000037

TABLE IX

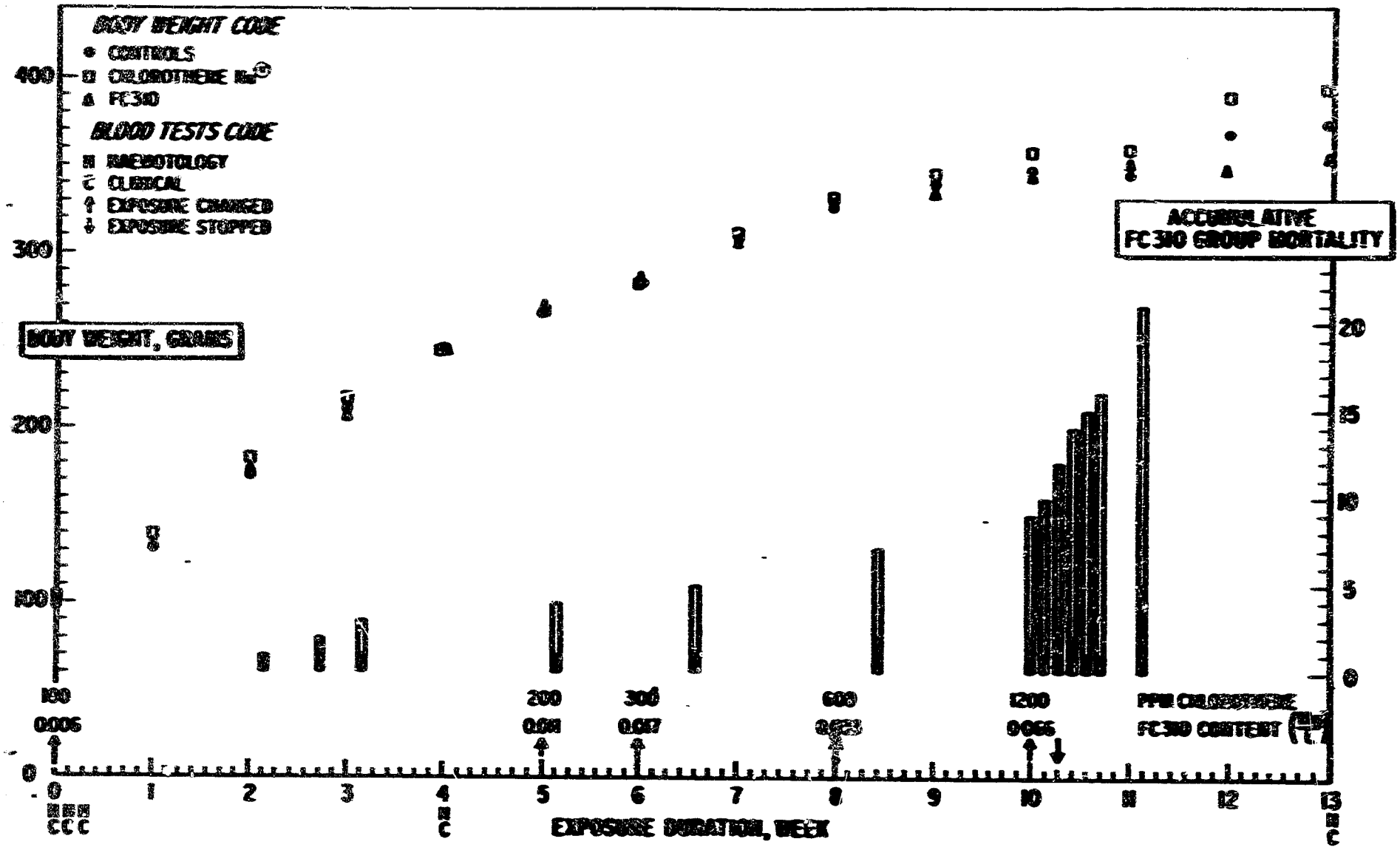
MEAN TERMINAL BODY AND ORGAN WEIGHTS AND BODY TO ORGAN WEIGHT RATIOS OF RATS EXPOSED TO AIR, XYLOL OR XYLOL + PC 310 AEROSOLS FOR 2 MONTHS

Specimens	Treatments					
	Control		Xylol		Xylol + PC310 (1% w/w)	
	Weight, g	g/100g	Weight, g	g/100g	Weight, g	g/100g
Terminal Body Weight	426 ± 49	—	362* ± 46	—	428 ± 46	—
Lung	1.93 ± 0.28	0.45	1.98 ± 0.49	0.56	2.09 ± 0.32	0.49
Heart	1.32 ± 0.19	0.31	1.18 ± 0.17	0.33	1.34 ± 0.16	0.32
Liver	9.46 ± 1.73	2.21	8.25 ± 1.45	2.27	11.62 ± 1.58	2.74
Kidney	2.43 ± 0.35	0.57	2.11 ± 0.24	0.58	2.60 ± 0.23	0.61
Spleen	0.66 ± 0.09	0.16	0.62 ± 0.09	0.17	0.69 ± 0.23	0.16
Testes	3.57 ± 0.25	0.85	3.42 ± 0.25	0.95	3.60 ± 0.21	0.85

*Replacement animals of slightly younger age

FIGURE 1

EFFECTS OF CHLOROTHENE-NU®, & [CHLOROTHENE-NU® + 1% W/W FC310] AEROSOLS ON RATS



000038

EFFECTS OF XYLOL VAPOR & [XYLOL + 1% W/W FC310] AEROSOLS ON RATS

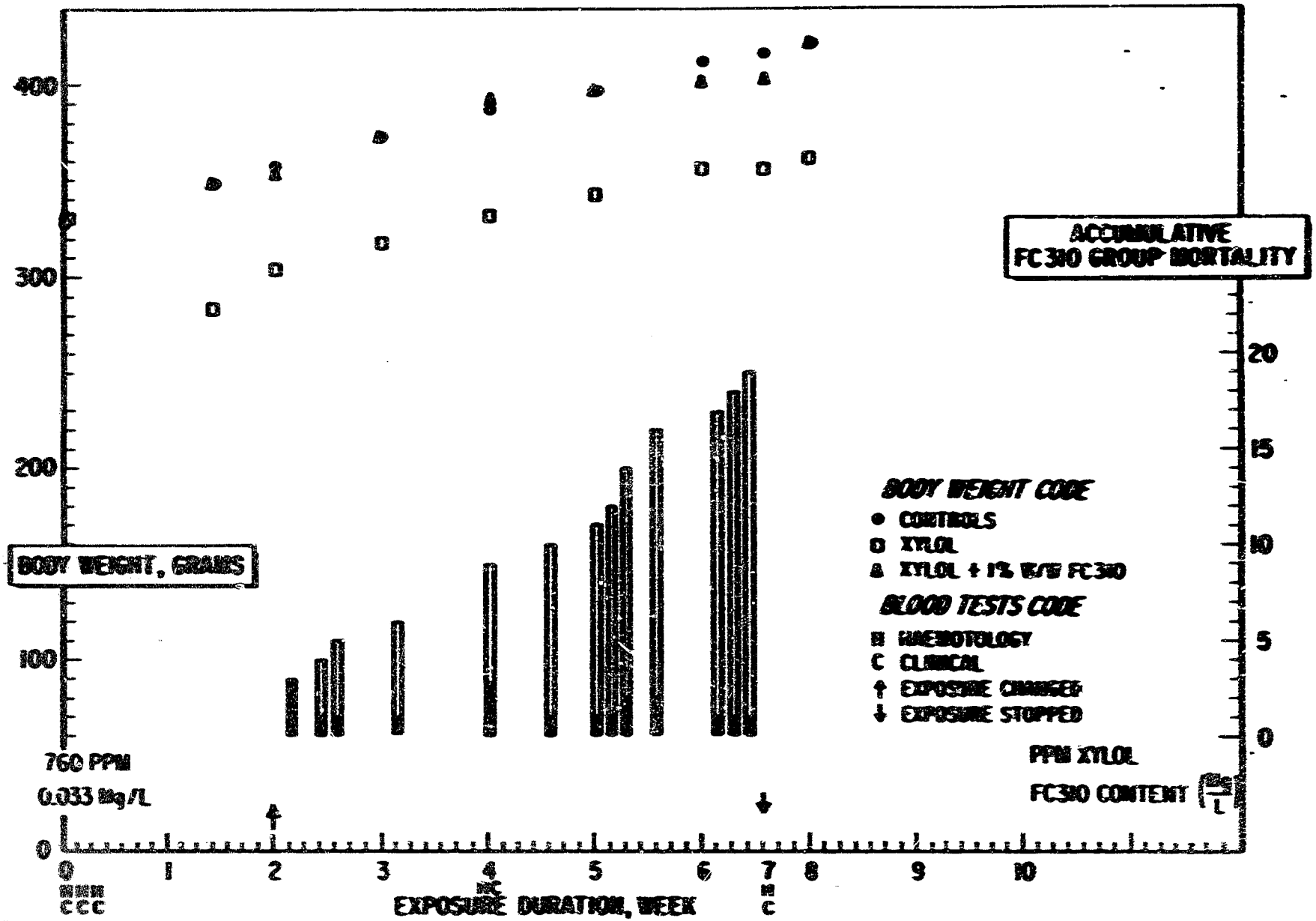


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FIGURE 3

LOGARITHM-PROBABILITY PLOT OF CUMULATIVE % MORTALITY VS (Ct) FOR RATS EXPOSED TO CHLOROTHENE NU® + FC310 AEROSOLS

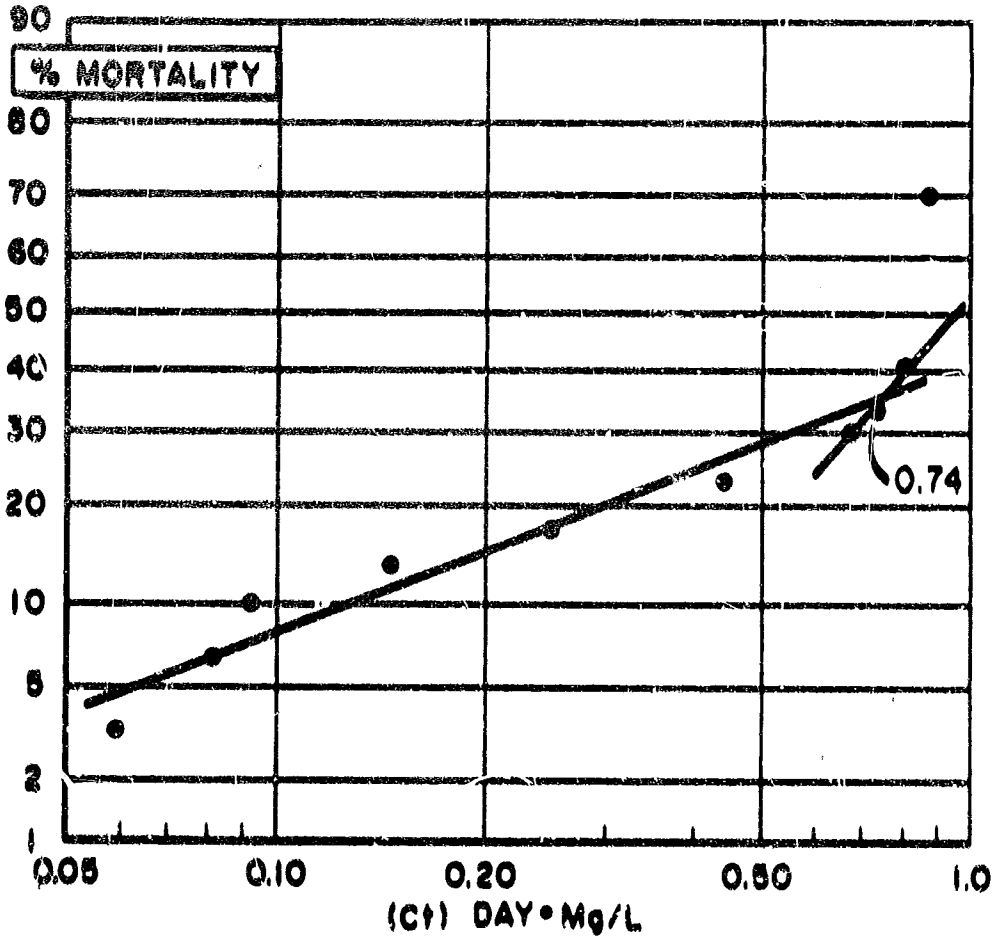

FIGURE 4

LOGARITHM-PROBABILITY PLOT OF CUMULATIVE % MORTALITY VS (Ct) FOR RATS EXPOSED TO XYLOL + FC310 AEROSOL

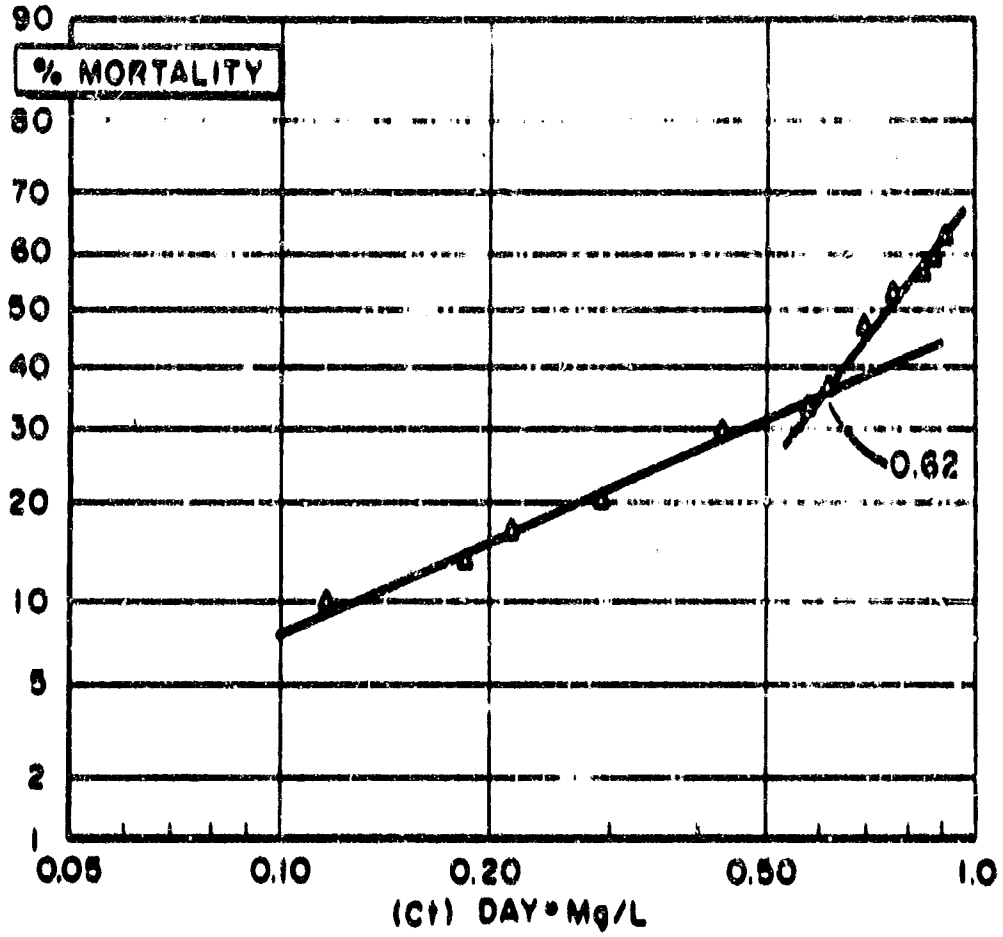

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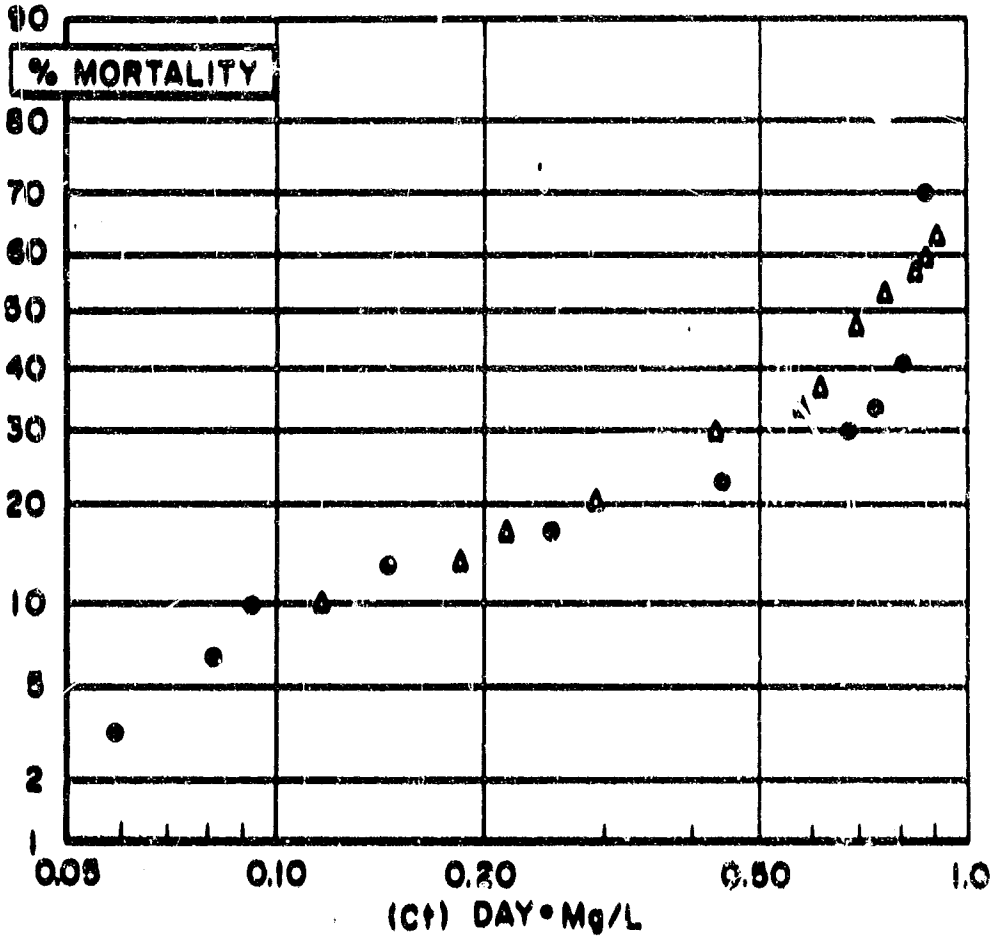
FIGURE 5

LOGARITHM-PROBABILITY PLOT OF CUMULATIVE % MORTALITY VS (Ct) FOR RATS EXPOSED TO CHLOROTHENE NU® + FC31C, & XYLOL + FC310 AERSOLS



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Doc Title 23

PRODUCTS FROM TROPOSPHERIC

PHOTODECOMPOSITION OF 1,1,1 - TRICHLOROETHANE

Chemical Name (300 per name)	25	CAS No. (10)	24
1,1,1 - TRICHLOROETHANE		71-55-6	

A.K. 5/27/83

D-323

8782106 99

DOW CHEMICAL U.S.A.

MIDLAND, MICHIGAN 48640

Environmental Sciences Research

Letter Report

August 10, 1978

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V. L. Stevens
Inorganic Chemicals T&D
2020 Dow Center

Products from Tropospheric Photodecomposition of 1,1,1-Trichloroethane

1,1,1-Trichloroethane (methylchloroform, CH_3CCl_3) is thought to photodecompose in the troposphere with a half-life of several years, according to current theories and data. The decomposition probably is initiated by the reaction of CH_3CCl_3 with hydroxyl radicals. Knowing the nature of the decomposition products is important from an environmental standpoint. This report summarizes the experimental data which are available on this subject. Two studies, which employed different techniques, have been reported on the products formed from CH_3CCl_3 under simulated tropospheric conditions.

Pearson and McConnell found CO_2 and HCl were the major products along with a small amount of Cl_2.[1]

$$CH_3CCl_3 \xrightarrow[\text{Ambient air}]{h\nu,\ >290\ nm,\ \text{Xe arc}} CO_2 + HCl + Cl_2$$

(100%) (99%) (1%)

Phosgene ($COCl_2$) was an intermediate in this reaction, but rapidly hydrolysed on contact with water.[1] The ambient air used in these experiments contained an unknown quantity of water vapor.[1] The authors did not state explicitly whether the $COCl_2$ hydrolysed during the photolysis or after the addition of liquid water to the gaseous reaction mixture. The water was added as part of the analytical procedure. $COCl_2$ is known to hydrolyse to CO_2 and HCl rapidly in liquid water; a half-life of 0.1 second at 25°C has been reported.[2] $COCl_2$ is not expected to persist in the troposphere because it probably will rain out and adsorb or dissolve in aqueous aerosols (clouds, etc.) and other water bodies.

Spence and Hanst used chlorine atoms (from 10 ppm Cl_2) to initiate the photooxidation of CH_3CCl_3 (10 ppm).[3] They used dry, purified (<0.1 ppm total hydrocarbons) air and found CO, HCl, $COCl_2$, and CO_2 were the products.

$$CH_3CCl_3 + Cl_2 \xrightarrow[\text{Dry air, 22.5°C, 6 minutes}]{h\nu,\ \text{Simulated sunlight}} CO + HCl + COCl_2 + CO_2$$

2 ppm reacted	1.5 ppm	6.0 ppm	2.0 ppm	~0.5 ppm

CO is a natural component of the troposphere and is oxidised to CO_2 under tropospheric conditions. The persistence of $COCl_2$ in this experiment may be the result of using a dry system. Otherwise the two studies gave similar results.

Initiation of the photooxidation of CH_3CCl_3 with Cl· or ·OH should lead to the same products except for the HCl and H_2O formed in the initiation step. The overall tropospheric decomposition of CH_3CCl_3 probably involves at least the following reactions?

$$\underline{CH_3CCl_3} + \cdot OH\ (Cl\cdot) \longrightarrow \cdot CH_2CCl_3 + \underline{H_2O}\ (HCl)$$

$$\cdot CH_2CCl_3 + O_2 \longrightarrow \cdot O_2CH_2CCl_3$$

$$\cdot O_2CH_2CCl_3 + NO \longrightarrow \cdot OCH_2CCl_3 + NO_2$$

$$\cdot OCH_2CCl_3 \longrightarrow \cdot CCl_3 + HCHO$$

$$\cdot CCl_3 + O_2 \longrightarrow \cdot O_2CCl_3$$

$$\cdot O_2CCl_3 + NO \longrightarrow \cdot OCCl_3 + NO_2$$

$$\cdot OCCl_3 \longrightarrow COCl_2 + Cl\cdot$$

$$COCl_2 + H_2O \longrightarrow \underline{CO_2} + \underline{2\ HCl}\ \text{(condensed phase reaction)}$$

$$Cl\cdot + CH_4 \longrightarrow \underline{HCl} + \cdot CH_3$$

$$HCHO + \cdot OH \longrightarrow \dot{H}CO + \underline{H_2O}$$

$$\dot{H}CO + O_2 \longrightarrow CO + HO_2\cdot$$

$$CO + \cdot OH \longrightarrow \underline{CO_2} + H\cdot$$

000004

Unresolved questions remain concerning the rates of some of these processes, but the ultimate decomposition products of CH_3CCl_3 are expected to be CO_2, HCl, and H_2O.

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2. W. H. Manogue, Diss. Abstr., 18, 904 (1958).

3. J. W. Spence and P. L. Hanst, J. Air Pollut. Contr. Assoc., 28, 250 (1978).

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EFFECT OF 1,1,1-TRICHLOROETHANE ON THE FORMATION OF OXIDANT OR OZONE IN THE TROPOSPHERE

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6

A.K. 5/27/83

D-334

878210700

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Environmental Sciences Research Laboratory

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April 28, 1970

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EFFECT OF 1,1,1-TRICHLOROETHANE ON THE FORMATION OF OXIDANT OR OZONE IN THE TROPOSPHERE

Scientists and regulators have been concerned for more than ten years over the contribution of 1,1,1-trichloroethane (methylchloroform, CH_3CCl_3) to the formation of photochemical oxidant or ozone (O_3) in the troposphere. This report summarizes the experimental data which are available on this subject. Three studies have been reported, all of which show that, compared to other compounds, CH_3CCl_3 contributes little to the formation of photochemical oxidant or O_3.

Fuller reported work carried out by the Los Angeles County Air Pollution Control District (1). The experimental conditions were not specified, but CH_3CCl_3 was reported to cause the formation of 0.11 ppm O_3. Under the same conditions, trichloroethylene ($ClHC{=}CCl_2$) caused the formation of 0.48 ppm O_3. The amount of O_3 generated in the absence of any organic material was not reported.

Work carried out at Dow showed small amounts of total oxidant were formed in the presence of CH_3CCl_3 (2). Total oxidant includes any compound which will oxidize neutral buffered potassium iodide solution. These experiments were performed with much higher concentrations of CH_3CCl_3 and nitrogen dioxide (NO_2) than one would normally find in the troposphere. CH_3CCl_3 (10-50 ppm) and NO_2 (2.5-12 ppm) were irradiated in purified air at 43% relative humidity and 24-25°C with sunlamps. The sunlamps had a total intensity, in the ultraviolet range, ~2.6 times that of natural sunlight at noon on a summer day in Freeport, Texas. Pertinent results are shown in Table I. Again the amount of oxidant formed in the presence of CH_3CCl_3 was considerably less than the amount formed in the presence of $ClHC{=}CCl_2$.

Table I. Formation of Total Oxidant in the Presence of CH_3CCl_3 and $CHCl=CCl_2$

Compound (conen, ppm)	Conen of NO_2 (ppm)	Conen of oxidant (as O_3, ppm) at 1 hr	2 hr	>2 hr
CH_3CCl_3 (10)	2.5	0.2	0.1	0 (4 hr)
CH_3CCl_3 (50)	12	0.6	0.5	0.1 (20 hr)
$CHCl=CCl_2$ (50)	12	2.7	0.8	0.7 (15 hr)
None	12	0.6	0.3	0 (6 hr)

Work reported by the U.S. Environmental Protection Agency showed that CH_3CCl_3 in air with NO_x did not produce more O_3 than was produced in the absence of CH_3CCl_3 (Table II) (3). These experiments were carried out with purified air at 30°C. The light intensity was roughly equivalent to the average sunlight intensity on a clear summer day in Los Angeles [$k_1(NO_2) \sim 0.34$ min^{-1}].

Table II. Formation of O_3 in the Presence of CH_3CCl_3

Reactant (conen, ppm)	Maximum O_3 conen (ppm)	Time to reach max. O_3 conen (hr)
CH_3CCl_3 (4), NO (~0.18), NO_2 (~0.02) + background air	0.04	4.6
NO (~0.18), NO_2 (~0.02) + background air	0.04	--
Background air	0.03	--

Copies of these references are available from the author.

References

1. L. J. Fuller, presented at Hearing on Petition to Exempt Trichloroethylene from Rule 66 of the Los Angeles County Air Pollution Control District before the Los Angeles County Air Pollution Control Board, April 2, 1968.

878210700

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2. W. L. Dilling, C. J. Bredeweg, and N. B. Tefertiller, Environ. Sci. Technol., 10, 351 (1976); with M. J. Mintz, J. P. Arrington, and V. L. Stevens, Supplementary Material for the Microfilm Edition.

3. S. D. Joshi and B. Dimitriades, Preprints of Papers Presented at the 173rd National Meeting, ACS, Div. of Environ. Chem., 17, No. 1, 20 (1977).

Wendell L. Dilling

Wendell L. Dilling

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Doc Title — 23

SOIL ADSORPTION OF 1,1,1-TRICHLOROETHANE

Chemical Name (300 per name)	25	CAS No. (10)	24
1,1,1-TRICHLOROETHANE		71-55-6	

A.K 5/21/83

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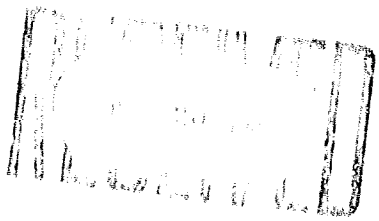
SOIL ADSORPTION OF 1,1,1-TRICHLOROETHANE

October 31, 1979



by

Wayne L. Rhinehart
Environmental Sciences Research Laboratory
Dow Chemical U.S.A.
Midland, Michigan

Date Test Started: June 18, 1979
Date Test Completed: August 24, 1979

ABSTRACT

The soil adsorption of 1,1,1-trichloroethane was measured using 2 soils, a Cecil sandy loam and a Commerce loam from Georgia and Mississippi, respectively. The average sorption coefficient for the Cecil sandy loam, 0.53% C, was 2.27 and for the Commerce loam, 0.54% C, was 3.42. The K_{oc} for 1,1,1-trichloroethane with these two soils averaged 427 and 633, respectively. The values indicate some mobility of the compound in soil.

006002

INTRODUCTION

Chemicals applied to soil will partition between the solid and aqueous phases. Determination of soil adsorption coefficients indicates the relative potential for movement of a chemical with water in soil. The purpose of this study was to determine the adsorptivity of 1,1,1-trichloroethane (TCE) on soil for registration as a pesticide. A commercial formulation of TCE, CHLOROTHENE* VG, was used in this study.

EXPERIMENTAL

The soils used for this study were obtained from H. Bidlack, Dow Agricultural Chemicals Department.

Cecil Sandy Loam

Plant Science Farm
University of Georgia

Located about 15 miles from Athens in Oconee County

Designated Soil A for this study

Commerce Loam

Dow Farm
Wayside, Mississippi

Designated Soil B for this study

---

*Trademark of The Dow Chemical Company

Analysis by H. Bidlack

	Soil A	Soil B
pH	5.62	7.70
Organic Carbon	0.53%	0.54%
Sand	66.0%	40.0%
Silt	18.0%	48.0%
Clay	16.0%	12.0%

The sample of 1,1,1-trichloroethane (TCE) was obtained from

CHLOROTHENE VG Lot Number TA042591

The drum from which this sample was obtained was analyzed and found to be 92.92%[1] 1,1,1-trichloroethane.

The soils were screened through 2 mm openings and stored "as is" at 4°C until used. The soils were oven dried at 110°C for 4.5 hours and stored in a dessicator until used.

A stock standard solution of the TCE was made on a volume/volume basis using a density factor of 1.35 to convert volume to weight. Two liters of a 10 mg/L concentrations was prepared. This solution was diluted to concentrations of 1 mg/L and 0.1 mg/L.

These solutions were stored at 25°C before and after the experiment and used for the standards against which the experimental analysis were based.

Aliquots of each soil, 4 g, were placed in a Corex 11 8446 centrifuge tubes sealed with aluminum lined caps. Sixteen mL each stock solution of TCE, 10 mg/L, 1 mg/L and 0.1 mg/L, were added to duplicate tubes of each soil. Solutions were placed in empty centrifuge tubes for blank values. An additional blank of soil with distilled water was shaken to detect analytical interference from the soil.

The tubes were placed on an Eberbach back and forth shaker, in a 25°C incubator and shaken for 24 hours. The rate of shaking was adequate to move the soil bed. The tubes had about 10% head room that allowed a sloshing motion of the contents. After the shaking, the tubes were centrifuged on a duPont Sorvall® RC-5B Refrigerated Superspeed Centrifuge at 3000 rpm (1500 x g), 25°C, for 45 minutes for Soil A samples. Soil B samples had a high silt content and were spun at 8000 rpm (10,400 x g), 25°C, for 45 minutes. Samples were stored at 25°C until analysed. An aliquot, 5 mL, of the supernatant from each tube was placed in a Tekmar LSC-1 Liquid Sample Concentrator.

Purge Time - 20 minutes
Purge Gas - Nitrogen
Trap Desorption Temp. - 200°C
Trap - Tenax/Chromsorb 102

This unit was connected directly to a Tracor 222 gas chromotograph with a Tracor Model 310 Hall Detector. This unit is specific for chlorinated compounds. The GC was temperature programmed, 4 min. at 40°C, heated 10°/min up to 200°C and held for 10 min. Each tube was analyzed once except one sample was spilled before analysis.

RESULTS

The results of the GC analysis of the supernatant solutions and derived values are reported in Table I. The values were calculated as follows:

Total Chemical in Solids (no break down)

$$G_s = G_a - T$$

G_s = Total quantity of chemical sorbed to solids, µg

G_a = Total quantity of chemical added, µg

T = Total quantity of chemical left in water, µg

Calculations of Sorption Coefficient, K_d

$$K_d = \frac{G_s/B}{C_s}$$

K_d = Sorption Coefficient

B = Oven dry weight of solids, g

C_s = Concentration of chemical in the water, µg/mL

TABLE I.

CECIL SOIL (SOIL A)

	Peak Height cm²	Instrument Parameters Conc. %	Instrument Parameters Atten.	Peak Height Value	TCE Conc. mg/L	TCE Added mg (C_o)	TCE in Soln. mg (T)	TCE Adsorbed mg (C_a)	K_d mg/g/ml	K_d Avg.	K_{oc} Avg.
Stock Conc. 10 mg/L											
A1	9.5	1000	1	9500	5.0	160	80	80	4.0	4.07	754
A2	SAMPLED SPILLED			--			--	--			
Initial Conc. 1.0 mg/L	16.9	1000	1	16900							
A1	15.5	100	1	1550	0.97	16	15.5	0.5	.120		
A2	11.1	100	1	1130	0.71	16	11.4	4.6	1.61	.87	160
Initial Conc. 0.1 mg/L	15.9	100	1	1590							
A1	10.9	10	1	104.5	0.073	1.6	1.16	.44	1.50	1.95	368
A2	9.4	10	1	89.5	0.063	1.6	1.00	.60	2.41		
Initial Conc.	14.3 14.3	10	1	143							
Blank	0.45	10	1	4.5							
								Soil A Avg.		2.27	427

COMMERCE SOIL (SOIL B)

Stock. Conc. 10 mg/L											
B1	9.9	1000	1	9000	5.20	160	84	76	3.62		
B2	8.4	1000	1	8400	4.44	160	71	89	5.01	4.31	799
Initial Conc. 1.0 mg/L	18.9	1000	1	18900							
B1	12.7	100	1	1270	.79	16	12.6	3.4	1.07		
B2	8.7	100	1	870	.55	16	8.8	7.2	3.27	2.17	402
Initial Conc. 0.1 mg/L	15.9	100	1	1590							
B1	8.4	10	1	78.5	0.055	1.6	.88	.72	3.27		
B2	7.4	10	1	68.5	0.048	1.6	.77	.83	4.32	3.79	697
Initial Conc.	14.3	10	1	143							
Blank	0.55	10	1	5.5							
								Soil B Avg.		3.42	633

Calculation of Sorption Constant, K_{oc}

$$K_{oc} = \frac{k_d \times 100}{\% \text{ O.C.}}$$

% O.C. = Percentage of organic carbon in solids

The sorption constant K_{oc} is a useful value because once the constant is determined and the organic carbon content of any soil is known, the sorption coefficient (K_d) can be estimated. Isotherms for TCE adsorbed on both soils are shown in Figure 1.

CONCLUSIONS

Data on the soil adsorption of TCE indicate it can be considered somewhat mobile in soil.[2] Using the correlation of Kenaga and Goring[3] between water solubility and K_{oc}, the K_{oc} is estimated to be 117.

$$\log K_{oc} = 3.64 - 0.55 \ (\log WS)$$

WS = water solubility in mg/L.

The water solubility of 720 mg/L was taken from Dilling.[4]

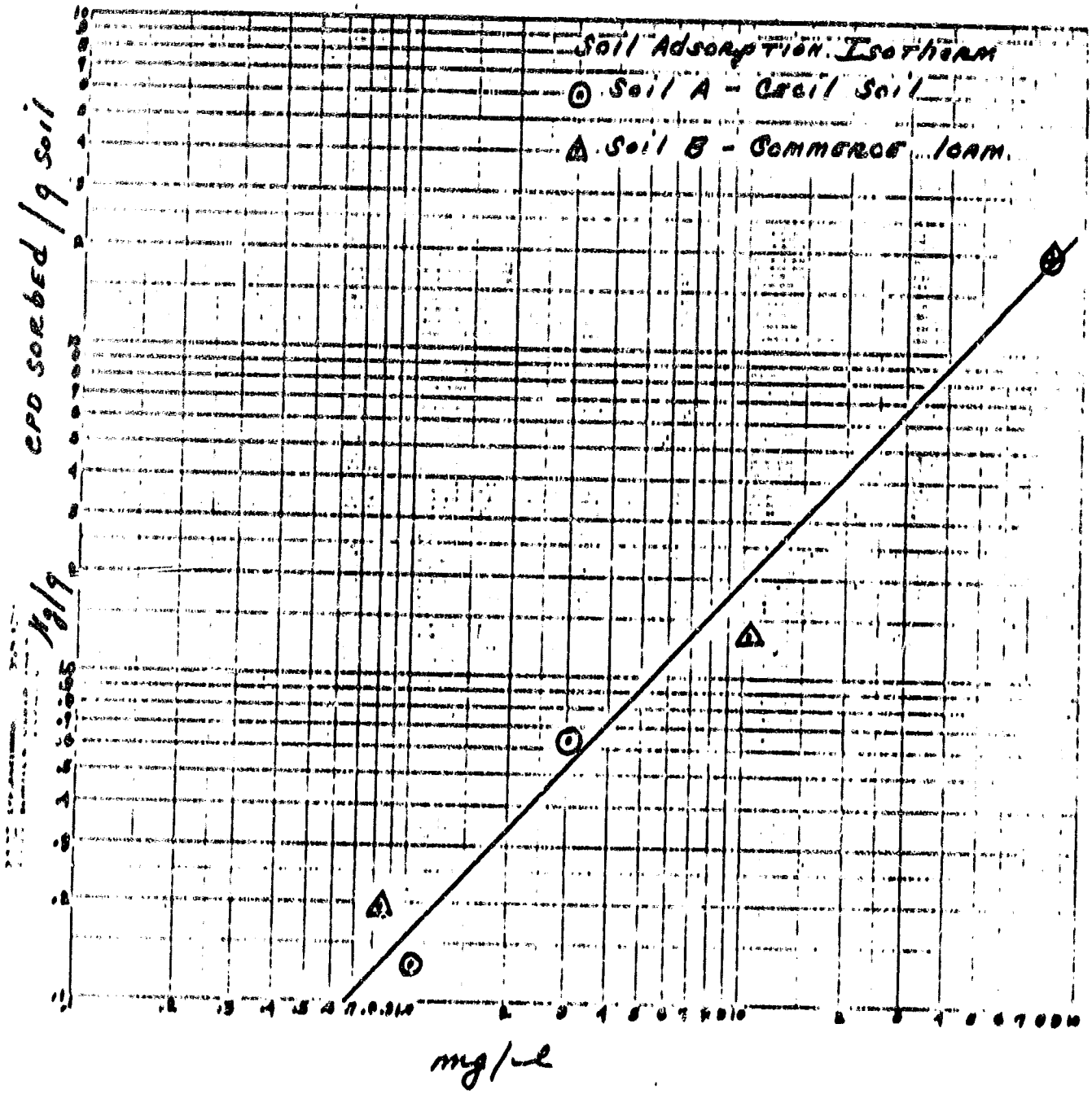


Figure 1.

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This report, ES- , has been written and approved by the personnel listed below.

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Doc Title 23

THE INHIBITION OF ANAEROBIC SLUDGE GAS PRODUCTION BY 1,1,1-TRICHLOROETHANE METHYLENE CHLORIDE

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6
METHYLENE CHLORIDE	75-Ø9-2

A.K.

878210702

D-340

R & D REPORT

DOW CHEMICAL U.S.A.

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CRI NUMBER

LABORATORY REPORT CODE

DATE ISSUED: January 27, 1977

LAB NO. | PROBLEM NO.

DEPARTMENT: Environmental Sciences Research

TITLE: THE INHIBITION OF ANAEROBIC SLUDGE GAS PRODUCTION BY 1,1,1-TRICHLOROETHANE, METHYLENE CHLORIDE.

32 PAGES IN FULL REPORT

AUTHOR(S): W. C. Hayes R. E. Bailey

AUTHOR(S) SIGNATURE(S): W. C. Hayes (RE) [signature]

REVIEWER'S SIGNATURE: R. J. Moolenaar

This report is: [ ] INTERIM [X] FINAL and mainly: [X] NEW [ ] REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS: (Include in this space references to data books, and to earlier related reports, patents and publications.)

The shock addition of chlorinated C_1 and C_2 hydrocarbons to anaerobic sludge from an operating municipal sludge digester showed inhibitory effects after 48 hours at the following concentrations:

Compound	20% Inhibition of Gas Production	50% Inhibition of Gas Production
Methylene chloride	3 mg/ℓ	50 mg/ℓ
1,1,1-Trichloroethane	1.4 mg/ℓ	25 mg/ℓ

This data is consistent with much of the prior literature and indicates a lower level of toxicity than claimed by some. The inhibitory levels are not strongly affected by the solids content of the sludge. Gas chromatographic analyses indicate somewhat less solvent in the vapor phase than is predicted from Henry's Law Constant.

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INTRODUCTION

Chlorinated hydrocarbons are widely used by industry, laboratories and dry cleaners. They may enter the sewage system either through spills or discharge of waste. Some of the compound will volatilize and leave the aqueous phase. Any chlorinated compound traveling down a sewer as small droplets, as well as that adsorbed onto solids in the sewer, will appear at the wastewater treatment plant in the primary clarifier or settling basin. This sludge will commonly be either aerobically, anaerobically or physically treated to improve the sludge stability and handling properties. The focus of the report is what happens when sludge containing methylene chloride, 1,1,1-trichloroethane,

is added to an anaerobic sludge digester.

In general, a chemical is not exclusively inhibitory or stimulatory to a mixed microbial culture such as this. If there is a concentration range over which gas production or other biological activity increases with increasing concentration, the chemical is stimulatory If above a particular point biological activity decreases with increasing concentration, the chemical is inhibiting biological activity. In the case of the chlorinated C_1 and C_2 hydrocarbons investigated in this work, the inhibitory range starts at less than 1 mg/ℓ. Other chemicals which can serve as food sources may not show inhibition until the concentration reaches 10% or more The concentration at which a particular chemical inhibits anaerobic microorganisms is dependent on many factors, including the presence of other chemicals which may display antagonistic or synergistic effects.

Kugelman and Chin (7) describe much information about toxicity of metals to anaerobic digester microorganisms and show one way this work could be extended for use by sanitary engineers.

A few failures of anaerobic digesters due to chlorinated C_1 and C_2 hydrocarbons have been previously reported (10). Several laboratory investigations on the effects of these compounds have been carried out (1,2,3,6,9,10,12). The methods and results of these studies are not consistent. It is the purpose of this study to gather data on the inhibition of gas production by anaerobic sludge in the presence of chlorinated hydrocarbons (methylene chloride, and 1,1,1-trichloroethane) to better define the inhibitory levels. Initial attempts were also made to test the effects of sludge solids concentration on inhibition and to determine the fate of the chlorinated hydrocarbons through gas chromatography.

Anaerobic digestion involves two biological processes. Two groups of anaerobic bacteria are required for complete digestion. The first group is reponsible for degrading polysaccharides, fats, and proteinaceous material to CO_2, H_2, acetate and other short-chain aliphatic compounds. The second group (methanogenic bacteria) transforms the products of the first group into methane and CO_2 and appears to be most susceptible to inhibition by heavy metals and chlorinated hydrocarbons (6). There is evidence to indicate that chlorinated hydrocarbons may inhibit the enzymes responsible for the cobamid-dependent (vitamin B_{12}) methyl-transfer reactions leading to the formation of methane (8,13).

Halomethanes form relatively stable complexes with vitamin B_{12}, removing it from its normal function of transferring methyl groups prior to the formation of methane.

LITERATURE SURVEY

Table I summarizes the data reported on inhibition of anaerobic microorganisms by several chlorinated hydrocarbons. An attempt was made to include the sludge source and concentration used in the work, but this was not given in all cases. It can be readily

TABLE I

SUMMARY OF LITERATURE DATA ON INTERACTION OF CHLORINATED HYDROCARBONS WITH ANAEROBIC MICROORGANISMS

Author	Sludge Source	CH_2Cl_2	CH_3CCl_3
Benson (3)	Marlboro Psy. Hospital N.J., No indust. waste, 80 mℓ/ℓ settable solids/2.5ℓ bottle	—	0.6 mg/ℓ, 41% inhibition
Jackson (6)	—	1 mg/ℓ in sewage	1 mg/ℓ in sludge
Sunswick (10)	Different municipal sewage sludge digesters	—	2 mg/ℓ (60 mg/kg dry solids), 50% inhibition
Thiel (12)	60 ℓ digester mixed bacterial population — synthetic substrate	100 mg/ℓ, 50% inhibition	>50 mg/kg, 20% inhibition
Barrett (1)	Exeter Sewage	—	—
Bauchop (2)	Rumen contents from cow	2.0 mM, 50% inhibition (170 mg/ℓ)	—
Montgomery (5)	As low as 0.01 mg/ℓ in sewage or 1 mg/ℓ in sludge may be toxic		
Stickley (9)	2-3% solids	—	—
This Work	Mt. Pleasant, MI, primary sludge, predominately domestic	1 mg/ℓ, 3% inhibition; 10 mg/ℓ, 34% inhibition	1 mg/ℓ, 15% inhibition; 10 mg/ℓ, 41% inhibition

000005

observed that there is some variation in results and sludges used. A major cause of confusion appears to be what constitutes a significant level of inhibition. Levels in the literature range up to 50%. Some investigators also express inhibitory levels in terms of mg/kg dry solids as opposed to mixed liquor or wet sludge.

The variations in reported data combined with the vague safety statements by Montgomery (5) led to our undertaking this project. We tested chemicals of interest to Dow: methylene chloride, 1,1,1-trichloroethane,

EXPERIMENTAL

The apparatus used in this work is shown in Figure 1. It consists of a 250 ml flask with an injection port and a 100 ml gas collection cylinder and water trap.

Anaerobic sludge was obtained from the Mt. Pleasant, Michigan, Wastewater Treatment plant. Their anaerobic digester is fed with primary sludge predominately from domestic wastes. This 50,000 gal digester simply accumulates solids for about 6 mos at which time the sludge is put onto sand drying beds. The sludge could be stored at 4°C for up to one week without apparent loss of activity.

The flasks were inoculated with 150 ml sludge. Originally, this was done in a glove bag filled with plant nitrogen gas. This procedure was discontinued in favor of flushing the apparatus with nitrogen after it was found that no significant difference in gas production occurred. Distilled water was added to the gas collection cylinder and a syringe was used to pull the water level in the graduated cylinder up to the top.

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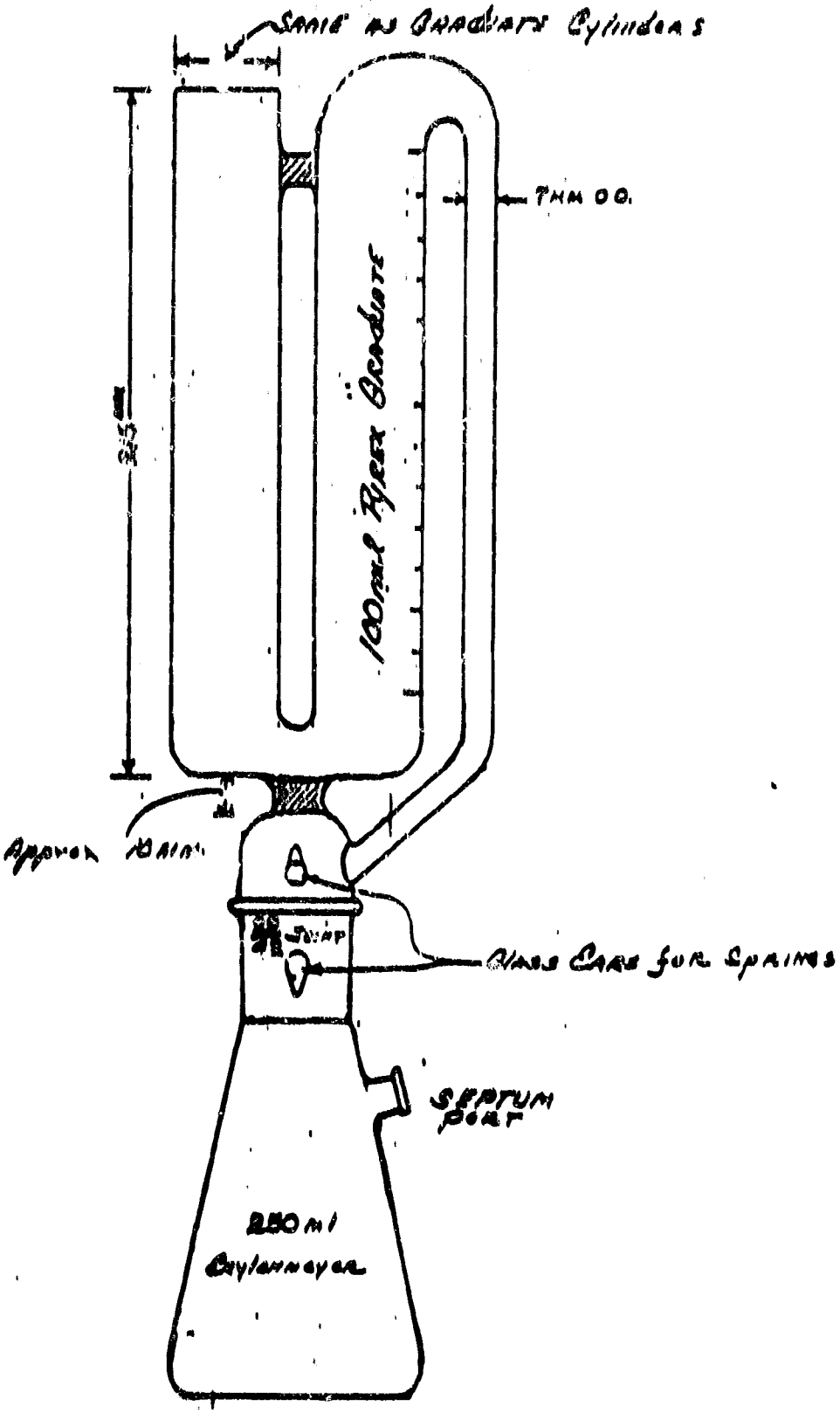

FIGURE 1. ANAEROBIC DIGESTER AND GAS MEASURING APPARATUS.

006007

The inoculated flasks were placed in a Lab-Line Incubator-Shaker at 35°C (temperature of Mt. Pleasant sludge digester) and swirled at 80 rpm. The window of the incubator was covered to prevent the growth of algae.

Gas volumes were read frequently, and periodically, the volumes were reset to the top of the cylinder so that gas would not escape and go unrecorded.

At the completion of a chlorinated compound test, pH and suspended solids (total and volatile) of the sludge were determined using a MILLIPORE® 0.45 µm filter.

Chlorinated hydrocarbons were tested in this manner: methylene chloride, and 1,1,1-trichloroethane. Commercial, inhibited 1,1,1-trichloroethane was used.

A preliminary test using all compounds at 0, 10, and 100 mg/ℓ gave an indication of their inhibitory ranges. Concentrations were calculated on the basis of dry sludge weight, as well as by volume.

During the preliminary tests, it was found that for better gas production, the digesters needed a source of nutrients. Ethanol (100 mℓ of formula 30 denatured ethanol) was chosen because it was simple and the sludge responded well to it. The ethanol could be consumed in as little as 48 hr. Ethanol was added at the start of the test series and again at 48 hr, if the test was to be extended.

000008

Analyses were carried out on a Tracor 222 Gas Chromatograph with a Hall Electrolytic Conductivity Detector in the chloride mode. The analyses were carried out under the following conditions:

Column	9 ft, 1/4" O.D., 2 mm I.D. glass column silanized with GLASS TREAT® before packing, on column injection
Packing	20% OC-200 on 100-120 mesh chromosorb WAW-DMCS
Carrier Gas	20 cc/min N_2
Reaction Gas	5 cc/min H_2
Conductivity Solvent	50:50 n-propanol:distilled water
Column Oven Temp.	CH_2Cl_2:60°C isothermal;
Injection Port Temp.	CH_2Cl_2:100°C;
Transfer Line Temp.	250°C
Furnace Temp.	800°C
Recorder	HP 3380A Integrator

Gas from the digesters and standards were injected directly; 40 µl from CH_2Cl_2 treated digesters

Analysis of the sludge phase was accomplished by hexane extraction. A 10 ml aliquot of sludge was drawn out with a syringe and injected into 2 ml hexane. After vigorous shaking and centrifugation to separate the layers, the hexane was removed. A second extraction was combined with the first for analysis. A third extraction of the CH_2Cl_2 sludge recovered only 4% as much as the first and second extractions. A 1 µl injection was used. No interferences were observed using the Hall detector.

To test for recovery of digesters with special treatment after inhibition, 3 flasks which displayed some inhibition from perchloroethylene were treated with 0.15 g powdered activated carbon. Similarly, 3 flasks were purged with 1.5 % (10 sludge volumes) of nitrogen.

Each specially treated sample was paired with a control at identical concentrations of solvent and sludge to make any improved operation apparent.

RESULTS

Gas production for a period of 48 hours for various chemical concentrations is shown in Figures 2-5. Two compounds were tested beyond the 48-hour period. Figures 6 and 7 combine the data for all four solvents showing gas produced, as a percent of the control, versus concentration. Table II lists the data on gas production. Sludge concentrations which were used in the different runs are listed in Table III. Table IV lists the pH values observed in different flasks. The double numbers are from duplicates which were set in nearly all cases. The pH varied slightly and stayed within the optimum range (6.4-7.5) (Ref. 11) for methanogenic bacteria for all tests. Table V lists some physical properties of the four chlorinated hydrocarbons tested which are significant in the aquatic environment.

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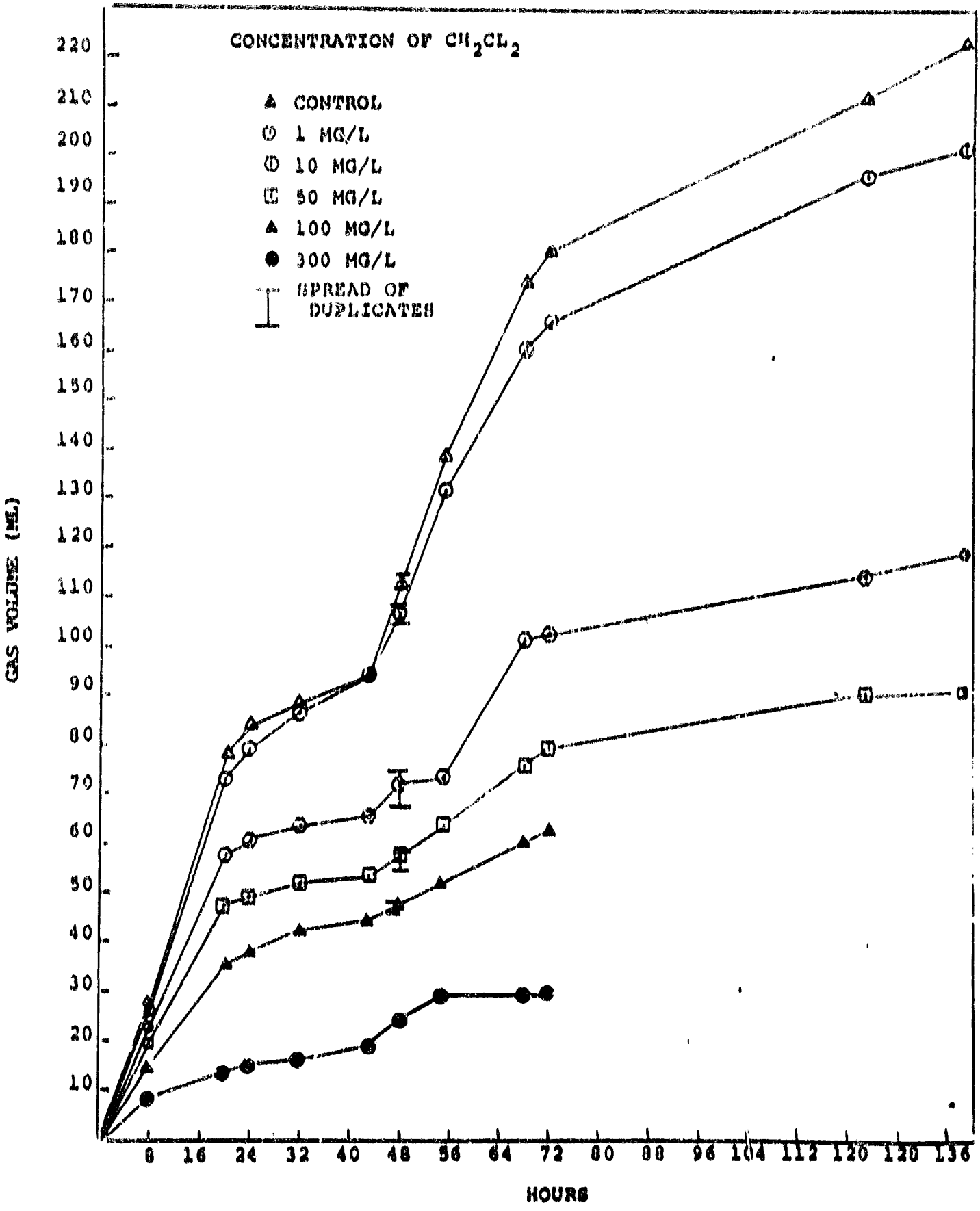

FIGURE 2. GAS PRODUCTION OF METHYLENE CHLORIDE (CH_2CL_2) INHIBITED SLUDGE.

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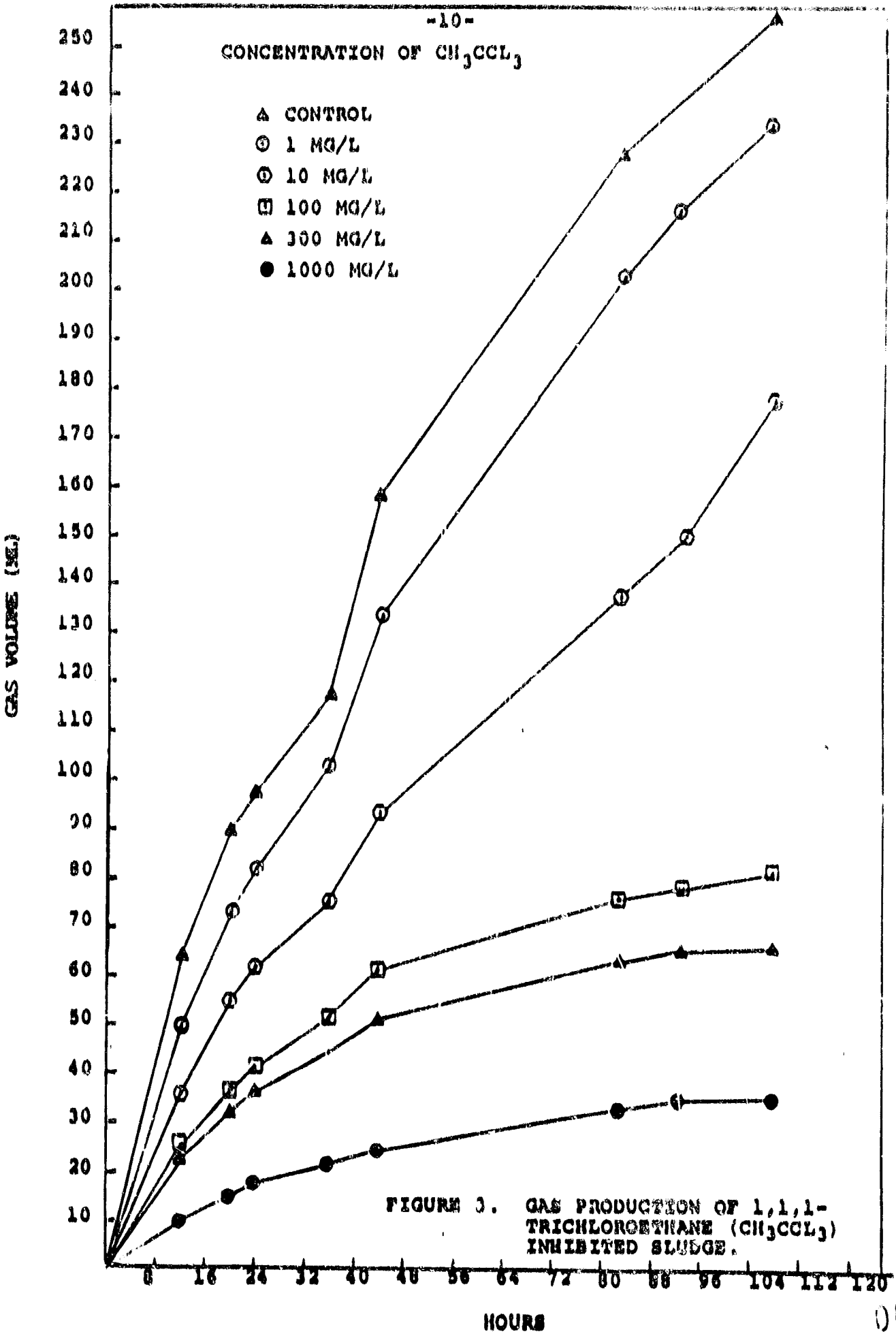

FIGURE 3. GAS PRODUCTION OF 1,1,1-TRICHLOROETHANE (CH_3CCL_3) INHIBITED SLUDGE.

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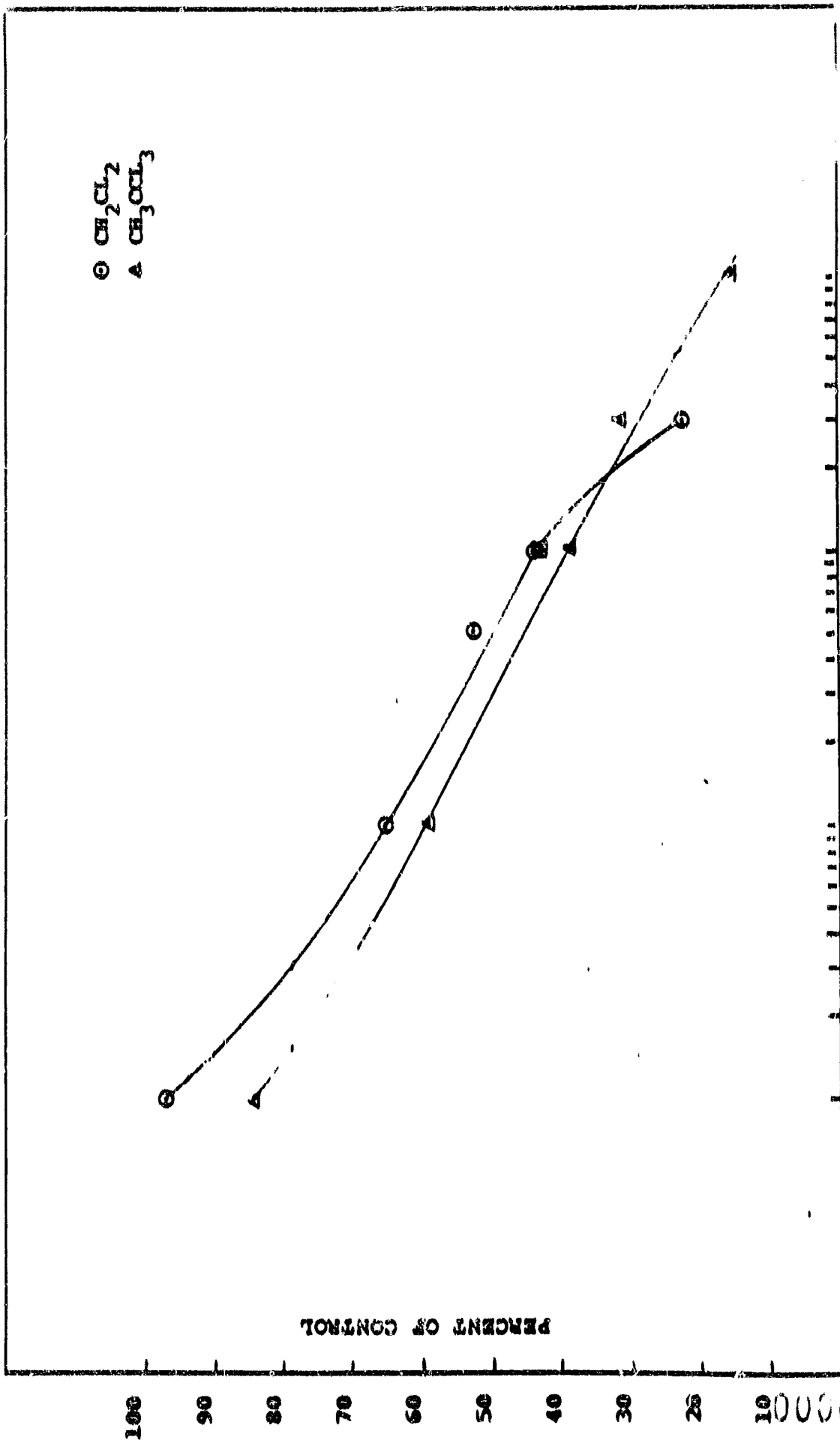

FIGURE 6. GAS PRODUCTION AS PERCENT OF CONTROL VERSUS CONCENTRATION IN MG/L MIXED LIQUOR.

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FIGURE 7. GAS PRODUCTION AS PERCENT OF CONTROL VERSUS CONCENTRATION IN MG/KG DRY SLUDGE.

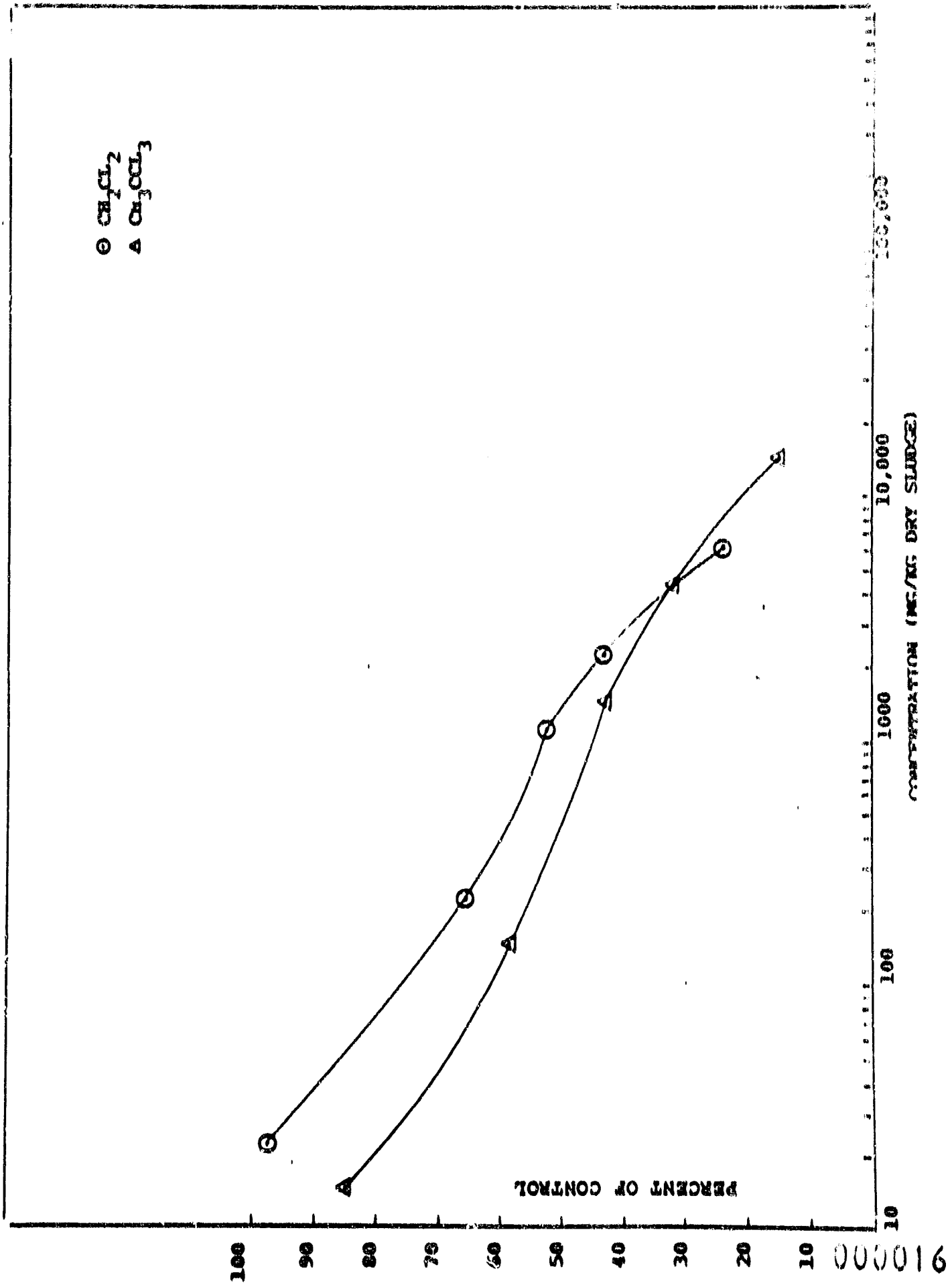

TABLE II

TOTAL GAS PRODUCTION AND PERCENT OF CONTROL AT VARIOUS CONCENTRATIONS OF CHLORINATED HYDROCARBONS AFTER 48 HOURS*

Compound	Control	1 mg/l	10 mg/l	50 mg/l	100 mg/l	300 mg/l	1000 mg/l
CH_2Cl_2	109 ml	107 ml, 98%	72 ml, 66%	57.5 ml, 53%	47.5 ml, 44%	24 ml, 22%	—
CH_3CCl_3	150 ml	133 ml, 84%	93 ml, 59%	—	61 ml, 38%	50.5 ml, 32%	24.5 ml, 16%

*Concentrations are in mg/l mixed liquor; gas volumes are the average of two flasks.

TABLE III

SOLIDS CONTENT OF SLUDGES USED WITH VARIOUS COMPOUNDS

Compound	Mixed Liquor Solids, g/l	Volatile Solids, g/l
CH_2Cl_2	40.35	23.55
CH_3CCl_3	69.6	35.3

TABLE IV

FINAL PH OF DIGESTERS AT EACH COMPOUND CONCENTRATION*

Compound	Control	1 mg/l	10 mg/l	50 mg/l	100 mg/l	300 mg/l	1000 mg/l
CH_2Cl_2	7.4, 7.4	7.35, 7.4	7.25, 7.25	7.2, 7.2	7.2, 7.2	7.15	—
CH_3CCl_3	7.3, 7.4	7.4, 7.4	7.4, 7.1	—	7.05, 6.9	6.9, 6.7	6.7

*Two digesters were run at each concentration unless only one number is shown.

TABLE 7

PROPERTIES OF CHLORINATED HYDROCARBONS

Compound	Molecular Weight	Solubility in Water, mg/l		Vapor Pressure, Torr		Henry's Law Constant	
		25°C	35°C	25°C	35°C	25°C	35°C
CH_2Cl_2	84.94	19400	19000	438	600	0.10	0.14
CH_3CCl_3	133.42	720	720	124	180	1.2	1.7

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Methylene chloride inhibited gas production 3% at 1 mg/ℓ (22 mg/kg dry solids) and 34% at 10 mg/ℓ (220 mg/kg dry solids). After 139 hours, inhibition at 1 mg/ℓ and 10 mg/ℓ had increased to 10% and 46%, respectively.

1,1,1-Trichloroethane inhibited gas production by 15% at a concentration of 1 mg/ℓ (14.1 mg/kg dry solids) and 41% at 10 mg/ℓ (144 mg/kg dry solids).

Effect of Feeding

It was observed that periodic feeding of digesters maintained or improved gas production overall. Digesters were fed with 100 µℓ ethanol at the start of each test. After 46 hours, the rate of gas production had slowed. Feeding at 48 hours, the rates again increased with the controls responding best. Feeding did not appear to significantly reduce the effects of the solvents. About 90 mℓ of gas can theoretically be produced from 100 µℓ ethanol and this was observed in some controls.

Effect of Sludge Concentration

Gas production is directly related to sludge concentration; that is, the more concentrated the sludge (in dry solids/ℓ) the faster the gas production provided other factors remain constant. The sludge used in these experiments, although from the same municipal digester, was not uniform. Dry solids varied from 9 g/ℓ to 60 g/ℓ with lumps, hair and other solids.

Each run included controls from the same stock of sludge at approximately the same concentration of solids. It would be beneficial to maintain a more consistent sludge concentration in the experimental digesters.

In the experiment using methylene chloride, where the sludge was diluted to 33%, gas production was inhibited to a somewhat greater degree with diluted sludge. The results are shown in Table VI and Figure 8.

TABLE VI

INHIBITION OF GAS PRODUCTION BY METHYLENE CHLORIDE AT TWO SLUDGE CONCENTRATIONS

Sludge and Chemical Concentrations	Total Gas Production in 48 hr (mL)	Percent of Control	Total Gas 96 hr	Percent of Control
100%[a], control	117	--	184	--
100%, 3 mg/L	88	75	116	63
100%, 30 mg/L	62	53	88	48
33%, 0 mg/L	60	b	114	c
33%, 3 mg/L	38	63	44	39
33%, 30 mg/L	29	51	35	31

[a]Sludge 45 g/L solids, 24 g/L volatile solids.

[b]The 33% control produced 51% of the 100% control.

[c]The 33% control produced 62% of the 100% control.

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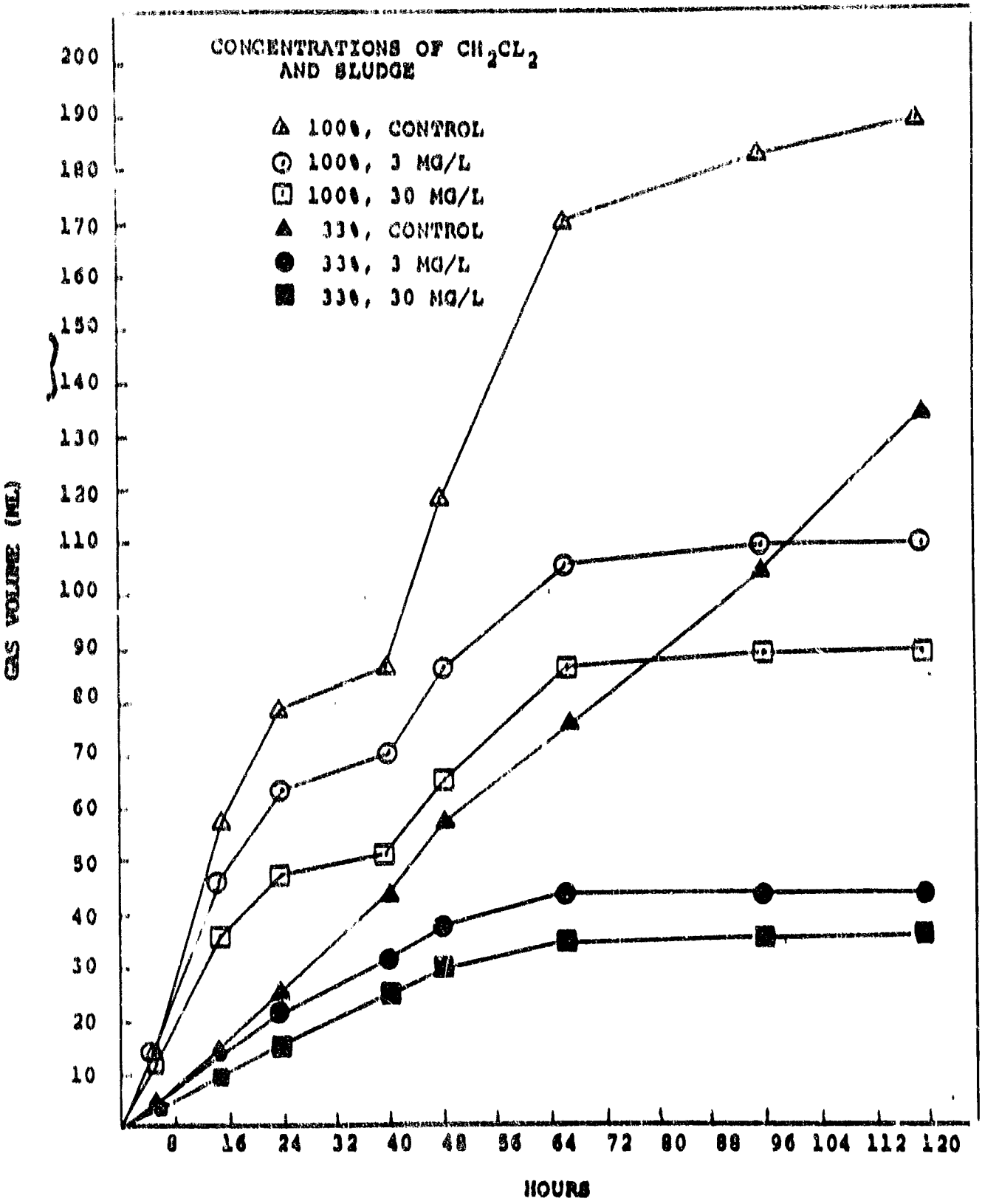

FIGURE 8. GAS PRODUCTION OF METHYLENE CHLORIDE (CH_2CL_2) INHIBITED SLUDGE AT TWO SLUDGE CONCENTRATIONS.

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Analyses

Methylene chloride were analyzed by gas chromatography. The results of the analyses of gas and sludge phases are shown in Tables VIII and IX. Accountability for methylene chloride was approximately 50%.

An atomic emission analysis of one sludge sample for heavy metals is shown in Table X. The analysis revealed no concentrations of metals which would be expected to affect the results with the possible exception of zinc.

DISCUSSION

The following concentrations (mg/l mixed liquor) of chlorinated C_1 and C_2 hydrocarbons were inhibitory to anaerobic sludge after 48 hours in this study.

Compound	20% Inhibition of Gas Production	50% Inhibition of Gas Production
Methylene chloride	3 mg/l	50 mg/l
1,1,1-Trichloroethane	1.4 mg/l	25 mg/l

TABLE VIII

FATE OF METHYLENE CHLORIDE IN ANAEROBIC SLUDGE BY GAS CHROMATOGRAPHIC ANALYSIS

Concentration mg/l Mixed Liquor, Sludge Concentration*	Concentration in Gas, mg/l	Total Gas Evolved, ml	Recovery from Gas, µg	Concentration in Sludge, mg/l	Recovery from Sludge, µg	Total Recovery, µg	Accountability, %
Control, 100%	0	189	0	0	0	0	—
3 mg/l, 100%	0.11	66.5	12.55	1.36	204	216.5	48
30 mg/l	1.75	88.3	154.5	15.2	2280	2424.5	54
Control, 33%	0	134	0	0	0	0	—
3 mg/l, 33%	0.181	45	8	1.44	216	224	50
30 mg/l, 33%	3.85	56	185.5	13.4	2010	2195.5	49

*Sludge, 100%, 45 g/l solids, 28 g/l volatile solids

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TABLE X

ATOMIC EMISSION ANALYSIS OF
ANAEROBIC SLUDGE FROM MT. PLEASANT
WASTEWATER TREATMENT PLANT*

Metal	Percentage
Fe	0.14
Cu	0.0082
Al	0.060
Ca	0.22
Mg	0.054
Mn	0.0018
Cr	0.0006
Zn	0.015
Ti	0.010
Sn	0.0027
Pb	0.003
Ni	0.0006
Sr	0.001
Cd	Not detectable, limit 0.00005
V	Not detectable, limit 0.0005
Sb	Not detectable, limit 0.001
Co	Not detectable, limit 0.0002

These levels were determined under the specific conditions of this test, i.e., 48 hr duration, single dose of solvent, 35°C, mild agitation, ethanol as the only food source added, and primary sludge from the Mt. Pleasant wastewater treatment plant. Referring to the inhibitory levels observed by other workers in Table I, there is a great deal of variation. Some of the factors which may affect the results are: time scale of experiment, solids concentration, composition of solids, active microbial concentration and any acclimation to chlorinated compounds. Nutrients added during the test may also affect the results.

Any other chemicals present in the sludge which could display a synergistic or antagonistic effect in combination with a chlorinated hydrocarbon may drastically affect the results from a study such as this.

For comparison with other chemicals toxic to anaerobic digester microorganisms, 1000 ppm magnesium or potassium would lead to about 20% inhibition (7). Heavy metals are much more toxic but are precipitated as sulfides and thus 99% removed from the biological system. Reported toxic total concentrations of copper, nickel, zinc and chromium range from 200 to 2000 mg/ℓ (7). Thus, the effective concentration is a few mg/ℓ for heavy metals, comparable to the most toxic of chlorinated C_1 and C_2 hydrocarbons.

We explored the effect of solids concentration on sensitivity to methylene chloride Tables VI and VII and Figure 8 show that at 48 hours there is relatively little difference between the percent of inhibition for the diluted and undiluted sludge. Continuing the observation period with more ethanol added at 48 hours, reveals a larger inhibitory effect in dilute sludge. The dilute sludge control is slowly working away on the ethanol food provided and appears as if it will catch up with the 100% control with both of them converting all the ethanol to gas.

The gas chromatographic analyses of sludge and vapor phase for methylene chloride show the extent to which adsorption on the sludge solids reduces the volatility of the solvent. Table XI takes the measured concentration data from Tables VIII and IX to calculate Henry's Law constants. Adsorption in a heavy grease or oil phase, or lipoids in the sludge microorganisms, could lead to the reduced concentrations of solvent in the gas.

One of the most important considerations in a digester which is operating poorly due to inhibition by a chlorinated hydrocarbon is recovery. Benson and Hunter (3) carried their inhibition experiments out 1200 hours and found that their most severely inhibited digester, 8 ppm, 1,1,1-trichloroethane, recovered in about 1100 hours. It was not possible within the time frame of this project to use such long periods.

Purging the digester with several volumes of nitrogen is one option, particularly with those compounds which are most volatile from aqueous solution, 1,1,1-trichloroethane However, vigorous gas purging of the anaerobic sludge will remove much of the dissolved CO_2 and may reduce the bacterial action for some time. The use of CO_2 as a component of the purge gas would alleviate this situation.

TABLE XI

COMPARISON OF OBSERVED HENRY'S LAW CONSTANTS WITH CALCULATED VALUES

	Conc. in Gas, mg/l	Conc. in Sludge, mg/l	H[a]	H[b]
CH_2Cl_2 3 mg/l 45 g/l sludge	0.11	1.36	0.08	0.14
CH_2Cl_2 30 mg/l 45 g/l sludge	1.75	15.2	0.12	--
CH_2Cl_2 3 mg/l 15 g/l sludge	0.18	1.44	0.13	--
CH_2Cl_2 30 mg/l 15 g/l sludge	3.85	13.4	0.29	--

[a]Apparent Henry's Law constant from gas chromatographic analysis.

[b]Calculated for 35°C, the temperature of the experiment, from data in (14).

Pretreatment, gas stripping of the primary sludge before it is added to the anaerobic digester, would be much more effective than stripping the digester contents if it is known that solvent has been spilled into a sewer system. Prior to the digester, air instead of nitrogen could be used to purge the sludge, without upsetting the microbial population. On a large scale, the volume of gas containing H_2S could present a potential air pollution problem if the digester were to be purged.

More appealing for removal of solvent from the sludge is addition of an adsorbent such as activated carbon or polymer beads. Simple adsorption on the solid will reduce the concentration of any chlorocarbon in a biochemical equilibrium. Removal of the adsorbent would then take the solvent out of the system.

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Doc Title 23

RESULTS OF SKIN ABSORPTION

STUDIES ON

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6

A.K. 5/10/83 1H

D-204

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The Dow Chemical Company

RESULTS OF SKIN ABSORPTION STUDIES ON

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Date March 29 1956 Date March 22, 1956

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PROBLEM

These materials are generally considered to cause dermititis due in part to their defatting action when in contact with the skin. However, little is known concerning the comparative toxicity of these materials by skin absorption. Browning in her book, "Toxicity of Industrial Solvents," reports that carbon tetrachloride and trichloroethylene have been found to be absorbed through the intact skin. However, there is apparently no reported data on the other materials. The study reported here was undertaken to establish the comparative toxicity of these materials by this route.

CONCLUSIONS

Animal experiments indicate that all of these materials are low in acute toxicity by skin absorption with

and the most toxic and

and chlorothene being the least toxic. The estimated LD_{50} for rabbits in gm. per kilogram of body weights were found to be as follows:

chlorothene and of the order of 10. When confined to the skin of rabbits for 24 hours under an impervious covering, all the materials burned the skin to a greater or lesser degree. Under the usual conditions of handling, this should not occur because these materials will evaporate readily.

SAMPLE INFORMATION

C.R.I. Name: Ethane: 1,1,1,-trichloro-,

Common Name: Methyl chlorcform; chlorethene

Source:

K No.:

Physical State: Water clear liquid

Molecular Formula: $C_2H_3Cl_3$

Structural Formula: $CH_3C\ Cl_3$

SUMMARY OF TOXICOLOGICAL DATA

Skin Absorption (24 hr. exposure)

Animal	Preparation Fed	Dose (gm./kg.)	No. Died / No. Treated	Response-Remarks
Rabbit	Undiluted	3.98	0/4	Slight edema and denaturation.
Rabbit	Undiluted	7.95	3/8	Slight edema and denaturation.
Rabbit	Undiluted	15.8	1/8	Skin severly burned with severe edema.

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UNIT INDEX

Results of skin absorption studies are given. A toxicological comparison is made of the materials.

INDEX HEADINGS

C.R.I. Name:

Common Name: Ethane: 1,1,1,-trichloro-,

Methyl chloroform; chlorethene

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COMPARATIVE SKIN AND EYE IRRITATION STUDIES ON CHLOROTHENE NU REFLUXED WITH WATER, IRON, GALVANIZED IRON AND ZINC AND SAMPLES OBTAINED FROM VAPOR DEGREASING EQUIPMENT

Chemical Name (300 per name) 25	CAS No. (10) 24
CHLOROTHENE NU	71-55-6

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The Dow Chemical Company

COMPARATIVE SKIN AND EYE IRRITATION STUDIES ON CHLOROTHENE NU REFLUXED WITH WATER, IRON, GALVANIZED IRON AND ZINC AND SAMPLES OBTAINED FROM VAPOR DEGREASING EQUIPMENT

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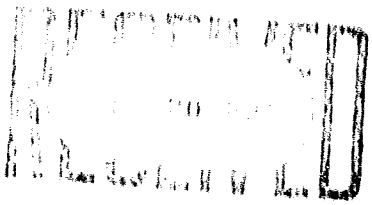

PROBLEM

Chlorothene NU is being proposed for uses involving prolonged contact with various metals and water. Information was desired regarding the effect that such use might have upon the ability of the product to cause irritation of the eyes and skin.

CONCLUSIONS

Slight to moderate increases were seen in the ability to cause irritation of the eyes and skin in samples which were refluxed with water and var-

CONCLUSIONS (continued)

ious metals. The irritation which developed may have been due to the acidity resulting from hydrolysis. The injury which resulted was typical of primary irritation. Chlorothene NU which had been in commercial use for up to 39 days was no more irritating than unused material.

These data together with the report from Dr. Klauder (T3.4-19-18) that unused Chlorothene NU has only a slight effect upon human skin are very encouraging. Consideration should probably be given to sensitization studies on humans using Chlorothene NU which has been in actual use for a long period of time.

PROCEDURE

The first eight samples received from TS and D were prepared in the following manner. Chlorothene NU (1000 ml) and water (500 ml) were refluxed for 44 hours in the presence of metal strips. The metal strips, 1/2" by 5" in size, were partially in the water and partially in the Chlorothene NU. One Chlorothene NU-water mixture had no metal strip and was used as a control. After refluxing the liquid phases were separated and sent to the Biochemical Research Laboratory and tested in the usual manner for irritating effects on the skin and eye. In addition, three samples which were taken from actual industrial applications involving the use of Chlorothene NU were also submitted. The first sample was from the boiling sump of a vapor degreaser run for 36 days. The sample contained approximately 30% mineral oil contamination. The second sample was from the boiling sump of a vapor degreaser running with approximately 30% mineral oil and after 39 days of operation. An excess of water had been added to this vapor degreaser 24 hours prior to the taking of this sample. The third sample was taken from the warm dip area of the same treatment as sample 2. The results are summarized in the following table.

SUMMARY OF RESULTS OF SKIN AND EYE IRRITATION STUDIES ON SAMPLES OF CHLOROTHENE NU REFLUXED WITH WATER AND VARIOUS METALS

Sample	Skin Irritation; ten 24-hour applications on the ear and belly	Eye Irritation; one application
Chlorothene NU, (See	Slight hyperemia and moderate sheet-like exfoliation.	Moderate pain with slight conjunctival irritation. Required several days to heal.
Chlorothene NU refluxed with water	Slight to moderate hyperemia, edema, and exfoliation.	Slight conjunctival irritation healed in 1-2 days. Moderate pain.
Water refluxed with Chlorothene NU	Essentially no response.	Slight conjunctival irritation; healed in 1-2 days.
Chlorothene NU refluxed with water and iron	Moderate hyperemia and exfoliation. Slight edema. Healing was delayed in the abraded area.	Slight edema, slight conjunctival irritation. Healed in 1-2 days.
Water refluxed with Chlorothene NU and iron	Slight exfoliation.	Slight conjunctival irritation healed in 1-2 days.
Chlorothene NU refluxed with water and galvanized iron	Moderate hyperemia, necrosis and exfoliation with a scar. An abraded area was also adversely effected. Only 6 applications were made.	Slight conjunctival irritation healed in 1-2 days.
Water refluxed with Chlorothene NU and galvanized iron	Very slight hyperemia and exfoliation.	Slight conjunctival irritation; required two days to heal.
Chlorothene NU refluxed with water and zinc*	Moderate exfoliation slight hyperemia and edema. Very slight necrosis with a scar.	Slight conjunctival irritation persisted for a week.
Water refluxed with Chlorothene NU and zinc*	Slight hyperemia and exfoliation	Slight conjunctival irritation healed in 1-2 days.

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Chlorothene NU from the boiling sump* Sample A	Slight hyperemia and exfoliation	Not studied.
Chlorothene NU from the boiling sump* Sample B	Very slight hyperemia.	Not studied.
Chlorothene NU from the warm dip Sample C	Slight hyperemia moderate sheet-like exfoliation	Not studied.

*These samples were slightly yellow in color.

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PRELIMINARY TOXICOLOGICAL EVALUATION OF THE SKIN IRRITATING AND SKIN ABSORBING PROPERTIES OF CHLOROTHENE IN RABBITS

Chemical Name (300 per name) 25	CAS No. (10) 24
CHLOROTHENE	71-55-6

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April 18, 1957

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PRELIMINARY TOXICOLOGICAL EVALUATION OF THE SKIN IRRITATING AND SKIN ABSORBING PROPERTIES OF CHLOROTHENE IN RABBITS

Results of range finding toxicological tests indicate that Chlorothene, when applied to the skin at the rate of 2 gm./kg./day for 20 days* produces no more than a slight to moderate irritation in rabbits.

The laboratory skin irritation tests were designed to simulate use conditions as nearly as practical. The animals were clipped and secured in a stock, belly up, during the working day. The daily doses were converted from a weight to a volume basis and applied in 4 aliquots, always to the same skin area. Body weights and skin thickness measurements were recorded weekly. Skin irritation was recorded daily. Following the 20th application the animals were sacrificed and observed for gross pathology. The liver and kidneys were weighed and saved for histopathological examination.

The animals on 0.5 gm./kg. and 2.0 gm./kg. of Chlorothene all showed essentially the same degree of skin irritation; that of slight hyperemia and slight to moderate exfoliation. For the most part the larger dose simply covered a proportionally larger area of skin. There was no gross pathology observed on these rabbits at autopsy. In spite of the strenuous experimental conditions all Chlorothene animals gained weight. From the accompanying table it should be noted that one of the water controls lost weight.

In parallel with the above study hair lacquer with and without Chlorothene was applied to one rabbit each. The pressure cans were discharged into a flask and the concentrated liquid residue was applied to the skin at a dose of 2.0 gm./kg./day. The skin irritating properties of this material under these conditions was similar to Chlorothene itself but the animal showed peritonitis, gray liver and congestion of the kidney medulla at autopsy. These reactions may or may not be due to the Chlorothene. Here again it should be noted that one of the control animals had what appeared to be a parasitic condition of

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* 5 days per week for 4 weeks.

REPEATED TOPICAL APPLICATION OF CHLOROTHENE TO RABBITS

R. No.	Material	Wt. (Kg.) I(1)	Wt. (Kg.) F	Dose/Day Gm./Kg.	Dose/Day cc(2)	Skin Thick. (mm) I(1)	Skin Thick. (mm) F	Organ Wt. Gm./Kg. Body Wt. L(3)	Organ Wt. Gm./Kg. Body Wt. K's	Response-Remarks
474F	Water (Control)	2.06	1.85	2.0	4.1	1.2	0.8	22.0	6.0	No irritation observed. Pathology negative.
473F	Water (Control	1.91	2.26	2.0	3.8	1.2	1.2	36.0	8.7	No irritation observed, liver parasitic.
472F	H. Lacquer (No Chlor.)	2.47	2.37	2.0	4.3	1.6	1.8	25.2	6.5	Slight exfoliation, pathology negative.
471M	H. Lacquer (Chlor.)	1.62	1.58	2.0	3.1	1.0	2.4	36.9	5.7	Slight hyperemia and exfoliation. Gray liver congestion of kidney medulla. Extensive peritonitis.
470M	Chlorothene	2.05	2.40	2.0	3.2	1.6	2.4	36.8	8.2	Slight hyperemia and moderate exfoliation. Gross pathology negative.
468F	Chlorothene	1.80	2.30	2.0	2.8	1.1	2.3	44.4	8.4	Same as above.
522F	Chlorothene	1.70	1.82	0.5	0.66	1.6	1.8	25.8	6.8	Slight hyperemia and slight exfoliation.
477F	Chlorothene	1.8	2.5	0.5	0.70	1.5	1.8	59.0	10.0	Slight hyperemia and moderate exfoliation. Gross pathology essentially negative.

(1) I = initial, F = final

(2) One fourth of the dose was given 4 times daily for 20 days.

(3) L = liver, K's = kidneys

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the liver. The experimental conditions of this study do not simulate or in any way resemble use conditions of hair lacquer containing Chlorothene.

In the opinion of the writer the data summarized in the accompanying table indicates if good care and cleanliness are exercised there should be no serious effects from skin irritation and absorption resulting from use conditions of Chlorothene. In order to further support this view, however, it is recommended that the data be expanded to include a greater variety of exposure conditions, larger numbers of animals and perhaps other species.

K. J. Olson

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Doc Title 23

A STUDY OF THE FATE OF METHYL CHLOROFORM IN RABBITS

Chemical Name (300 per name)	25	CAS No. (10)	24
METHYL CHLOROFORM		71-55-6	

A.K.
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Biochemical Research Department

The Dow Chemical Company

A STUDY OF THE FATE OF METHYL CHLOROFORM IN RABBITS

File
K No.
Chg.
Rept. By Samuel E. Allerton

Signed Samuel E. Allerton Checked V K Rowe

Date 10-25-56 Date 10-25-56

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For the investigator interested in the relation between chemical structure and function, the various chlorinated short-chain hydrocarbons present a challenging problem; the problem gains added interest from the fact that comparatively little information is yet known about the metabolism of these non-aromatic compounds, making deduction by analogy almost impossible. Yet experimental investigation is not easy, since these compounds are often rather resistant to sensitive chemical analysis,[1] and there are variations among the species in the way these substances are metabolized.[2]

The investigator is confronted with a wide spectrum of toxicity, even among members of a homologous series,[3] and he may be inclined to relate the degree of toxicity to certain chemical changes occurring in the living organism (although the importance of purely physical changes has been justifiably stressed by Stuber).[4] These chemical changes harm the organism as a result of interference with normal body processes by the compound or its metabolites,

and one can usually only guess as to the nature of that interference. It is somewhat misleading to assume that a metabolic scheme based on the isolation of a few intermediates provides an adequate biochemical explanation of toxicity data; that explanation must lie in the effect of the production, accumulation, and excretion of these compounds on the living organism. Thus, the metabolism of trichloroethylene results in the production of a variety of different compounds, but the intervening steps are none too clear;[5] monochloroacetic acid has been identified as a trace metabolite, but in what way and to what extent does that compound fit into the toxicology picture on trichloroethylene?[6]

This paper will describe a short-term study of the metabolisms of methyl chloroform, a solvent marketed as Chlorothene[7] by The Dow Chemical Company for industrial use in degreasing, etc. A previous paper reported vapor toxicity data for a number of different species of animals.[8] It was found that methyl chloroform is much less toxic than its relative, carbon tetrachloride; for example, repeated daily exposure of rabbits to 5000 ppm produced no ill effects. von Oettingen reports that methyl chloroform is readily absorbed through the lungs, but that nothing is known about its fate or excretion.[9]

EXPERIMENTAL

1. Development of Method of Analysis for Methyl Chloroform.

A. The Fujiwara reaction.

Approximately 800 ml. of methyl chloroform was redistilled, the fraction collected having a boiling point of 73.8-74.4 at 760 mm. (K1716-19).

A Fujiwara color test was carried out,[10] using reagent grade sodium hydroxide and redistilled pyridine. Methyl chloroform does give a positive reaction, but it is not sufficiently sensitive for detection at concentrations of 30 mg.% and below, even though proportions of reagents and solvent, heating time and heating temperature be varied. Turbidity and color fading occur at various times, despite closing off of the system and shortening of the heating period. It was found that the pink color obtained with high concentrations of methyl chloroform was destroyed irreversibly by acid on standing. Although the pink solutions often had strong absorption maxima in the ultraviolet (~365 mμ.), this was not always observed and cannot be used for routine analysis. Methyl chloroform is quite resistant to chemical attack, hydrolysis occurring very slowly, even with the use of alkali. Silver nitrate produces a very slight turbidity, this inertness being characteristic of compounds having accumulated halogen on one carbon atom.

B. Volhard determination of volatile halide.

Alford's modification of a method for determination of volatile halide[11] is superior to the Fujiwara colorimetric method for the following reasons: the reactions which occur are known, it can be easily adapted to analysis of any biological material (of which large samples can be used), the colors of blood and urine are of no consequence, only oil-soluble, volatile halide is determined so that chloride ion and polar organic chlorine compounds cannot interfere, and the method takes full advantage of the abundance of chlorine in methyl chloroform.

The principle of this method is the removal of volatile halide (and some water) from mineral oil (used in extraction) by a warm current of purified air (silica gel and soda lime drying towers), passage through a straight reflux condenser and burning to hydrochloric acid on platinum gauze in a hot quartz combustion tube; the hydrochloric acid is absorbed in a 1% sodium carbonate - 0.2% arsenious oxide solution after passage through a sintered glass bubbler.

The procedure followed was that proposed by Alford, with the following modifications: silica gel and soda lime drying towers were used to purify the laboratory air; a 1-liter boiling flask with electric heating mantle was used

to volatilize the methyl chloroform; ball-joint glass tubing was used to convey the vapors to the combustion furnace, and an electrically heated platinum-filled quartz tube wrapped in asbestos tape was used to thermally decompose the vapors of methyl chloroform.

When a known amount of methyl chloroform was made up in mineral oil and analyzed as though it were a blood sample, recovery was excellent:

Actual concentration = 1.05 mg.%

Concentration found = 1.07 mg.%

When a methyl chloroform-ethanol solution was added to blood and extraction with paraffin oil was attempted, the blood and oil emulsified easily and separation was difficult. Xylene was tried as an extractant, but although separation of layers occurred readily, recovery was never greater than 75%, perhaps because the considerable vapor pressure of xylene interferes in the complete volatilization of methyl chloroform. Transformer oil, noted for its failure to emulsify with water, was then tried as an extractant for a 32.9 mg.% methyl chloroform solution in blood (with ethanol).

Concentration found = 29.2 mg.% (89% Recovery)

This recovery was boosted to over 90% by several changes in procedure: in addition to the initial 15 ml. of oil,

three 5 ml. volumes of oil were used; water was used in the last two extractions, along with the oil; aeration was prolonged for one hour and fifteen minutes, with a vigorous air flow during this last fifteen minutes. A thermometer resting between the warm flask and the heating mantle was not allowed to exceed 140°C. in temperature.

2. Blood and Urine Levels of Methyl Chloroform after Intravenous Administration.

A. Procedure.

0.25 ml. methyl chloroform was injected into the marginal ear vein of each rabbit. After various intervals, 3 ml. blood samples were collected via cardiac puncture, placed under oil, and shaken. Urine samples were obtained by applying abdominal pressure, 5 ml. for methyl chloroform analysis (under oil) and 1 ml. for trichloroacetic acid (TCA) - chloral hydrate detection. The method of Waldman and Krause was used for the latter, the Fujiwara reaction being quite sensitive for these oxygenated compounds.[12] The rabbits used were:

#917 2.68 kg. male
#929 2.66 kg. male

B. Results.

No methyl chloroform was found in the blood of #917 15 minutes after injection; none was found in the urine

sample taken 1 1/3 hours after injection. Neither this urine sample nor one collected 19 1/3 hours after injection of #929 revealed the presence of TCA or chloral hydrate. In a later experiment, a 2.57 kg. male rabbit received 0.25 ml. methyl chloroform intravenously and blood samples taken 2, 3, and 5 minutes after injection contained no detectable methyl chloroform. A large female rabbit (3.26 kg.) died rapidly after receiving 0.50 ml. methyl chloroform intravenously.

3. Blood and Urine Levels of Methyl Chloroform after Chronic Oral Administration.

A. Procedure.

Female rabbits were given methyl chloroform and corn oil via stomach tube. The rabbits used were:

#985 2.61 kg.

#997 2.67 kg.

The feeding schedule was as follows:

Time:	0 hrs.	1 5/6 hrs.	18 1/4 hrs.	20 1/4 hrs.
Dose:	1 gm./kg.	1 gm./kg.	2 1/2 gm./kg.	1 gm./kg.
Accum. Dose:	1 gm./kg.	2 gm./kg.	4 1/2 gm./kg.	5 1/2 gm./kg.

Blood samples were analyzed for methyl chloroform; urine samples were analyzed for methyl chloroform, TCA and chloral hydrate.

B. Results.

One hour after the final feeding, the blood level of methyl chloroform in #985 was 2.8 mg.%; two hours later it had risen to 3.7 mg.%. The urine contained no detectable methyl chloroform after the first two feedings and 1.2 mg.% 3 hours after the final one. The rabbits seemed drowsy and docile one hour after the third feeding. A standard test for urine glucuronides (hydrolysis, naphthoresorcinol, ether extraction, Klett reading at 570 mμ.) revealed glucuronide levels 1 1/2 times that of one control rabbit, but this might be accounted for by normal fluctuation in glucuronide excretion. No TCA or chloral hydrate was found in the urine.

4. Vapor Exposure Experiments.

This is the type of exposure most often encountered industrially. Female rabbits were given repeated daily exposures at two levels: 4000 ppm and 10,200 ppm in a chamber similar to those described in the report on vapor toxicity.[13] Dow Chlorothene was used instead of pure methyl chloroform.

A. Results.

(1) 4000 ppm.

(a) Rabbits used: #990 (2.65 kg.) and #991 (2.00 kg.). #990 received eight 7-hour exposures. Blood level of methyl chloroform varied from 1.4-2.8 mg.%,

averaging 2.0 mg.%. No methyl chloroform was found in the urine at the end of the first exposure. TCA and chloral hydrate were repeatedly absent in blood and urine. At the end of the eight exposures, tissues were removed and analyzed for methyl chloroform content:

Fat	120 mg.%
Liver	14.9 mg.%
Lungs	7 mg.%
Brain	5.2 mg.%

#991 received nine 7-hour and one 7 1/2-hour exposures. Blood level of methyl chloroform after the ten exposures was 1.3 mg.%. Tissue analysis was as follows:

Fat	86.5 mg.%
Liver	9.3 mg.%
Lungs	4.1 mg.%
Brain	3.1 mg.%

(2) <u>10,200 ppm</u>.

(a) Rabbits used: #160 (2.38 kg.), #162 (2.20 kg.), and #163 (2.37 kg.).

#160 received three 7-hour exposures and then died of an unsuccessful heart puncture. At the end of the first exposure, the blood level of methyl chloroform was 4.5 mg.%, and the urine

level was 1.8 mg.%. After the third exposure, the urine level was 2.5 mg.%. No TCA or chloral hydrate was found in the blood or urine.

#162 received five 7-hour exposures. Blood levels of methyl chloroform at the end of these exposures ranged from 4.4-8.4 mg.%. The urine level was 5.6 mg.%. No TCA or chloral hydrate was found in blood or urine. At the end of the five exposures, tissues were removed for analysis:

Fat	155 mg.%
Liver	10.9 mg.%
Lungs	6.4 mg.%
Brain	8.3 mg.%

#163 received three 7-hour exposures. The blood level of methyl chloroform at the end of the third exposure was 4.3 mg.%; in the urine it was 5.0 mg.%. The results of tissue analysis:

Fat	208 mg.%
Liver	8.2 mg.%
Lungs	7.8 mg.%
Brain	5.4 mg.%

No TCA or chloral hydrate was detected in blood or urine, although in all these tests, 1 mg.% would have been detected.

5. Blood Level of Methyl Chloroform During Acute Narcosis.

A 2.34 kg. male rabbit was put into deep narcosis by intranasal exposure to large amounts of methyl chloroform vapor. The reactions of the rabbit were as follows: licking, grinding of teeth, heart rate increase, heart rate decrease, steadying of respiration, and narrowing of pupils. The rabbit began screaming, so exposure was stopped and a 5 cc. blood sample taken five minutes later. The rabbit was still in a heavy stupor, but was not screaming. The pupils of the eye remained narrow for some time. The concentration of methyl chloroform in the blood was 2.1 mg.%.

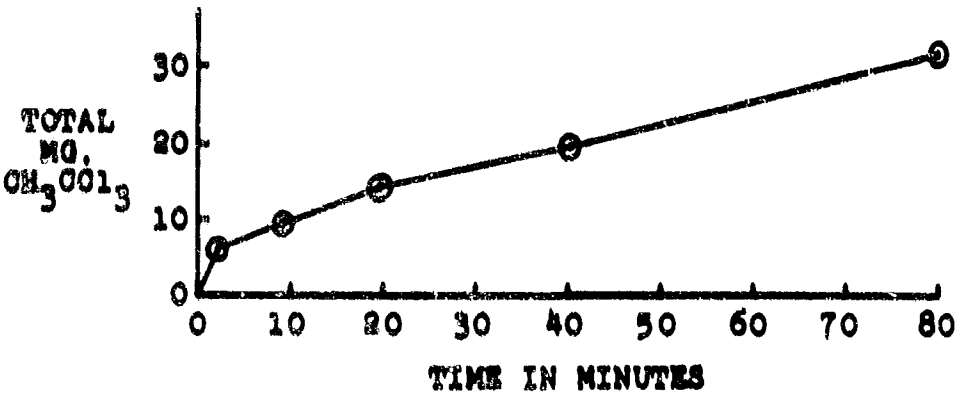
6. Elimination of Methyl Chloroform via Lungs.

A. Procedure.

A white female 3.10 kg. rabbit was placed in a wooden stocks. A latex glove with plastic tubing connected to one finger was placed over the rabbit's head and its left ear was exposed by drawing it through one of the fingers of the glove. The sleeve of the glove was drawn snugly around the neck of the rabbit and the tubing was connected to the combustion apparatus. Another piece of tubing was taken in through the sleeve and into one of the fingers to allow adequate ventilation. The vacuum pump was started up and 0.25 cc. of methyl chloroform was injected (330 mg. into the marginal ear vein).

B. Results.

The time course of methyl chloroform exhalation is shown below:



It was impossible to continue collecting the expired air, so that it is not known what fraction of the injected material was removed by the lungs; however, about 10% of the injected dose was eliminated by the lungs within 80 minutes.

DISCUSSION

It appears that this work fits into a general outline of methyl chloroform metabolism, as shown below:

CH_3CCl_3

↓

blood ⟶ lungs ⟶ CH_3CCl_3 and metabolites

blood ⟶ kidney ⟶ CH_3CCl_3 and metabolites

blood ↓↑ body tissues

1. $CH_3CCl_3 \longrightarrow Cl_3CCH_2OH \longrightarrow Cl_3CH(OH)_2 \longrightarrow Cl_3CCOOH$
2. $CH_3CCl_3 \longrightarrow CH_3COOH$, etc.
3. $CH_3CCl_3 + RCH_2R' \longrightarrow CH_3CHCl_2 + RCHClR'$

Blood levels of methyl chloroform have been determined in rabbits during repeated oral and vapor exposures. The lungs are an important route for the elimination of methyl chloroform, but are not sufficiently effective to account for its rapid disappearance from the blood stream after intravenous administration. Methyl chloroform has been found in the urine after repeated vapor exposure at 10,200 ppm, the concentration increasing to about 5 mg.%. The distribution of methyl chloroform among several types of tissue after vapor exposure has been determined for four rabbits; the fat-soluble chlorinated hydrocarbon is especially abundant in fatty tissue, although it does not seem to accumulate in large amounts in the brain.

The fate of methyl chloroform in the body tissues has not been established. The tissues may, in part, act only as a reservoir, gradually releasing methyl chloroform into the blood stream. It is likely, however, that some of the methyl chloroform is transformed chemically along one or more pathways, such as those indicated in the above outline.

The first pathway, to 2,2,2-trichloroethanol, chloral hydrate, and trichloroacetic acid, is quite plausible, but no evidence has been found of chloral hydrate or trichloroacetic acid in blood or urine of rabbits during our work. Blood or urine have not been examined for 2,2,2-trichloroethanol or urochloralic acid, however. Butler is correct in his assertion that 2,2,2-trichloroethanol gives a negative Fujiwara reaction (no pink color), but a

color is produced varying from light yellow to orange-brown in more concentrated solutions.[14] A suitable colorimetric method for detection of this compound is based on its conversion to formaldehyde by alkali.[15]

The second pathway, to acetic acid, looks more promising in view of a radioactive tracer study performed with a relative, carbon tetrachloride.[16] Some of the labeled carbon was found to have entered the 1-carbon pool as carbon dioxide, etc. It is entirely possible that methyl chloroform can enter the 2-carbon pool as active acetate, a normal important biochemical intermediate.

The third pathway is described as a "peroxide-induced exchange of hydrogen and chlorine between saturated hydrocarbons and polychloroalkanes" by West and Schmerling, who describe a number of such reactions occurring <u>in vitro</u>.[17] The yields are excellent when the catalyst is present; however, there is no evidence at all for an <u>in vivo</u> reaction as a result of exposure to methyl chloroform.

SUGGESTIONS FOR FURTHER WORK

1. A radioactivity recovery experiment with C-labelled methyl chloroform could be performed, with particular attention given to substances participating in or resulting from the "2-carbon pool." The use of Cl-labelled methyl chloroform would provide information on the lability and subsequent fate of the chlorine atoms.

2. If methyl chloroform competes with normal reduced substrates for dehydrogenase or other enzymes active in oxidation, the enzymatic activity in such preparations as cell extracts from liver should be correspondingly affected.
3. Whether or not 2,2,2-trichloroethanol or urochloralic acid participate in methyl chloroform metabolism should be decided one way or the other.
4. The site, means, and extent of production, accumulation, and excretion of any intermediates should be examined with respect to the stress thereby placed upon the "host" organism, so that ultimately these findings may adequately support the toxicity data.
5. More needs to be learned about the chemistry of the Fujiwara reaction, so that it may be used to better advantage in the study of these polychlorinated short-chain hydrocarbons.

SUMMARY

1. No methyl chloroform could be detected in the blood of rabbits 2-3 minutes after intravenous injection of 125 mg./kg. body weight. No TCA or chloral hydrate was found in the urine.
2. The blood level of methyl chloroform after a cumulative oral dose of 3.5 gm./kg. was approximately 2-4 mg.%. No TCA or chloral hydrate was found in the urine.
3. Repeated vapor exposure at 4000 ppm resulted in blood methyl chloroform levels of 1-3 mg.%. At 10,200 ppm, blood levels

varied from 4-8 mg.%. Tissue analysis of four rabbits revealed an appreciable amount of methyl chloroform in depot fat and much lesser amounts in liver, lungs, and brain. No TCA or chloral hydrate was found in blood or urine.

4. After intravenous injection of about 100 mg./kg. of methyl chloroform in a rabbit, about 10% of this dose was eliminated via the lungs within 80 minutes.

The author gratefully acknowledges the excellent cooperation and assistance of members of the Biochemical Research Department.

NOTES

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UNIT INDEX

Results of studies to elucidate the metabolism of methyl chloroform are given. The material rapidly disappears from the blood; is not converted to trichloroacetic acid or chloral hydrate; is slowly eliminated from the lungs; and deposits in the depot fat to a greater extent than in any other tissue. It is postulated that the material may be converted to the acetate and enter the 2-carbon pool.

INDEX HEADINGS

C.R.I. Name: 1,1,1-Trichloroethane
Methyl chloroform
Chlorothene

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Doc Title 23
OAK RIDGE SPECIFICATIONS FOR
CHLOROTHENE

Chemical Name (300 per name)	25	CAS No. (10)	24
CHLOROTHENE		71-55-6	

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D-2.1

8782/0973

THE DOW CHEMICAL COMPANY

MIDLAND MICHIGAN

November 21, 1955

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SUBJECT: OAK RIDGE SPECIFICATIONS FOR CHLOROTHENE

Since my last letter to you (October 19, 1955) on this subject, we have run some acute vapor toxicity tests that were designed to give information suitable for making recommendations for changes in the first draft of the subject specifications. The results of these tests are outlined briefly below:

Groups of 20 rats (10 male and 10 females per group) were given single exposures to atmospheres containing 10,000, 12,000, or 14,500 ppm of Chlorothene vapor for periods of 7.0, 7.0, and 4.0 hours, respectively. At 14,500 ppm by volume (equivalent to approximately 65,000 ppm by weight) all of the rats were unconscious within one hour. One rat died after an exposure lasting for 1 hour and 10 minutes. A total of 4 animals had died at the end of 2 1/2 hours; and at 4 hours, when the experiment was terminated, 6 rats of the total of 20 had died in the chamber.

At the 12,000 ppm level, some animals were still conscious at the end of 3 hours; however, one male and one female died before the end of the 7-hour exposure. At the 10,000 ppm level, the rats were all conscious at the end of 3 hours and none of the animals died although some animals showed a decrease in body weight.

Based on the above information and a discussion on the subject given in my previous letter, it is suggested that a rewording in the draft on Chlorothene specifications for Oak Ridge be made as follows.

8788109 73

In Table 1 - Physical Requirements, change approximate allowable concentration in air to 500 ppm (minimum) rather than 550 ppm (maximum) as now stated.

Section 4.3.1 - Allowable Concentration in Air. A glass-wall chamber or similar chamber having a capacity of approximately 200 liters shall be used. A constant air flow shall be maintained through the chamber and the air metered at the rate of 20 liters per minute through a Precision Wet Test meter, or other suitable device such as an accurately calibrated rotometer. The vapor concentration shall be obtained by metering the material at a constant rate into a suitable container for complete volatilization into the main air flow. The apparatus used may consist of a burette discharging into a Phipps and Bird glass Nebulizer, or by another suitable device such as a gear driven syringe pump apparatus, into a volatilization flask. A minimum of heat shall be applied at the point of addition to effect complete volatilization. A group of 10 rats (5 of each sex) shall be introduced into the chamber and observed for a period of 7 hours. All of the rats shall be healthy albino rats and shall weigh approximately 200 grams each. The concentration in the chamber shall be 10,000 ppm by volume (55 milligrams of material per liter of air calculated at 25°C. and 760 mm. Hg.) At the end of 7 hours, all animals shall be alive and at least one of the animals shall not be comatose at the end of 3 hours.

Please advise us if you have any further questions or if we can be of any additional help in this matter.

Very truly yours,

D. D. McCollister
Biochemical Research Department
12-694 Building
Phone - 6988

DDM/jwm

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Doc Type 22
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Doc Title 23
AN INVESTIGATION OF THE POTENTIATING EFFECTS OF ETHANOL ON A CHRONIC EXPOSURE OF RATS TO 1,1,1-TRICHLOROETHANE

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6

A.K. 5/27/83 1A

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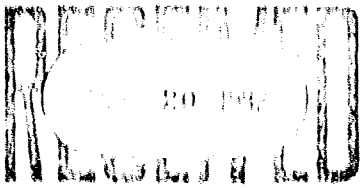
AN INVESTIGATION OF THE POTENTIATING EFFECTS OF ETHANOL ON A CHRONIC EXPOSURE OF RATS TO 1,1,1-TRICHLOROETHANE



A Thesis

by

DEWAYNE HAROLD WALKER

Submitted to the Graduate College of
Texas A&M University in
partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

August 1973

Major Subject: Laboratory Animal Medicine

000002

AN INVESTIGATION OF THE POTENTIATING EFFECTS OF ETHANOL ON A CHRONIC EXPOSURE OF RATS TO 1,1,1-TRICHLOROETHANE

A Thesis

by

DEWAYNE HAROLD WALKER

Approved as to style and content by:

(Chairman of Committee)

(Head of Department)

(Member)

(Member)

(Member)

August 1973

000003

ABSTRACT

An Investigation of the Potentiating Effects of Ethanol on a Chronic Exposure of Rats to 1,1,1-Trichloroethane. (August 1973)

DeWayne Harold Walker, D.V.M.,

University of Minnesota

Directed by: Dr. Archie Flowers

Accidents represent a disease entity which has reached epidemic proportions in our society. This unfortunate status holds true even in an industrial environment where great emphasis has been placed on preventive medicine. One contributing factor is alcoholism, which can mediate its effects in the form of direct damage to the body and/or by predisposing workers to accidental injuries. In certain industrial environments workers are chronically exposed to other chemical agents, such as chlorinated hydrocarbon compounds used in degreasing operations, which can likewise cause direct or indirect injuries. In this investigation the hypothesis was made that chronic ethanol intoxication in conjunction with a supposedly safe exposure to a chlorinated hydrocarbon, 1,1,1-trichloroethane, could result in injuries more extensive than is seen with either agent individually.

Four groups of 12 rats each were exposed to one of the following combinations: 350 parts per million (ppm) 1,1,1-trichloroethane and ethanol treatment, 350ppm 1,1,1-trichloroethane and no ethanol treatment, ambient air and ethanol treatment, ambient air and no ethanol treatment. The appropriate groups were exposed to 350ppm 1,1,1-trichloroethane vapors, 24 hours per day for 56 days. Ethanol was administered daily to rats at a level of 1.8 g/kg body weight by intraperitoneal injection.

Three parameters were selected to evaluate the postulated synergistic effect of ethanol treatment and 1,1,1-trichloroethane exposure. The body weight gains or losses of the rats over the 56 day period represented the first. No indication of a synergistic effect was evident over the 8 week period, however, reduced weight gains were demonstrated in ethanol treated rats, ethanol x time interaction ($P < .025$).

The tilting plane and swimming to endurance tests were selected as the psychophysiologic tests in evaluating coordination, equilibrium and endurance. No synergistic effect was reflected in the results of either test. As expected, an increasing decrement in performance was observed in the animals receiving ethanol, ($P < .001$) for the tilting plane ethanol x time interaction and ($P < .005$) for the swimming test ethanol x time interaction.

Surprisedly, increased performance was seen in both tests as a consequence of 1,1,1-trichloroethane exposure. The significance of the atmosphere x time interaction was ($P < .001$) and ($P < .05$) for the tilting plane and swimming tests, respectively.

The direct bodily injury effect of the agents was evaluated by serum glutamic pyruvic transaminase (SGPT) determinations, liver weights, and gross and histopathologic examination of various organs. Pre and post experimental SGPT values were evaluated for all 4 groups. The only significant, ($P < .005$), SGPT increase was reflected in the ethanol treated groups.

Liver weights were reported both as actual weight and as liver weight relative to body weight. The overall average liver weight of the 1,1,1-trichloroethane exposed rats was significantly higher than control atmosphere rats, ($P < .001$). No significant ethanol effect or synergistic effect was noted.

Finally, gross and histopathologic examination of the lungs, kidneys and liver of each rat was conducted. Chronic respiratory disease (CRD) was noted in all rats; however, a higher incidence of moderate CRD involvement was seen in rats exposed to the 1,1,1-trichloroethane. Kidney lesions were evenly distributed within the 4 groups. Liver necrosis was noted to be nonexistent in

the 1,1,1-trichloroethane exposed ethanol treated rats but present in the other 3 groups. The control rats had a higher incidence of liver lipid infiltration than the treated rats.

This investigation did not demonstrate a synergistic relationship between ethanol treatment and 1,1,1-trichloroethane exposure. The investigation did, however, demonstrate the following 3 previously unreported findings concerning 1,1,1-trichloroethane exposure; an apparent psychomotor stimulatory affect, significant liver weight gains, and augmentation of the severity of existing lung lesions.

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INTRODUCTION

The American Conference of Governmental Industrial Hygienists have established on a yearly basis threshold limit values (TLV) for numerous industrial solvents. These values represent maximum airborne concentrations at which no adverse effects are expected for repeated human exposure to a substance. The Committee establishing these guidelines, however, also noted that because of a wide variation in individual susceptibility to like substances, a small percentage of workers may experience discomfort from some substances at concentrations below the threshold limit. Likewise, a small percentage of workers may be affected more seriously by development of an occupational illness or by aggravation of a pre-existing condition (1). The excessive intake of alcohol represents a preexisting condition which has been shown to enhance the injurious properties of various substances (2,3).

Health statistics demonstrate the fact that alcoholism is not a rare entity among the industrial ranks and that alcohol related industrial accidents and deaths represent a major health problem in America (4,5). The

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The citations on the following pages follow the style of the journal of Laboratory Animal Science.

effects of alcohol intoxication and exposure to an industrial substance are two-fold since such a combination can predispose an individual to both direct and indirect injuries to the body. Direct injury implies the straightforward cause and effect relationship of the exposure producing frank pathologic visceral lesions. Indirect injury refers to the possible increased likelihood of an accidental injury as a consequence of loss of consciousness, equilibrium and/or coordination due to the exposure.

The purpose of this investigation was to evaluate on the direct and indirect effects of a combination of exposures of ethanol intake and 1,1,1-trichloroethane inhalation on a chronic basis. This combination of exposures on a chronic basis has not been investigated at the realistic low levels one would encounter routinely in an industrial degreasing operation. The assumption that such a working situation exists is not too remote a possibility since the solvent 1,1,1-trichloroethane is being utilized by the millions of gallons annually and since the prevalence of alcoholism in industry is calculated to be as high as 3% of the American working force (5,6,7).

During the course of the experiment 350ppm 1,1,1-trichloroethane, the TLV, was administered through inhalation to rats 24 hours each day for 56 days. Ethanol

was administered daily to members of designated groups at a level relative to body weight. The direct effects of the exposure were evaluated by weight gain/loss, serum glutamic pyruvic transaminase levels (SGPT), and gross and histologic examination of the visceral organs of the subjects. The effect of the combination of exposures on equilibrium, coordination and endurance of the subjects was evaluated by two psychophysiologic tests, the tilting plane and swimming to endurance.

The results this study may encourage other investigators to evaluate further the apparent stimulatory properties of 1,1,1-trichloroethane at low level exposures. It is also hoped that the results may assist in establishing future guidelines for the use of 1,1,1-trichloroethane in industry.

REVIEW OF LITERATURE

ACCIDENTS AND HEALTH

In 1965, as in the past, accidents in the United States were the fourth most important cause of death for all age groups, exceeded only by heart disease, cancer, and vascular lesions of the central nervous system (8,9). Between 1951 and 1965 accidents represented the leading cause of death among all persons aged 1 through 36 years. In actual numerical figures the needless cost of human lives becomes even more apparent. In the United States in 1965, over 107,000 persons, or the equivalent of 55.2 persons out of every 100,000 persons, died as a result of injuries from accidents (8).

The number of accidental injuries of a nonfatal variety is also a staggering figure. In 1965 there were over 10.8 million disabling accidents. Included in this figure were 400,000 accidents that resulted in some degree of permanent disability ranging from the partial loss of a finger to blindness or complete crippling (8). Kiefer (9) who considers accidents as an epidemic or a pandemic or a great accumulation of endemics, estimates that at any given time patients injured in accidents occupy 65,000 American hospital beds. According to the National Health Survey, if injuries of a lesser severity are included in

the total to include injuries causing 1 or more days of restricted activity or requiring medical attention, the total would be about 56,000,000 injuries, 20% of the nation's population. Although it is impossible to place a price tag on death or human suffering, Recht (5) estimates that accidents cost this nation about $17.3 billion annually in lost wages, medical expenses, insurance, property damage and indirect costs of injuries resulting from work accidents. This figure represents about one-third of the current U.S. defense budget or about 40% of the total U.S. corporate profits after taxes. The statistical consideration of the subject of accidents makes it most obvious that this disease is a major health problem in America.

ACCIDENTS IN THE INDUSTRIAL ENVIRONMENT

Like the medical profession, industry has made tremendous advancements in safeguarding the health and welfare of its population. Permanent in-shop physicians, nurses and preventive medicine officers have contributed immensely in minimizing accidents and providing a healthful industrial environment. These precautions have no doubt resulted in the saving of numerous lives throughout the years; however, the statistics of industrial injuries

and deaths still parallel the figures previously cited (8,10).

In 1965 over 14,000 deaths occurred as a consequence of injuries from work condition accidents. Industrial accidents also account for about one-fifth of all disabling injuries (8). In American industry over 8,400,000 workers were injured annually between 1960 and 1970, with 2,300,000 of these being disabling injuries. The principal types of nonfatal work injury accidents include: handling objects, struck by falling or moving objects, machinery and falls. These 4 types account for over 66% of the total work injuries (10). Falls represent the greatest single cause of accidental deaths, accounting for 18% of such deaths in 1965 (11).

Once again in industry as with individuals, it is impossible to equate human suffering or the loss of a life with a price tag. It is estimated, however, that in 1971 work injuries cost industry $9.3 billion, a cost to industry of $120.00 per worker (10).

ALCOHOLISM AND HEALTH

Alcoholism like accidents is considered by several authors to be a disease (4,7,12). The dictionary defines chronic alcoholism as a, "pathological condition, affecting chiefly the nervous and gastro-enteric systems,

caused by the habitual use of alcoholic beverages in poisonous amounts" (13). There is, however, an obvious difference between the disease of accidents and the disease of alcoholism. Accidents are normally considered a disease of acute and sudden onset, whereas true alcoholism is a progressive disease which develops to a serious state only after 2 to 15 years of habitual intoxication (4). Alcoholism also differs considerably from accidents in terms of recognition. An accident of any consequences will usually appear in the statistics eventually in one form of data or another, whereas alcoholism is a disease entity which is often hidden and protected from surveys and questionnaires by involved individuals.

ALCOHOLISM IN THE INDUSTRIAL ENVIRONMENT

Alcoholism is a disease which thrives as successfully in an industrial environment as the disease of accidents. The recognition of the disease entity in an industrial situation, however, is not always an easy task. Maxwell (5) has noted from informal observation and inference from other studies that many problem drinkers are effectively hidden from the general recognition of management by supervisors and fellow workers, by the employee's own efforts, and by the widely held stereotype of how disorganized an alcoholic is supposed to be. Statistics as

a whole, therefore, probably run well below the true estimate of alcoholism in American industry. Although accurate figures are difficult to obtain, it has been estimated that there are at least 2 million problem drinkers within the labor force of America (7). Other authors have calculated from surveys and statistical sampling that the incidence of severe cases of alcoholism in industry runs between 1.1% and 3% of all employees (4,14).

ALCOHOLISM AND ACCIDENTS IN THE INDUSTRIAL ENVIRONMENT

The relationship of alcoholism and accidents is a most obvious one which is usually exemplified by noting the daily automobile death toll. It is an unfortunate but well known fact that approximately 50% of all highway accident deaths involve a drunken driver (10). The predisposition to accidents as a consequence of alcohol intoxication is also recognized in industry. Maxwell (5) in a study of a large company employing over 10,000 employees, compared the activities of 40 problem drinkers against 96 matched nonalcoholic employees and noted that problem drinkers experienced 3.6 times more on the job accidents than the nondrinking group. Winslow et al (15) in a similar study concurred with the results of Maxwell and added that in their study problem drinkers

were noted to be rated lower by superiors, paid less salary, and worked only 77% as efficient as a comparable control group. One can, therefore, conclude that alcoholism not only affects the financial and job security of a worker but more importantly has an indirect effect upon his personal safety and health by predisposing him to possible injury.

THE DIRECT EFFECTS OF ALCOHOL CONSUMPTION

The effects of ethanol ingestion originate in actions on neural, hormonal and metabolic systems. Ethanol modifies each system and also alters the complex interactions between various physiologic effector and regulatory systems. The difficulties in disentangling this profusion of intertwined phenomena are further accentuated by the differences in the action of alcohol caused by variations in dosage and in rate of consumption. The infinite number of possible combinations of ethanol consumption and resulting types and degrees of pathologic injury makes it an almost impossible task to even begin to review the subject. It is, however, a medical fact that in addition to the classical liver involvement associated with chronic excessive drinking, there has also been reported damage to the pancreas, gastrointestinal

tract, brain, cardiac and skeletal muscle systems (15,17, 18,19).

ETHANOL AND LIVER DAMAGE

The association of ethanol consumption and resulting liver lesions has been reviewed and argued for well over a century. The first suggestion that overindulgence in alcoholic beverages may damage the liver was made by Thomas Addison, a physician to Guy's Hospital, London in 1836 (20). His observation of repeated cases of fatty degeneration of the liver among alcoholics stimulated early interest in the liver specificity of ethanol. This early interest in this relationship was later reinforced when metabolic studies showed that the elimination of ethanol from the organism is almost entirely due to its oxidation in the liver. In man, the liver is responsible for removal of about 95% of the ethanol from the body (21). However, the etiological role of alcohol predisposing directly to liver damage, as if it was a hepatotoxin, has been and still is a highly controversial subject.

One of the first serious works to suggest a direct relationship between ethanol consumption and corresponding liver lesions was performed on dogs by MacNider in 1933 (22). Although his experiment was based upon high dosages

administered acutely, he did show that when dogs were intoxicated for a period of 12 hours to such a degree as to become semi-anesthetized that liver damage did develop The injury consisted of edema of the peripheral cells of the liver lobules accompanied by an accumulation of stainable lipoid material. A second group of animals intoxicated in the same manner for 24 hours exhibited an extension and increased severity of liver lobular edema involving the cells of the entire lobule. Berman et al (23), utilizing dogs with biliary fistulas, noted that the appearance and odor of bile collected from these dogs following ethanol intoxication, resembled that obtained from humans with hepatitis or partial biliary obstruction and that the depressing action of ethanol on cholic acid and total pigment spoke very forcibly for the hepatotoxic action of ethanol.

These early studies stimulated considerable interest in the possible hepatotoxic properties of ethanol; however it soon became apparent to many workers that this relationship is not a straightforward one but rather one containing numerous variables. The most important variable interrelated with ethanol and accompanying liver damage is that of nutrition. A considerable number of investigations have been conducted which have dealt with liver injury as a consequence of excess carbohydrates, excess

fat intake, lack of lipotropic factors, starvation, a combination of these variables or still other newly recognized nutritional factors. The extent to which ethanol effect on the liver is modified by these dietary factors, especially dietary protein and fat, still needs exploration; thus, most of the questions remain unanswered at this time. To date only the relationship of ethanol and lipotropic factors has been adequately studied (24,25,26).

1,1,1-TRICHLOROETHANE

1,1,1-Trichloroethane (1,1,1-trico) or methyl chloroform has become an increasingly popular agent for use in cold cleaning, dip cleaning, and bucket cleaning of metals for the removal of greases, oils and waxes. In recent years inhibited 1,1,1-trico, inhibitors being other additives designed primarily to control corrosion of common metals, has found increasing use in vapor degreasing operations (27,28). In addition, the volatility and solvent power of 1,1,1-trico closely resembles that of carbon tetrachloride and consequently in recent years it has come into wider use as a substitute product (28). Overall, the industrial use of 1,1,1-trico now exceeds 35 million pounds per year (6).

The threshold limit value (TLV) for 1,1,1-trichloroethane is presently 350 parts per million (ppm) versus much lower values of 10 to 100ppm for most of the commonly utilized industrial organic solvents. The TLV for any given agent, as adopted by the American Conference of Governmental Industrial Hygienists, refers to the air concentrations of substances, and represents conditions under which it is believed that nearly all workers may be repeatedly exposed 8 hours per day, 40 hours per week, without adverse effect (1).

The recent popularity of 1,1,1-trico has brought about a considerable number of studies to evaluate its toxicity. Adams et al (20), in one of the first investigations, reported that the LD50 for 1,1,1-trico for rats was 18,000ppm for 3 hours or 14,000ppm for 7 hours. The principal toxic effect produced by single exposures at these levels was a depression of the central nervous system such as that typical of an anesthetic agent. Relatively minor organic injury, consisting of fatty changes and some necrosis of the liver, was produced only by exposures having strong anesthetic effects. The results of repeated 7 hour exposures, given 5 days a week for 4 to 10 weeks were as follows: At 5,000ppm rats and rabbits exhibited a slight retardation of growth, while guinea pigs had moderate fatty changes of the liver,

testicular degeneration and poor growth. At 3,000ppm rats and monkeys suffered no adverse effects whereas guinea pigs had slight liver damage and some depression of growth. At 1,500ppm and at 650ppm guinea pigs exhibited no organic injury but a slight retardation of growth.

Torkelson et al (30) ran an acute and chronic study involving several species of laboratory animals and several different routes of administration. The administration of single oral doses of 1,1,1-trico yielded an LD50 range of 5.6 g/kg for rabbits to 12.9 g/kg for rats. The agent, when placed in the eyes, was shown to cause slight transitory irritation with little likelihood of permanent injury. The toxicity from skin absorption was found to be low with doses of 4 g/kg failing to kill any rabbits exposed for 24 hours. Repeated multiple daily applications of 500 mg/kg to the skin of rabbits caused no effect other than reversible local irritation. A series of inhalation exposures was also conducted on various laboratory animals at 500, 1,000, 2,000 and 10,000ppm. Rabbits, guinea pigs, rats and monkeys were unaffected after 6 months of repeated exposures, 7 hours per day, 5 days per week at 500ppm. Female guinea pigs were found to be the most sensitive in previous experiments but in this investigation they were able to

tolerate 1,000ppm for 0.6 hours per day and 2,000ppm for 0.1 hours per day for 3 months with no detectable adverse effects. Male rats tolerated exposures of 16,000ppm for 0.5 hours per day with no organic injury.

Prendergast et al (31), in a continuous 90 day exposure of laboratory animals at 370ppm, noted that there was less body weight gain in dogs and rabbits exposed to the vapors of 1,1,1-trico than in control animals. Gross and histopathologic examination of these subjects, however, did not reveal any abnormalities attributable to exposure. In exposures of animals to 2,200ppm 1,1,1-trico for 8 hours per day, 5 days per week for 30 days, no significant hematologic differences were noted in the exposed rats, guinea pigs, rabbits and dogs.

HEPATOTOXIC AND NEPHROTOXIC PROPERTIES OF 1,1,1-TRICHLOROETHANE

One of the areas of study which has been given considerable priority is that of evaluating the hepatotoxicity and nephrotoxicity of the agent. Such investigations with 1,1,1-trico have undoubtedly been prompted by the infamous past histories of closely related chlorinated hydrocarbons such as carbon tetrachloride, 1,1,2-trichloroethane and chloroform. These compounds, in several investigations, have been shown to manifest mild

to severe pathologic effects in the liver and kidney even at very low concentrations and for very short exposures (32,33,34,35,36,37,38,39). In contrast to these chlorinated hydrocarbons, 1,1,1-trico has been reported to have minimal pathologic effects upon the liver and kidney.

Plaa et al (34) evaluated subcutaneous injections of 7 halogenated hydrocarbons, including carbon tetrachloride, 1,1,2-trichloroethane, chloroform, trichloroethylene, tetrachloroethylene, tetrachloroethane and 1,1,1-trichloroethane, in regards to liver damage in the mouse. The study, utilizing prolongation of pentobarbital sleeping time, bromsulfalein retention and microscopic examination revealed that 1,1,1-trico had the least hepatotoxic potency. In a later study these same agents were evaluated following intraperitoneal injections. Sulfobromophthalein retention and serum glutamic pyruvic transaminase (SGPT) activity were utilized as the hepatic tests. It was found again that 1,1,1-trico was the least toxic agent with only mild hepatic dysfunction noted even when the agent was administered at near lethal doses (36)

An investigation of the potential nephrotoxic properties of a group of chlorinated methane, ethane and ethylene derivatives by Plaa and Larson (38), showed that the nephrotoxic properties of 1,1,1-trico were as minimal as the hepatotoxic properties of the agent. Mice given

intraperitoneal injections of the agent at levels high enough to cause 70% mortality still showed neither necrosis nor tubular swelling at necropsy.

HUMAN EXPOSURES TO 1,1,1-TRICHLOROETHANE

Torkelson et al (30) conducted the first investigation dealing with the effects of human exposures to 1,1,1-trico vapors. Table 1 (p. 18) contains the results of his series of human exposures.

Stewart et al (40) conducted a series of 6 controlled human exposures to 1,1,1-trico and obtained results which quite closely paralleled those of Torkelson and his associates. The study, with emphasis on forensic toxicology, established that 1,1,1-trico had a prolonged exponential decay in the expired air which was predictable enough to allow an estimation of the magnitude of exposure 0.5 to 3 hours following the exposure.

Several years later Stewart et al (40) evaluated the effects on humans of exposure to 1,1,1-trico on a more prolonged basis. Eleven subjects were exposed to 500ppm 1,1,1-trico for periods of 6.5 to 7 hours per day for 5 days. The overall effects were of doubtful clinical significance. The only adverse response was an abnormal modified Rhomberg test (the subject stands on one foot with his eyes closed and his arms at his sides) observed

TABLE 1[a]

Results of single exposures of humans to the vapors of 1,1,1-trichloroethane

Concentration (ppm)	Exposure time (minutes)	Symptoms and response
840 (450-710)	90	No effect other than the smell of the chemical
500 (415-590)	450	Moderate smell which tended to disappear. Examination made before and after exposure found no significant changes in pulse, respiration, blood pressure, reflexes and equilibrium. Liver function tests were also negative.
1000 (890-1190)	30	Unpleasant odor, equilibrium apparently not disturbed.
920 (900-1000)	70-75	Transient eye irritation, mild degree of lightheadedness and a slight but definite loss of coordination and equilibrium as determined by the Flanagan and Rhomberg tests. Recovery appeared quite rapidly, 5 to 10 minutes after the end of the exposure session. Electrocardiograms were normal throughout the exposure. All liver function tests and hematology tests were normal.
1900 (1740-2100)	5	Very noticeable odor. Obvious disturbance of equilibrium with a positive Rhomberg test.

[a]Note: Taken from Torkelson et al (30).

in 2 of the subjects. No clinical laboratory test performed during or following the vapor exposures revealed any abnormality of liver or kidney function.

Salvini et al (42) evaluated psychophysiologic responses after two 4 hour exposures, separated by a 1.5 hour interval, to 450ppm 1,1,1-trico vapors. It was noted that the agent caused a 20% drop in the perceptive test, 6% drop in instantaneous memory, and a 2.5% decrease in reaction time. It was also concluded from further studies of the interaction of vapor exposure and psychophysiologic testing that a significant decrease in all around performance may occur when persons under mental stress are exposed to the vapors.

1,1,1-TRICHLOROETHANE METABOLISM

The favorable toxicologic picture found with both acute and chronic exposures of animals to 1,1,1-trico is due to the ability of the animal to excrete the agent rapidly and almost in entirety via the respiratory system. Hake et al (43), after intraperitoneal injections of radioactive labeled 1,1,1-trico into rats, found that 98.7% of the dose could be accounted for as unchanged 1,1,1-trichloroethane-1-C^{14} in the expired air. The only metabolite found besides CO_2 was 2,2,2-trichloroethanol, excreted as the glucuronide in the urine. The rapid and

almost total excretion of the 1,1,1-trico undoubtedly accounts for the low toxicity of the agent.

ACCIDENTS ASSOCIATED WITH 1,1,1-TRICHLOROETHANE

Although studies have shown that 1,1,1-trico is a benign agent under various controlled test conditions, overexposure to the agent can still be dangerous. Brief nonfatal vapor exposures and nonfatal accidental ingestion of the agent have been reported on occasions and there undoubtedly have been numerous nonfatal unreported episodes (8,44). In terms of fatal exposures there have been 10 deaths reported to date which were attributable solely to 1,1,1-trico poisoning.

Kleinfeld and Feiner (44) reported the first death due to industrial use of 1,1,1-trico. They reported an incident in which 4 workers were overcome by 1,1,1-trico vapors while degreasing conduit pipes in a confined underground vault. Three of the 4 workers recovered while the fourth worker collapsed and stopped breathing shortly after leaving the vault. It was estimated that the victim had spent approximately 10 minutes in the vault where the vapors had risen well in excess of 5,000ppm. Prior to this first industrial case there had been 2 nonindustrial deaths attributable to abusive inhalation of the agent (45). Stahl et al (46) reported on the toxicologic

analysis and pathologic findings of 6 deaths involving naval personnel utilizing 1,1,1-trico as a cleaning agent in confined spaces. Hatfield and Maykonki (47) reported on the fatal overexposure of a worker cleaning the inside of a 460 gallon wing-tip airplane tank with the 1,1,1-trico. In all of these deaths vapor exposures exceeded 5,000ppm with the level estimated in the latter case to be as high as 62,000ppm (47).

Although pathologic damage in each of the previously described fatal cases varied from individual to individual and was dependent upon exposure levels, the following lesions were consistently noted: congestion and moderate edema of the lungs, congestion of the liver, spleen, kidneys and mild edema of the brain.

POTENTIATING EFFECTS OF ETHANOL AND 1,1,1-TRICHLOROETHANE

Ethanol has previously been associated with the potentiation of hydrocarbons. Individuals recovering from an acute ingestion of ethanol are more susceptible to the liver damaging properties of the halogenated hydrocarbons than individuals not receiving ethanol (48). Cornish and Adefuin (3), in a study of ethanol ingestion in combination with acute inhalation exposures to a series of halogenated hydrocarbons, demonstrated ethanol potentiation of hepatic damage with carbon tetrachloride and

trichloroethylene inhalation. Kutob and Plaa (2) noted that pretreating mice with single and multiple intoxicating doses of ethanol before challenging with a minimal hepatotoxic dose of chloroform resulted in an increased incidence of liver damage. The mechanism of action for this synergistic effect is poorly understood. Kutob and Plaa (2) have hypothesized that ethanol increases liver lipids and that the increase results in an increased partitioning of the hydrocarbon into the liver, thereby exposing the liver cells to an increased amount of the agent. Other researchers have hypothesized that there is a paralleling relationship between increased hepatic damage and elevated triglyceride levels (49,50,51,52). Mallow and Block (53) reported that total liver lipids content reached maximum elevation 16 hours after the administration of ethanol. Brodie and co-workers (52) noted maximum triglyceride levels 18 hours after ethanol administration. Liver triglyceride levels reportedly remained at increased levels, approximately twice that of nonethanol treated controls, for up to 21 hours after ethanol consumption. Triglyceride levels eventually returned to a normal range approximately 40 hours after the ethanol consumption (2,52).

1,2,2-Trichloroethane is a hydrocarbon which has also been investigated in relation to ethanol potentiation.

Cornish and Adefuin (3) administered 5 g/kg ethanol to rats and 16 to 18 hours later exposed different groups of the treated rats to several exposure levels of 1,1,1-trico: 5,000ppm for 6 hours, 10,000ppm for 4 to 6 hours, 10,000-15,000ppm for 2 hours. It was reported that the exposures, even at a level just below the lethal range did not alter serum enzyme levels in the rats treated with ethanol. Negative findings were also noted upon examination of the lungs, liver, kidneys, spleen and adrenal glands of these rats. Similar but more extensive studies were performed in mice and dogs by Klaassen and Plaa (36,37). In their first study, utilizing mice, increases in sulfobromophthalein (BSP) retention time were observed in those mice given 3 days of pretreatment with ethanol followed by a LD50 intraperitoneal injection of 1,1,1-trico on the 4th day. The elevation of BSP retention was also noted in mice given a 12 hour pretreatment with ethanol. Liver damage was confined to the following observed lesions: enlargement of hepatocytes, cellular infiltration, vacuolation, and an occasional incidence of very mild hepatic necrosis. No significant variations were noted in the kidney function test, phenolsulfonephthalein (PSP), between mice pretreated with ethanol and controls injected with 1,1,1-trico. On necropsy no significant microscopic changes were noted in the kidneys

of the mice pretreated with ethanol. In their second investigation, utilizing dogs, Klaassen and Plaa (37) found no difference in SGPT or PSP levels in control animals versus test animals given 4 g/kg ethanol 24 hours prior to an intraperitoneal ED_{50} dose of 1,1,1-trico. Histopathologic examination of the liver and kidneys of the animals pretreated with ethanol revealed no demonstrable variations from those observed in the control animals.

Ethanol did not potentiate the toxicity of 1,1,1-trico to a point where measurable effects were noted under the acute experimental conditions reported in these investigations. To date no investigation has been conducted to evaluate the possibility of ethanol potentiation of 1,1,1-trico on a chronic basis.

EXPERIMENTAL EVALUATION PROCEDURES AND TESTS

Many diversified types of procedures and tests have been developed for use in evaluating the effects of exposure to various chemical agents. In addition, other standardized procedures and tests have been modified to fit the needs of a given subject under investigation. For this study the possible potentiating effects of the combination of 1,1,1-trico inhalation and ethanol administration were evaluated utilizing the following procedures

and tests: body weight gains or losses of test subjects, psychophysiologic tests, SGPT determinations, and liver weight determinations.

Previous studies involving ethanol consumption have shown that lower gains in body weight are seen in rats treated with ethanol versus controls (24,49,54). Mallov (26) observed that young chronically intoxicated rats gained from 34% to 75% as much weight as controls. Such a finding is still unexplained but it is suggested that alcohol in some way affects food utilization, increases caloric loss or that the calories from ethanol are less available for growth (26,49). Retardation of growth has also been reported with chronic 1,1,1-trico exposure (29).

One of the most important considerations in this investigation was the effect of the 1,1,1-trico vapor alone and in combination with ethanol consumption upon motor activity, in particular the effect upon the endurance, coordination, and equilibrium of the subject. To provide acceptable data covering these motor activities a number of tests are available, including the maze, rope climbing, treadmill, and the shutter box (55,56,57). However, careful scrutiny of these tests has revealed that learning and motivation differences often confuse the interpretation of the test results. Since evaluation of physical performance rather than psychologic responses

was the goal of this phase of the investigation, a test or tests involving an unlearned natural physical response would provide the described data. The investigator, therefore, selected the tilting plane test as the best procedure available for evaluation of equilibrium and coordination, and swimming to exhaustion as the best procedure for evaluation of physical endurance.

The tilting plane test is a procedure which permits rapid and easy measurement of motor capability, requires little or no practice by the animal, and is a relatively nonstressful test. The tilting plane test is highly suitable for repeated measurement in the same animals and for use in conjunction with other tests. The test consists of the subject being placed with all four feet resting on a flat surface which is then slowly tilted upward until the animal slides backwards. The angle at which the animal is no longer capable of holding onto the board surface, and thus begins to slide, is the measurement recorded. The tilting plane test to date has found its greatest application in evaluating equilibrium and motor coordination in ethanol intoxication studies (58,59,60,61,62,63). Kosa (61), however, has utilized the apparatus for evaluating the effects of barbiturates and related drugs, in addition to ethanol. Arvola et al (58) first introduced the test and found the procedure superior

to numerous other tests, including the maze, swimming time, and righting reflex, for distinguishing different levels of ethanol intoxication. He found that 4 or 5 separate statistically significant levels of impaired performance could be noted in rats tested on the plane following the oral consumption of 0 to 7.2 g/kg ethanol. The most important consideration for the test appears to be the material utilized as the board surface. Friedman and Ingalls (60) demonstrated that on a smooth surface the more intoxicated rats slid at steeper angles, which is in contrast to earlier findings. The correct surface for the accumulation of reliable data appears to be a rough surface which allows the animal to slightly dig into it with his claws. Standard Finnish hardboard, 80-mesh bronze netting or the rough side of masonite board have been utilized with great success (58,59,61,62,63).

Swimming has been utilized extensively as a test of performance or of exercise capacity following various exposures (63,64,65,66,67). In addition, forced swimming has been utilized to study the effect of stress upon body weight, the effect of stress in association with ethanol intoxication, and the ability or inability of forced swimming to cause pulmonary emphysema in test subjects (68,69,70,71,72).

In this phase of the investigation the objective was to evaluate the effect of the various combinations of ethanol and 1,1,1-trico exposure upon the stamina of the test subject. The test consists of releasing the rat into a tank of water, from which there is no escape, and allowing the rat to swim to a predetermined state of exhaustion. The elapsed time between the release of the rat into the water until the designated state of exhaustion is reached is the data recorded. The actual arbitrary sign of exhaustion selected by the investigator is not as important as the fact that this same end point be duplicated for all of the test subjects. The end point selected by investigators in previous studies have varied from a state of exhaustion in which coordinated muscular movement has ceased to the extreme end point of allowing the rat to remain submerged on the tank bottom for 60 seconds (73,74). Ershoff (66) found that rats submerged for 15 seconds below the water surface rarely returned to the surface. He found that of the 30 rats tested only 2 rats rose to the surface after remaining submerged for a period of 15 seconds. A period of 10 to 15 seconds has, therefore, become the end point chosen by most investigators.

Since it has been reported that unweighted rats in thermoneutral water may swim for several hours, one of

the important points concerning swimming to exhaustion is to reduce the swimming time of the test subjects to a practical duration of time (73,75). Investigators have attempted to reduce the swim time of test subjects and at the same time standardize the work performed by 2 means; alteration of the temperature of the water and/or addition of weights to the swimming animal.

It appears from the data of numerous studies that animals swim for their maximum period of time in water at or slightly below the normal deep body temperature. Wilbur and Hunn (73) found that in albino mice the swimming capability dropped from over 1 hour in water at 25°C to 6.5 ± 0.5 minutes in 15°C water. Matoush et al (76) found a similar effect in rats. He noted that rats swam one-third as long in 17°C water as they did in 25°C water. Past studies, therefore, indicate that adjustment of water temperature can shorten swimming times and perhaps reduce extreme variations among individuals.

The second means of reducing swimming time is by the addition of weights to the body of the test subjects. Normally, the weights applied are lead strips wrapped about the distal portion of the tail or attached by means of a harness about the abdominal region. Various amounts of weight have been applied to test subjects, including a constant load or a relative load based upon body weight

(76,77). The latter choice is probably the more desirable method of applying a load to the test subject as it is more impartial when animals of extreme size difference are compared for swimming capacity.

Serum glutamic pyruvic transaminase (SGPT) was selected as the liver function test of choice in this investigation to complement the necropsy findings of the study. Wroblewski and La Due (78) have concluded that SGPT is a more sensitive test than serum glutamic oxaloacetic transaminase (SGOT) in depicting acute hepatocellular damage. Cornish and Adefuin (3) found SGPT an acceptable means of noting toxic responses when rats were exposed to various halogenated aliphatic solvents following ethanol ingestion.

Significant increases in liver weight in comparison to body weight have been reported with 1,1,1-trico exposure in guinea pigs (30). Likewise, liver weight increases relative to body weight have been reported in chronic ethanol studies (25,49). Mallov (26) found that ethanol promotes the accumulation of liver lipids in the rat in as short a period of time as 1 month. He reported that in this short time span, rats treated with ethanol could develop livers weighing 36% more than those of isocalorically fed controls.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Male, Sprague Dawley rats (Rattus rattus norvegicus) weighing 200 ± 20 grams, obtained from Hilltop Laboratory, Philadelphia, Pennsylvania, were utilized as the animal model in this investigation. The rats were held in quarantine for 2 weeks observation and adjustment after which 40 animals were randomly selected as test subjects. The 40 animals were divided into 4 equal groups consisting of animals exposed to the following combination of agents: Group 1 consisted of animals exposed to ambient air. Group 2 consisted of animals exposed to ambient air but receiving a daily administration of ethanol. Group 3 consisted of animals exposed to 350 parts per million (ppm) 1,1,1-trico. Group 4 consisted of animals exposed to 350ppm 1,1,1-trico but also receiving daily administration of ethanol. Four additional animals were randomly selected as replacement rats to substitute for any subjects expiring during the preliminary testing period. Throughout the investigation the rats were housed individually in steel cages, had free access to water, and were maintained ad libitum on Purina Laboratory Chow (Ralston Purina Co., St. Louis, Missouri). All animals

were handled according to the "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research.

PRELIMINARY LABORATORY PROCEDURES

The following preliminary procedures were conducted during a 5 week period on each group member and on the 4 replacement rats: weekly weighing, weekly introductory swimming sessions, and baseline SGPT determination.

Test subjects were weighed during the preliminary period by the use of a triple beam balance (Harvard Apparatus Co., Dover, Massachusetts). Animals were weighed to the nearest gram. The purpose of this preliminary series of weighings was to determine whether there were any inherent group differences which might affect the experimental portion of the study. The series was also conducted to aid in eliminating unthrifty test subjects prior to commencing with the testing period with the result that 1 rat was culled and replaced with a suitable substitute from the replacement group.

To reduce the possibility that some rats may swim better than others, as a consequence of quicker adaptation to the stress, all of the rats were forced to swim prior to the chamber experimentation. The swimming sessions consisted of placing 4 test subjects at a time in a 20

gallon container of 21° ± 1°C water and allowing them to swim for a specific period of time. Commencing with the second week of the preliminary period each rat was forced to swim for 5 minutes. During the following weeks the swimming period for each rat was progressively increased to 7, 10, and finally 12 minutes.

The SGPT procedure conducted in this study began with the collection, via cardiac puncture, of 0.6 ml of blood from each of the 40 test animals. Blood was collected in 1 ml tuberculin syringes with 25 gauge 5/8" needles from animals lightly anesthetized with methoxyflurane (Penthrane, Abbott Laboratories, North Chicago, Illinois). Two-tenth of a ml of serum was squeezed from the fresh blood clots and SGPT levels determined by the modified Reitman-Frankel method read at 515 mu. Two test subjects died as a result of the cardiac punctures and were, therefore, replaced with 2 replacement rats.

Twenty-eight additional animals were randomly selected for use in a series of 4 preliminary ethanol studies. The first study was designed to determine basic background information concerning the metabolic breakdown time of ethanol following its administration. The main objective was to establish the fact that ethanol is metabolized in entirety within 24 hours postadministration; therefore, eliminating any concern that alcohol blood

levels may accumulate as a consequence of repeated daily administration of ethanol. The data collected from the 3 remaining studies was collected to demonstrate the approximate blood alcohol levels expected in animals being routinely tested 20 to 60 minutes after ethanol administration.

In the first study 10 rats were administered per os a 15% w/v solution of ethanol at a dose of 5.0 g/kg body weight. At 1, 2, 4, 8, and 24 hours postadministration a 0.2 ml blood sample was collected via cardiac puncture from each rat by means of a 25 gauge 5/8" needle and 1 ml tuberculin syringe, under light methoxyflurane anesthesia. Two animals died during the course of this study. The data collected from these animals was discarded and the procedure repeated with 2 replacem nt rats. In the remaining studies ethanol was administered to animals in each group in the following combinations: 15% w/v solution given orally at a dose of 2.5 g/kg body weight; and 10% w/v solution given by intraperitoneal injection at a dose of 1.5 g/kg body weight. In each of these studies blood was collected in the same manner as previously described; however, collections were made at 20, 40, 60, 80, and 100 minutes postethanol administration.

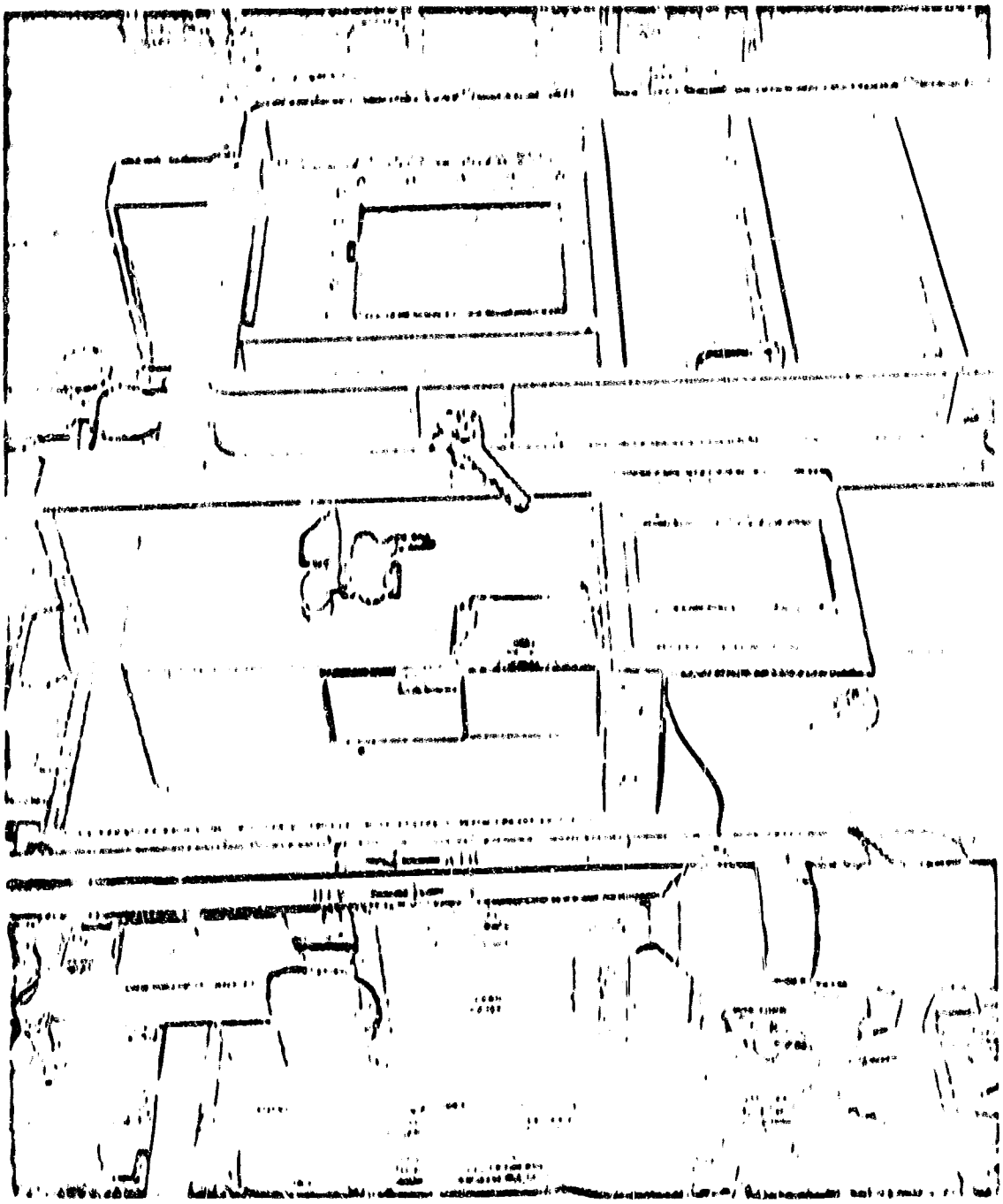
The 0.2 ml of blood collected from the animals in each of the study groups was immediately placed in a stoppered 25 ml Erlenmeyer flask containing sodium

chloride granules. Blood alcohol analysis was performed by gas chromatography taking advantage of the vapor phase of the gas-liquid equilibrium of ethanol as described by Wallace and Dahl (79).

EXPERIMENTAL CHAMBERS

The chambers utilized in this study were of approximately equal size, 8' x 8' x 8', although different in actual design. The hypobaric chamber utilized for the 1,1,1-trico atmosphere had a stainless steel interior with a 3' x 3' x 8' entrance lock. The chamber utilized for the ambient (control) atmosphere had a painted interior with no entrance lock (Figure 1, p. 37). Installed equipment in each chamber provided for automatic control and continuous recording of barometric pressure, temperature, and relative humidity. Each chamber was set to maintain a temperature range of 23° ± 1°C and a relative humidity range of 30% to 60%. During the course of the investigation a daily log of morning and afternoon temperature and humidity readings was maintained on both chambers. Lighting within the chambers was provided by fluorescent lights which were manually operated for 10 hours of light daily. The chambers were adjacent to one another and thus were subject to the same external lighting, noise, and other external stimuli.

FIG 1. AMBIENT ATMOSPHERE CHAMBER



The chamber utilized for the 1,1,1-trico vapors was modified to fit the needs of the investigator in the following fashion. Two human face masks were fitted to the inside of the chamber with an inlet and outlet hose to the outside air (Figure 2, p. 40). An additional hose, for the external sampling of the 1,1,1-trico level within the chamber, was fitted 3 feet off of the floor. All hose connections were stoppered from the outside of the chamber when not in use.

AIR-VAPOR GENERATION SYSTEM

Many dynamic systems have been developed for generating a known concentration of various vapors for human or animal experimentation. Unfortunately, a number of these systems are not suitable for vapor generation of all agents and many other systems do not provide for accurate maintenance of vapors at low concentrations. The system eventually selected was a modification of the system designed by Haskell Laboratory, E. I. duPont de Nemours and Co., Wilmington, Delaware, for the investigation of fluorocarbon 113 (80). The equipment utilized in the study is listed in Table 2 (p. 41) and is seen assembled in Figure 3 (p. 43).

The vapor generation system functioned basically in the following manner. 1,1,1-Trico, contained within a

FIG 2. INVESTIGATOR WITH FACEMASK WORKING WITHIN 1,1,1- TRICHLOROETHANE CHAMBER

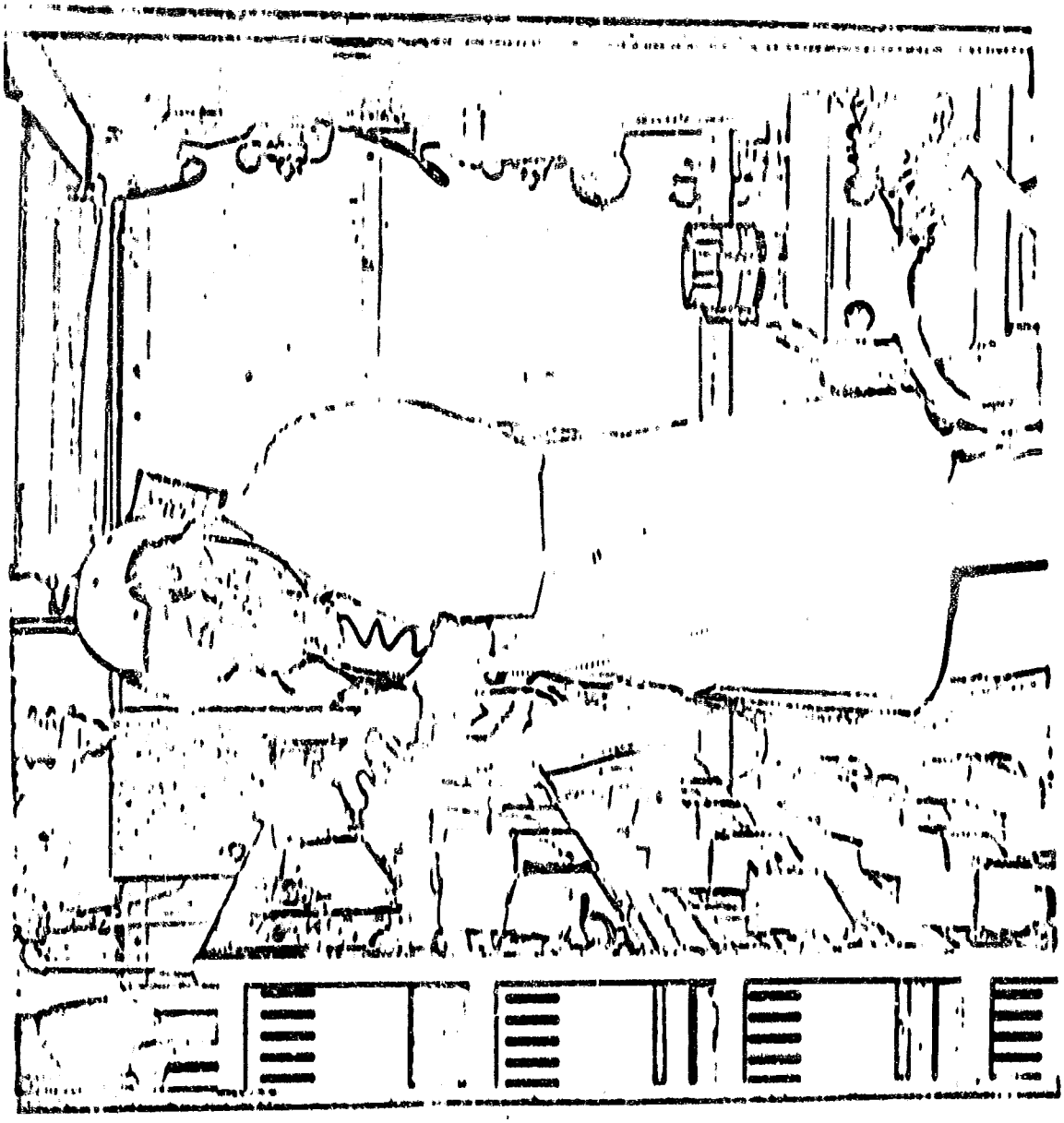


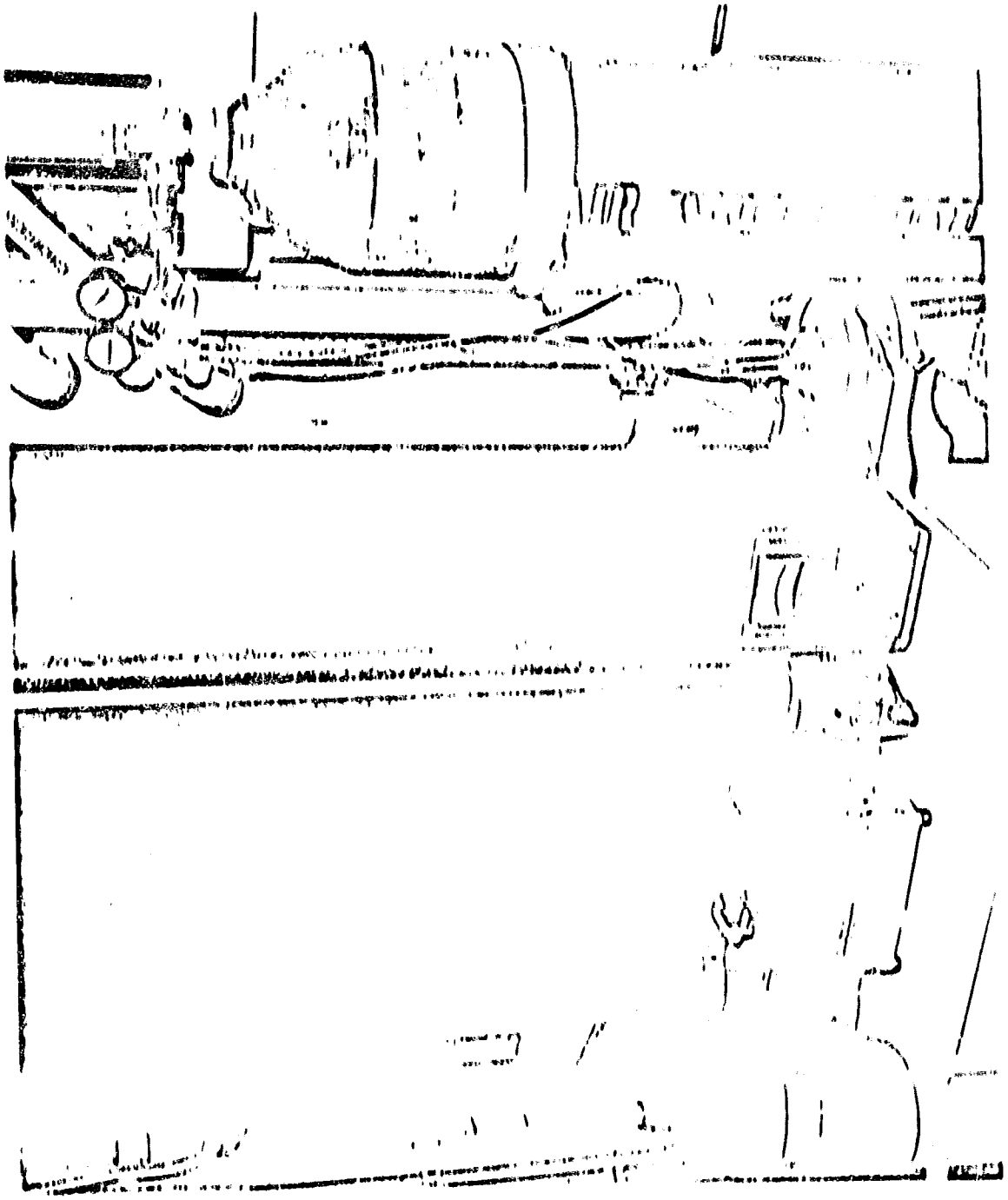
TABLE 2

Air-vapor generation system

Item	Source
Auto analyzer proportioning pump with voltage stabilizer	Technicon Corp., Terrytown, New York
Temperature controller with thermocouple	Electo-flux Heat Inc., Bloomfield, Connecticut
Univac rheostat	Standard Electrical Products Co., Dayton, Ohio
500 ml boiling flask (modified to have a capillary liquid inlet spout and a gaseous outlet spout)	Modified by the Glass Apparatus Shop, Brooks AFB, San Antonio, Texas
Heating basket	Glascon Apparatus, Terre Haute, Indiana
Compressed air tank and regulator valve	Oxequip Co., Chicago, Illinois
400 ml capacity flowmeter	Fischer & Porter Co., Warminster, Pennsylvania

Various sizes of silicon tubing (Bard-Parker Co., Rutherford, New Jersey), rubber stoppers, and necessary electrical connections to complete the system.

FIG 3. ASSEMBLED VAPOR GENERATION SYSTEM



standard 1 gallon bottle, passed through tubing from the bottle source through the auto-analyzer roller pump to the capillary liquid inlet spout of the boiling flask. Initial filling of the tubing was accomplished by creating a temporary negative vacuum on the boiling flask and by use of a large syringe. Once the auto-analyzer roller pump was engaged the tubing remained filled with 1,1,1-trico. The auto-analyzer roller pump, by a continuous massage of the tubing, provided a constant input of 1,1,1-trico into the boiling flask. The heating mantle maintained the boiling flask at a temperature of 70°C to 90°C. 1,1,1-Trico from the connected tubing formed droplets in the inlet capillary tube of the flask which immediately vaporized as the droplets struck the heated bottom of the flask. The vapors then escaped through the outlet spout of the flask through silicon tubing to the inlet circulation system of the chamber. Fresh air for the chamber was provided by a compressed air tank source attached in parallel with the 1,1,1-trico tubing to the capillary inlet spout. The compressed air was fed into the system at a rate of approximately 250 ml/minute as determined by a calibrated flowmeter located between the air source and the boiling flask. By the use of an E2 Oxygen Analyzer (Beckman Instruments Inc., Fullerton, California) the rate of fresh air noted above

had been found to be satisfactory for maintaining the rate within the chamber. Upon entering the inlet pipe of the chamber, the 1,1,1-trico vapor and fresh air circulated to the planum area in the ceiling of the chamber. A circulating fan provided for even mixing and distribution of the vapor within the chamber.

MONITORING OF 1,1,1-TRICHLOROETHANE

Monitoring of the in-chamber environment was provided by a combination of the readings of a Davis Halide Meter (Davis Emergency Equipment Co., Inc., Newark, New Jersey) and a gas chromatograph designed and constructed by personnel at Brooks AFB, Texas. Initially, the Davis Halide Meter, attached to an outlet hose from the chamber, was utilized for continuous readings during the preliminary trial runs of the generation system. Once the generation system became operational, the in-chamber levels of 1,1,1-trico were monitored by the more accurate gas chromatograph method. The gas chromatograph utilized in this study consisted of a Porapak Q (Waters Assoc. Inc., Framingham, Massachusetts) packed 6 foot, 0.55 cm diameter column heated to 197.7° ± 1°C Operational conditions included a 125 ml/minute flow of helium, 18 ml/minute hydrogen flow, and an air flow of 300 ml/minute.

During the course of the 5f day investigation, samples from the chamber containing the 1,1,1-trico vapors were collected on an hourly basis between the hours of 6 a.m. and 5 p.m. When personnel were within the chamber exposed to the vapors of the agent, samples were collected every 15 minutes. Although individuals within the chamber breathed ambient air from the face masks, the frequent sampling was advisable to safeguard against accidental high exposures should it have become necessary for personnel to breathe the in-chamber atmosphere. Four additional samples were collected each evening at 2 hour intervals between 6 p.m. and 12 p.m. During the weekend period the chamber environment was monitored a minimum of 6 times during the day. A daily log was maintained to record the daily samples of the chamber environment.

MATERIALS USED

The materials studied in this investigation were 1,1,1-trico and ethanol. The 1,1,1-trico selected for this study was an inhibited industrial grade (Sol Chem Co., Amoro Chemicals Inc., Durham, North Carolina). The term "inhibited" refers to the addition of various chemicals which improve the working properties of the solvent. Inhibitory agents are normally added to 1,1,1-trico to prevent corrosion of certain metals such as aluminum.

Because of the great variety of inhibitory agents which may be added to 1,1,1-trico and because of the variation in amounts of these agents which may be added by the different manufacturers, it is impractical to include characteristics of the agent applicable to all brands of the solvent. The following information represents the general physical properties of 1,1,1-trico: chemical formula- CH_3CCl_3, molecular weight- 133.42*, specific gravity at 25°C- 1.318- 1.320, boiling point- 72°- 88°C, vapor pressure at 20°C- 150mm Hg. The agent is further described as being clear with not too unpleasant odor. The solvent is noninflammable, insoluble in water but miscible with alcohol and ether (27,28,81,82,83).

Mass spectrophotography of the 1,1,1-trico was performed prior to chamber operation so as to provide background data for the Sci Chem Co. brand of the solvent. The solvent was found to contain in addition to 1,1,1-trichloroethane, approximately 2.5% 1,4 dioxane, 0.5% nitromethane, 0.5% 1,2 epoxybutane, and 0.5% 1,2 dichloropropane.

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*The molecular weight noted above refers to the uninhibited grade of 1,1,1-trico as the molecular weight of the inhibited form would be variable depending upon the type and amount of inhibitory chemicals added.

The ethanol utilized in this investigation was a reagent grade 95% solution (U. S. Industrial Chemical Co., Tuscola, Illinois). During the first 2 weeks of the investigation period ethanol was administered per os, by use of a stainless steel stomach tube, as a 15% w/v solution. The ethanol was prepared daily by the addition of 19.8 ml of the 95% ethanol to 90.2 ml of tap water. The solution was administered at a dose of 5 g/kg body weight to each animal from each appropriate group on a daily basis 20 to 30 minutes prior to commencing with the psychophysiological tests. After 2 weeks it became obvious that the volume of ethanol solution required to obtain the dose of 5 g/kg body weight was greater than could be accommodated by the stomachs of the growing rats. Between the tenth and fourteenth day of administration 3 rats from each of the groups treated with ethanol died. Four animals died from esophageal ruptures, 1 animal died as a consequence of aortic rupture, and 1 animal died from asphyxiation. It was, therefore, elected to begin ethanol administration via the intraperitoneal route by use of a 10% w/v solution at a dose of 1.5 g/kg body weight. The 10% w/v ethanol solution was prepared by the addition of 152 ml of 95% ethanol to 1000 ml of sterile lactated Ringer's solution. Each animal was held upside down and the solution placed in the left ventral

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abdominal quadrant by means of a 23 gauge 3/4" needle and 10 ml syringe. Five ml of sterile lactated Ringer's solution was administered in a similar manner to rats in the groups not receiving ethanol.

BODY WEIGHT STUDY

Each week during the experimentation period each test subject from each group was weighed to the nearest gram by use of a Torbal Electric Gram Scale (The Torsion Balance Co., Clifton, New Jersey). The weighing procedure was conducted each Monday morning so that the body weight data could be utilized for the weekly calculation of ethanol administration levels and swimming weight calculations. The question considered in this phase of the study was whether or not 1,1,1-trico would work synergistically with ethanol, resulting in a greater reduction in weight gain than is seen with ethanol administration or 1,1,1-trico inhalation alone. At the completion of the 56 day period the mean body weights of each group were compared statistically by means of the t-test.

TILTING PLANE TEST

For this investigation a tilting plane was constructed by staff members of the Mechanical Shop, Brooks AFB, Texas. The apparatus consisted of a masonite board 45 cm

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x 25 cm utilized as the plane with the rough surface up. The board was adjacent to a perpendicular piece of aluminum sheeting which had been calibrated in 2° divisions. In contrast to all previous studies this tilting plane apparatus was motor operated rather than manually operated (Figure 4A, p. 51). The apparatus was motorized so as to eliminate such human errors as inconsistent speed of tilting the board, difficulty in overshooting the correct angle of tilt at which the subject slides, and inconsistent ability to hold the plane at the correct angle while attempting to take the reading. The driving motor (Slo-Syn Motor, Superior Electrical Co., Bristo, Connecticut) was geared to traverse the tilt board through a 90° arc in a period of 4.8 seconds. This time was comparable to the approximate 5 seconds of tilting recommended in previous studies (58,60). The motor was operated by a hand operated toggle switch which allowed the plane to go up or down. A brake system was incorporated into the fulcrum which provided for instant braking of the plane upon releasing the toggle switch from the up or down position to the neutral position.

In the investigation, the tilting plane apparatus was handled entirely by an unbiased assistant who was not informed as to the status of each animal being tested. The procedure was conducted once weekly with each test

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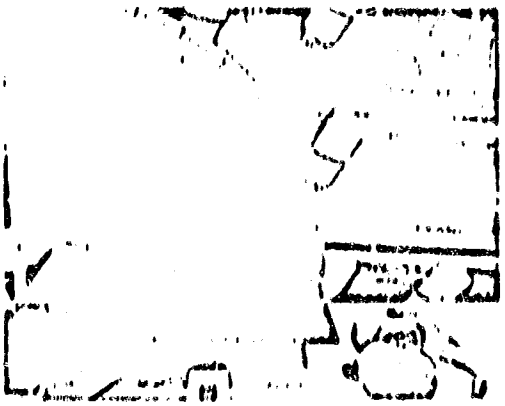
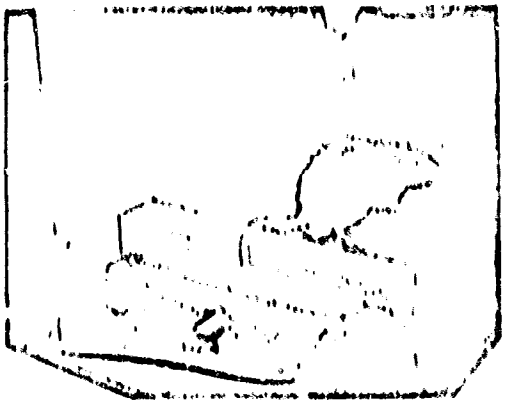
FIG 4. TILTING PLANE OPERATION

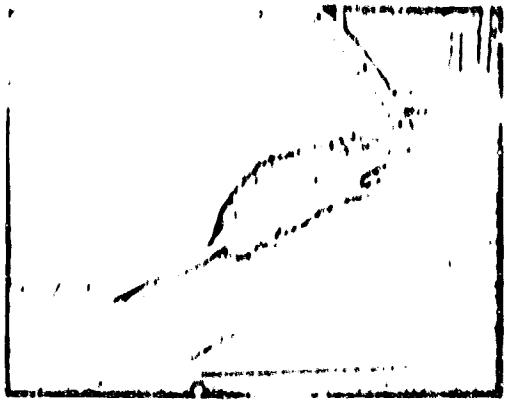
4A. TILTING PLANE EQUIPMENT



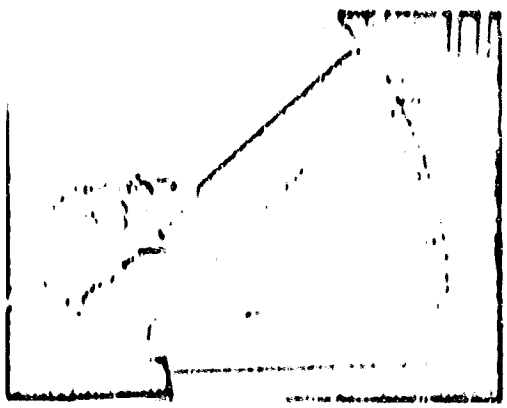
4B. POSITION OF SUBJECT



4C. ELEVATION OF PLANE



4D. END POINT OF OPERATION



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subject. Testing was conducted entirely within the respective chambers, with the tilting plane apparatus being transported between the 2 chambers as needed. Rats treated with ethanol and control rats were randomly placed on the tilting plane to keep the operation as unbiased as possible; however, the test procedure was performed no earlier than 20 minutes after ethanol administration and no later than 60 minutes after ethanol administration. This time span was the period in which approximately maximum severity of intoxication was expected following the administration of ethanol (62). In addition, ear plugs were worn by the assistant in both chambers so that the operator based his operation of the tilting plane strictly on sight.

Each rat from each group was placed on the board as many times as necessary to obtain 3 consistent readings. The assistant alone determined which 3 tilts were acceptable. Movement of the animal during the tilting of the board, other than by actual slippage, constituted an unacceptable tilt. At the end of the 56 day investigation there had been 8 tilting plane sessions. The data collected during this 8 week period was statistically evaluated by means of the t-test.

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SWIMMING TO EXHAUSTION

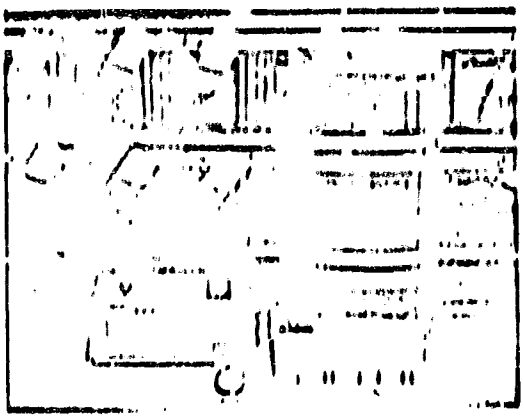
The materials utilized in this phase of the study included the following: 2 glass chromatography tanks 30 cm in diameter by 60 cm deep, a stopwatch accurate to 0.2 seconds, assorted bell shaped fishing sinkers varying in weight from 2 grams to 10 grams, a thermometer accurate to 1°C, and an elastic tie-down cord with an attached fishing swivel (Figure 5A, p. 54).

The swimming phase of this investigation was performed on a weekly basis. Each week each test subject was forced to swim once, with alteration of swimming group order from week to week to avoid establishing a bias pattern of expected results. Test subjects receiving ethanol were forced to swim 20 to 60 minutes after its administration. A relative load of 5% of the animals body weight was utilized to reduce maximum swimming times in this investigation. Thus, a 400 gram rat would swim with a 20 gram weight attached about his body. The appropriate load attached to each animal was supplied by use of standard weight fishing sinkers which were attached with elastic cord and fishing swivel about the chest of the rat (Figure 5B,D, p. 54).

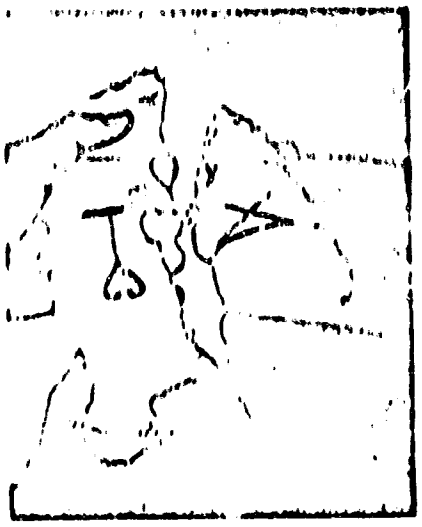
Each week the chromatography tanks were filled with fresh water at least 48 hours prior to the swimming sessions so that air bubbles within the water could

FIG 5. SWIMMING TO EXHAUSTION OPERATION

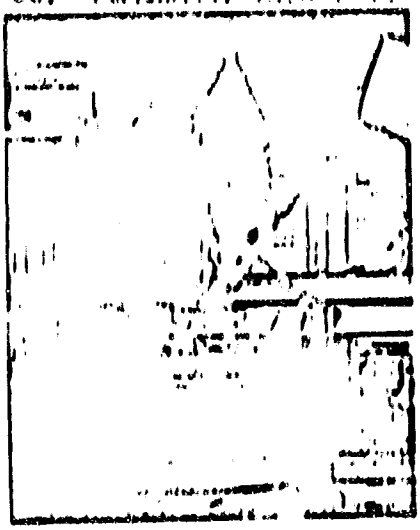
5A. SWIMMING EQUIPMENT



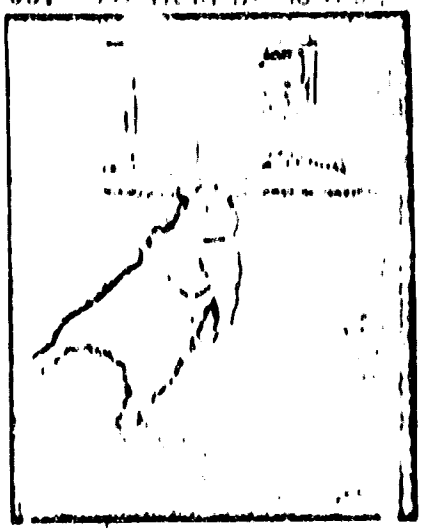
5B. HARNESS AND WEIGHTS



5C. PLACEMENT INTO TANK



5D. SWIMMING BY SUBJ



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escape. This was necessary as it has been reported that in freshly poured tap water even weighted animals may temporarily float on the water surface due to the accumulation of air bubbles in their fur (77). Small amounts of warm or cold water, however, were added just prior to the swimming sessions so that each tank contained water at a temperature of 20°C. Rats were carefully lowered into the tank of water by means of holding onto their tail (Figure 5C, p. 54). Such a procedure provided uniformity in the placement of rats into the water. A stopwatch was utilized in recording the time span between the placement of the animal into the water to the moment when the subject had remained submerged beneath the water for 15 seconds. At this end point the test subject was quickly retrieved by hand, dried completely with a towel, and returned to his cage.

During the 56 day investigation period each animal was forced to swim 8 times. The swimming times recorded for each group on each occasion was statistically evaluated by the t-test.

PATHOLOGY

At the termination of the 56 day test period each animal was sacrificed by decapitation utilizing a Harvard Decapitator (Harvard Apparatus Co., Millis,

Massachusetts). At that time blood was collected in test tubes for determination of the test animals final SGPT level. This SGPT level was then compared statistically by means of the t-test to the baseline SGPT level obtained prior to the chamber exposures. Each animal was allowed to drain head down for a period of 10 minutes following the decapitation. The intact liver of each animal was then carefully removed and weighed with a Mettler Electric Gram Scale (Mettler Instrument Corp., Hightstown, New Jersey). The t-test was utilized in comparing mean values of body weight and organ weight.

Gross pathology was noted during the harvesting of tissues. Sections of the liver, the kidneys, and the lungs were collected and placed in buffered 10% formalin solution for fixation. Histologic slides were prepared from these tissues for each test subject. All tissue sections were stained with hematoxylin-eosin. Frozen sections were prepared on selected liver sections utilizing Oil Red O as the stain. No statistical approach was applied to the histologic slides other than recording the presence or absence of pathologic lesions.

RESULTS AND DISCUSSION

PRELIMINARY ETHANOL STUDIES

The 10 animals randomly selected for the 24 hour ethanol metabolism study weighed an average of 200.8 g with a range of 184 g to 226 g. Individuals were administered an oral dose of 6.1 ml to 7.5 ml of a 15% w/v ethanol solution at a rate of 5 g of ethanol per kg body weight. It was noted that the animals exhibited no difficulty in consuming this amount of fluid. Approximately 20 minutes after administration and for a period of approximately 2 hours, all of the animals exhibited varying degrees of intoxication. Most animals displayed slight incoordination while walking whereas, a few animals remained in a prone position unless disturbed. During the course of blood collection, via cardiac puncture, 1 animal died at the 4 hour postethanol collection and a second animal died during the 8 hour collection session. Two rats of comparable weight were exposed to an identical sequence of procedures the following day. The data for the original 8 surviving rats and the 2 replacement rats is outlined in Table 3 (p. 50).

It is apparent from observing the data from the study that substantial blood alcohol levels in rats were maintained for at least 4 hours following consumption,

TABLE 3

Blood alcohol levels in 10 rats 1,2,4,6, and 24 hours after the per os administration of ethanol at 5 g/kg body weight

Time postethanol administration	Blood alcohol level mean and S.D. (mg%)	Blood alcohol range (mg%)
1 hour	202.0 ± 33.2	142.0 - 242.6
2 hours	202.4 ± 39.3	139.2 - 275.0
4 hours	151.6 ± 33.9	108.0 - 203.3
6 hours	30.8 ± 39.8	0.0* - 80.3
24 hours	0.0	0.0

*After 6 hours, 4 of 10 animals had metabolized all of the alcohol.

with the highest level of intoxication being noted between 1 and 2 hours postethanol administration. The blood alcohol levels noted in the study are comparable to that obtained in similar ethanol studies. Leikola (84) reported an average blood alcohol level of 210 mg%, 1.5 hours after the oral administration to rats of a 30% v/v solution of ethanol at 5 g/kg body weight. Kutob and Plaa (2), after orally administering a 25% solution of ethanol at 5 g/kg to mice, found a value of 215 ± 59 mg%.

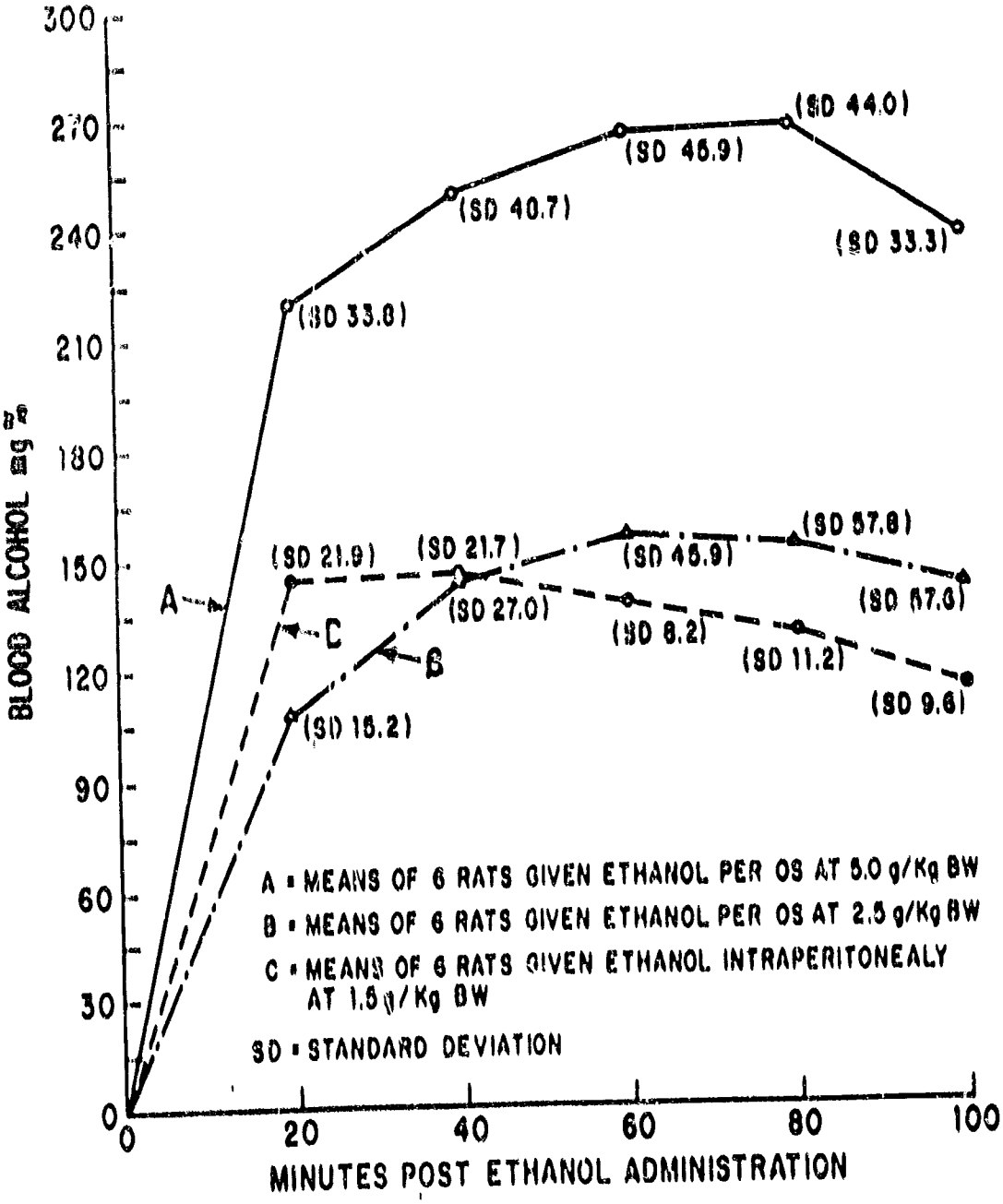
The study has also established the important fact that the ethanol was being metabolized in its entirety

in less than 24 hours. It has been reported that rats eliminate alcohol from their blood at a rate of 1 g/kg body weight every 3 hours and are capable of elimination 7.2 g/kg/day (.1). Theoretically, at the dose administered in this study the rats should have eliminated all of the ethanol in approximately 15 hours. Blood alcohol levels were not determined in these animals at the 15 hour time period but the figures for the 8 hour and 24 hour collection correlate well with the hypothetical schedule for ethanol metabolism.

Figure 6 (p. 60) depicts the blood alcohol levels obtained from administration of ethanol via different routes and different doses. Curve A represents the mean values of 6 rats weighing between 192 g and 224 g, average 208 g, who were given ethanol per os at 5.0 g/kg body weight. The mean blood alcohol level obtained in this study was higher than in the 24 hour study, 207.0 mg% versus 202.0 mg%, but figures were within the range of previously accepted data (2). The study complemented the first study by demonstrating that peak blood levels were not reached until at least 1 hour after ethanol administration.

Curve B shows the mean alcohol blood levels obtained following the oral administration of ethanol at 2.5 g/kg body weight. Larger rats, ranging in weight from 470 g

FIG 6 THE CORRELATION BETWEEN BLOOD ALCOHOL LEVELS AND TIME



to 543 g, average 511 g, were utilized in the study. The use of larger rats was based upon the availability of only large rats at the time, rather than by an scientific objective. The use of larger rats should have had no influence upon the data obtained since the ethanol dose was calculated relative to body weight. No difficulty was noted during the oral dosing of the rats. At the 2.5 g/kg dose level the rats exhibited only an occasional incoordinated gait while moving. It can be noted from the figure that the peak mean ethanol blood level of curve B occurs at 60 minutes versus 80 minutes in curve A; however, the difference is not significant and for all practical purposes the shape of curve A and curve B can be considered identical.

Curve C depicts the mean blood ethanol levels obtained by the administration of ethanol by intraperitoneal injection. Six large rats ranging in weight from 460 g to 580 g, average of 526 g, were administered a 10% w/v ethanol solution at a dose of 1.5 g/kg body weight. Following the intraperitoneal (ip) injection of 7.0 ml to 8.5 ml of the solution the rats were noted to be slightly more intoxicated than the rats given 2.5 g/kg ethanol orally, but not as intoxicated as the rats that received ethanol orally at a dose of 5.0 g/kg body weight. The rats were also noted to be visibly

intoxicated within 10 minutes after ethanol administration. The more rapid intoxicated state was also noted upon reviewing the results of the mean ethanol blood levels. Curve C is noted to peak within 20 minutes following ethanol administration. Kalant and Czaja (62) reported a similar rapid peaking of mean ethanol blood levels following the ip administration of ethanol at 2.0 g/kg body weight. Their study demonstrated that the peak blood ethanol level was obtained between 20 minutes and 30 minutes postethanol administration, and that the drop in blood alcohol was much more rapid than was seen by the oral administration of a comparable amount of ethanol.

The difference in shape between curves A and B versus curve C is explained by the fact that the ip route of ethanol administration circumvents the complications of gastrointestinal tract uptake, such as paralysis of the muscles of the stomach reportedly seen with the administration of ethanol per os. The ip route is also reported to provide more uniform blood alcohol levels than the per os route for the same reason cited above (21). The much lower standard deviations calculated from the mean values from curve C substantiate this claim.

ETHANOL ADMINISTRATION DURING THE EXPERIMENTATION PERIOD

After evaluation of the data collected from the preliminary ethanol studies, the investigator elected to administer ethanol orally to appropriate groups at a dose of 5.0 g/kg body weight for the duration of the experimentation period. The decision to administer ethanol orally and at this dose was based upon 2 reasons. Firstly, oral administration more closely duplicated the natural means of intake of ethanol in man. Secondly, the ethanol dose provided a moderate state of intoxication which was desired for testing purposes.

During the first 2 weeks of the experimentation period it became apparent that the oral administration of ethanol on a daily basis was too traumatic. Although no problems had been experienced at this ethanol dose level during the preliminary study, the experimental test subjects displayed obvious difficulty in handling the large daily volume of solution. Necropsy findings of 6 test subjects dying within the first 2 weeks of the testing period revealed 4 deaths from esophageal ruptures, 1 death from a ruptured aorta, and 1 death from asphyxiation. In addition to the probable daily trauma of the stainless steel stomach tube, it was observed that the calculated volume of fluid administered caused great distention of the stomach. Obviously, the preliminary

test subjects weighing 200 grams and thus given 7 ml of fluid had not experienced the discomfort of test subjects weighing 400 grams and having received 14 ml of fluid. To prevent the further loss of test animals it was necessary to change routes of ethanol administration. Since the oral administration of ethanol at 2.5 g/kg body weight would eliminate the problem of fluid volume but would still result in possible trauma due to the stomach tubing procedure, it was elected to administer ethanol via the ip route at 1.5 g/kg body weight.

Through the course of the remaining 6 weeks of the experimentation period, ip injections of ethanol solution were performed daily on appropriate test subjects without complications. No resulting complications were noted in those rats receiving, via the ip route, 5 ml of lactated Ringer's solution daily in place of ethanol solution.

EXPERIMENTAL CHAMBER CONDITIONS

During the course of the 56 day experimentation period a daily log was maintained on the humidity and temperature of each chamber, and the temperature of the water in each swimming tank in each chamber. The compiled figures for this experimentation period are summarized in Table 4 (p. 65).

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TABLE 4

Summarized chamber temperature, chamber relative humidity, and water temperature data for the experimentation period

Air temperature:	Ambient Air Chamber	1,1,1-Trico Chamber
AM mean and range (°C)	24.6 (23.4-27.2)	23.4 (22.0-26.6)
PM mean and range (°C)	24.8 (23.4-27.2)	23.5 (22.2-26.6)
Relative humidity:		
AM mean and range (%)	46.5 (40-62)	41.2 (26-58)
PM mean and range (%)	48.0 (42-65)	44.3 (20-62)
Water temperature: (°C)	(20-21)	(20-21)

Both chambers for all practical purposes were comparable in terms of overall internal environment. During the 50 day experimentation period no temperature or relative humidity extremes occurred with either chamber. The range of temperature and humidity readings, however, were wider for the 1,1,1-trico chamber. This outcome was probably due to the fact that the restricted ventilation system of the chamber could be more easily upset by subtle extrinsic factors. The only significant overall trend, which was evident in both chambers, was a slight elevation in temperature and relative humidity between 8 AM and 5 PM readings. The probable explanation for

this rise over the course of the day was the fact that the AM readings were obtained prior to penetration of the chamber by the investigator and assistants. Obviously, enough body heat was generated by the workers to affect the PM temperature readings. Likewise, it is probable that enough respiratory vapors were exhaled by the workers, while making face mask adjustments within the chamber, to affect the PM relative humidity readings. Although the swimming tank temperatures varied between 20°C and 21°C from week to week, the temperature for both tanks for any given week were identical.

1,1,1-TRICHLOROETHANE MONITORING

During the 56 day experimentation period over 1,000 samples were collected from the 1,1,1-trico chamber and analyzed by gas chromatography. To simplify the record keeping, only the recordings at 8 AM, 10 AM, 12 AM, 1 PM, 3 PM, and 5 PM were recorded for statistical purposes. Over the course of the experimentation period the level of 1,1,1-trico in the test chamber averaged 383.6 parts per million (ppm) with a range of 265ppm to 455ppm. During 35 of the 56 days the range varied from 300ppm to 390ppm. Normally, the level fluctuated less than 10% from the 8 AM to the 5 PM reading. The greatest number of high and low readings, deviating from the desired

350ppm 1,1,1-triac, were noted in the first AM readings. This is explained by the fact that no supervision of the air generation system was afforded between 12 PM and 8 AM and thus vapor levels could build up or fall over an 8 hour period.

BODY WEIGHT STUDY

Animal weights were recorded during both the 5 week quarantine period and the 8 week experimentation period. The collected data is reflected in Table 5 (p. 68) and in Figure 7 (p. 69). Table 5 contains several sets of means which are explained below. One set consists of the cell means. The cell means in this particular study is defined as the average weight of the animals under a given combination of atmosphere exposure and ethanol treatment at a given time. Another set contains the atmosphere x time means and the overall atmosphere means. An atmosphere x time mean is defined as the unweighted average of the 2 cell means at a given atmosphere and a given time. An overall atmosphere mean is defined as the unweighted average of all cell means at a given atmosphere. The third set consists of the ethanol treatment x time means and the overall ethanol treatment means. These means are defined in an analogue manner to those just discussed. Finally, one set contains the

TABLE 5 COMPILED DATA FROM THE WEIGHT (GRAMS) GAIN/LOSS STUDY

TABLE 5A: CELL MEANS

ATMOSPHERE	TREATMENT	QUARANTINE WEEK 1	2	3	4	5	EXPERIMENTAL WEEK 1	2	3	4	5	6	7	8	N
NO TRICO	NO ETHANOL	296.6	309.8	357.1	370.0	384.5	420.6	419.5	436.6	451.4	474.4	486.0	499.3	469.9	10
NO TRICO	ETHANOL	297.4	316.2	342.4	380.0	388.3	393.7	388.4	429.6	428.6	453.7	460.3	469.4	445.7	9
TRICO	NO ETHANOL	296.8	307.8	331.5	368.1	396.2	416.9	431.1	460.5	469.8	476.4	489.2	500.2	510.2	12
TRICO	ETHANOL	297.5	321.6	341.2	397.0	435.0	405.0	378.0	419.8	443.7	486.4	467.3	478.4	484.0	9

TABLE 5B: ATMOSPHERE × TIME MEANS

ATMOSPHERE	QUARANTINE WEEK 1	2	3	4	5	EXPERIMENTAL WEEK 1	2	3	4	5	6	7	8	OVERALL ATMOS MEANS
NO TRICO	297.0	312.8	359.8	375.0	393.4	407.1	404.0	443.1	438.8	464.0	477.7	484.4	487.8	447.1
TRICO	297.0	314.7	331.4	379.5	415.6	411.0	404.6	440.1	454.7	466.4	478.8	489.3	497.1	455.2

TABLE 5C: ETHANOL TREATMENT × TIME MEANS

TREATMENT	QUARANTINE WEEK 1	2	3	4	5	EXPERIMENTAL WEEK 1	2	3	4	5	6	7	8	OVERALL TREAT MEANS
NO ETHANOL	296.7	308.4	339.3	366.0	390.3	419.8	425.3	438.6	448.0	475.4	482.1	499.8	490.1	464.9
ETHANOL	297.3	316.9	351.8	388.5	408.7	399.3	383.2	424.7	434.6	455.1	463.8	473.9	464.8	427.4

TABLE 5D: OVERALL TIME MEANS

QUARANTINE WEEK 1	2	3	4	5	EXPERIMENTAL WEEK 1	2	3	4	5	6	7	8
297.0	312.6	345.6	377.3	399.5	409.0	404.3	441.6	446.9	465.2	478.0	486.9	477.8

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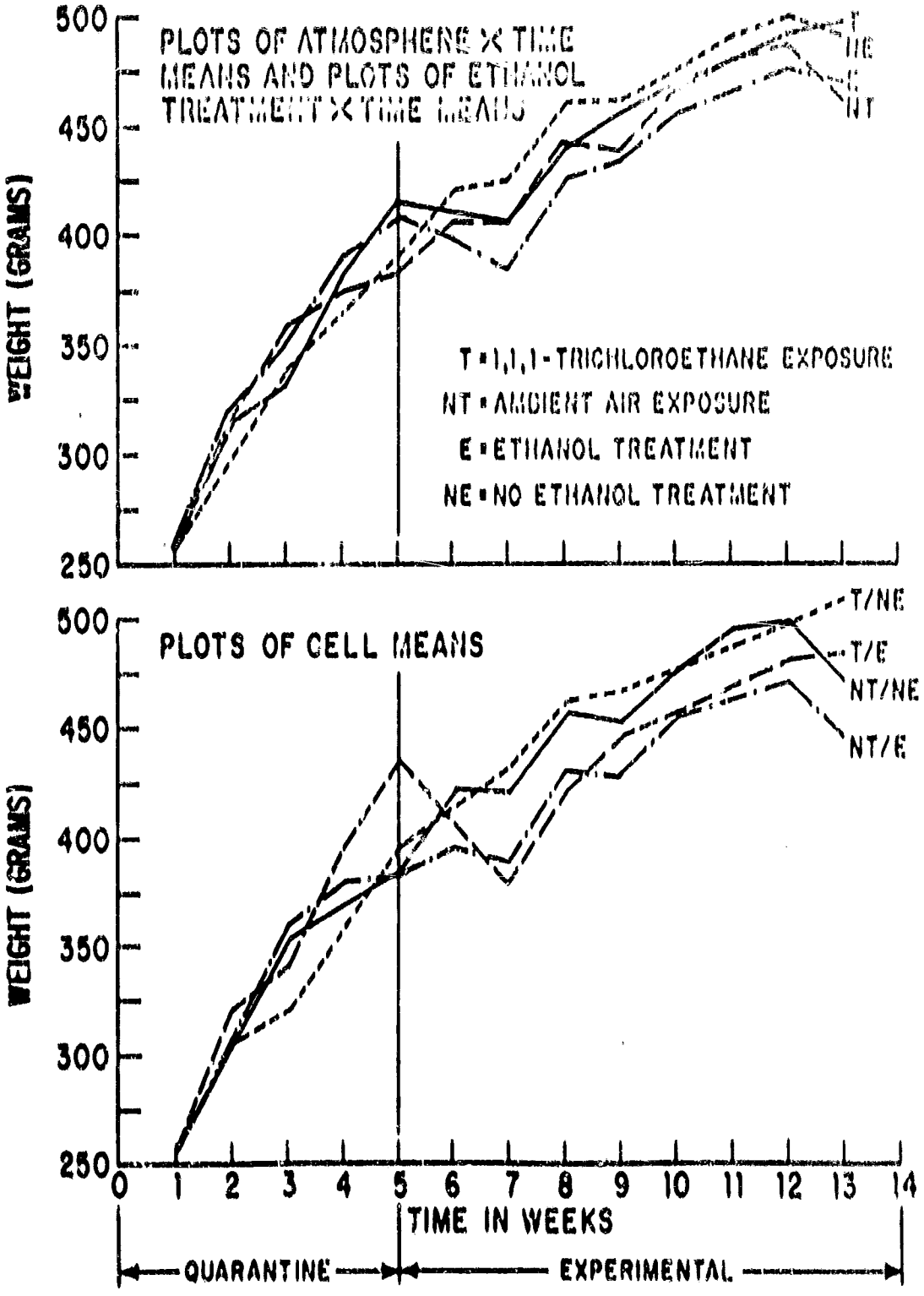
FIG 7 WEIGHT GAIN/LOSS STUDY



overall time means. An overall time mean is defined as the unweighted average of the 4 cell means at a given time.

A preliminary analysis was performed using only the quarantine data to determine whether there were any inherit group differences which might affect the experimental portion of the study. A significant interaction of atmosphere with time was detected ($P < .001$). It can be seen, however, from the data in Table 5B and in the top of Figure 7 that there is no definite trend to the 1,1,1-trico versus ambient air means, therefore, this result would not grossly affect the interpretation of the experimental data.

An analysis of the experimental data set yielded the following results. A significant atmosphere x time interaction was detected ($P < .001$). That is, there is statistical evidence that the average weight trend for the animals exposed to 1,1,1-trico was not the same as the average weight trend for the animals in the ambient air. From the top of Figure 7 and from Table 5B, there does not appear to be a pattern to the differences in trend. In other words, as one checks across time one notices that the means start close together, spread apart, become close again, and finally spread again at the last week. From a statistical point of view, this

pattern has no reasonable explanation. Since there is no apparent physiological explanation for this pattern, it is probably nothing more than the same type of fluctation that was seen during the quarantine period.

In addition to the above result, an overall ethanol treatment difference was detected ($P < .025$). Specifically, the overall mean weight for animals treated with ethanol was significantly lower than the overall mean weight for those animals not treated with ethanol (Table 5C). It is also noted in Figure 7 that the curves for the animals treated with ethanol and the animals not treated with ethanol tend to remain parallel from week to week rather than fluctuating as seen in the atmosphere x time means. Finally, it can be noted from either Table 5C or Figure 7 that the difference between the ethanol treatment means of the 2 groups becomes greater up to the second week of the study but thereafter the differences becomes less.

Although several authors have reported similar weight differences between controls and rats treated with ethanol, no author has established an acceptable answer to explain the phenomena (25,26,50,54). The investigator suggests that the weight differences seen in this experiment are a result of 2 distinct effects of the ethanol treatment. Since the preliminary ethanol study data revealed the fact that blood alcohol levels remained

high for at least several hours following administration of ethanol by either the oral or ip route, it seems feasible that the rats treated with ethanol may be depressed to the state of being refractile to food. Thus, while nonintoxicated rats are capable of feeding over a 24 hour period, rats treated with ethanol are perhaps only capable of feeding for 18 or 20 hours each day. The investigator also suggests that the ethanol solution taken orally produces a satiated state in the animal and thus again the subject is refractile to food intake. It has been reported that a concentration of alcohol in the stomach exceeding approximately 5% w/v begins to paralyze the muscles of the stomach wall and causes secretion of water for dilution into the stomach. Both of these factors slow gastric evacuation (21). Since ethanol was administered for the first 2 weeks of the investigation as a 15% w/v solution, in amounts capable of causing gastric distention, it seems feasible that the rats treated with ethanol remained in a physiologically satiated state daily for at least several hours after ethanol consumption. The investigator, therefore, postulates that the overall lower weight gains of the animals treated with ethanol could at least in part be due to the depressing affect of ethanol, and that the even poorer weight gains of the first 2 weeks

of the study were due to the additional satiety effect produced by the large volume of ethanol solution received per os.

Finally, the test for overall time differences was significant ($P < .001$). Generally, the means increased over time (Table 5D) which was expected because of the growth of the young animals.

TILTING PLANE STUDY

The tilting plane test was executed by placing an animal on the horizontal plane and tilting the plane through a 90° arc until the animal slid. The angle at which this occurred was determined, and the sum of the angles from 2 such trials recorded. The test was performed on each rat once each week during the experimental period. The data was compiled into mean tables, (Table 6, p. 74), as described in the last section. The means were also plotted graphically (Figure 8, p. 75).

Table 6A and 6D and Figure 8 show that a definite degenerative trend occurred through the course of the 8 week experimental period. The overall time means decreased significantly, ($P < .001$), and when the data is considered on an individual group basis it is seen that the downward trend occurred for each group. No reference could be found which describes the results

TABLE 6 ANGLE (DEGREES) MEANS FOR TILTING PLANE TEST

TABLE 6A: CELL MEANS

ATMOSPHERE	TREATMENT	WEEK								N
		1	2	3	4	5	6	7	8	
NO TRICO	NO ETHANOL	149.6	156.2	150.9	148.3	145.4	130.1	120.3	121.8	10
NO TRICO	ETHANOL	153.1	150.6	146.9	144.4	141.3	116.9	105.8	110.6	9
TRICO	NO ETHANOL	166.2	160.5	160.0	159.4	156.5	148.0	140.6	142.2	12
TRICO	ETHANOL	161.4	155.6	153.7	150.6	149.7	135.4	132.3	120.1	9

TABLE 6B: ATMOSPHERE × TIME MEANS

ATMOSPHERE	WEEK								OVERALL ATMOS MEANS
	1	2	3	4	5	6	7	8	
NO TRICO	151.5	153.4	148.9	146.4	143.4	123.5	113.0	116.2	137.0
TRICO	163.0	160.0	157.2	159.5	153.1	140.7	136.5	131.2	150.0

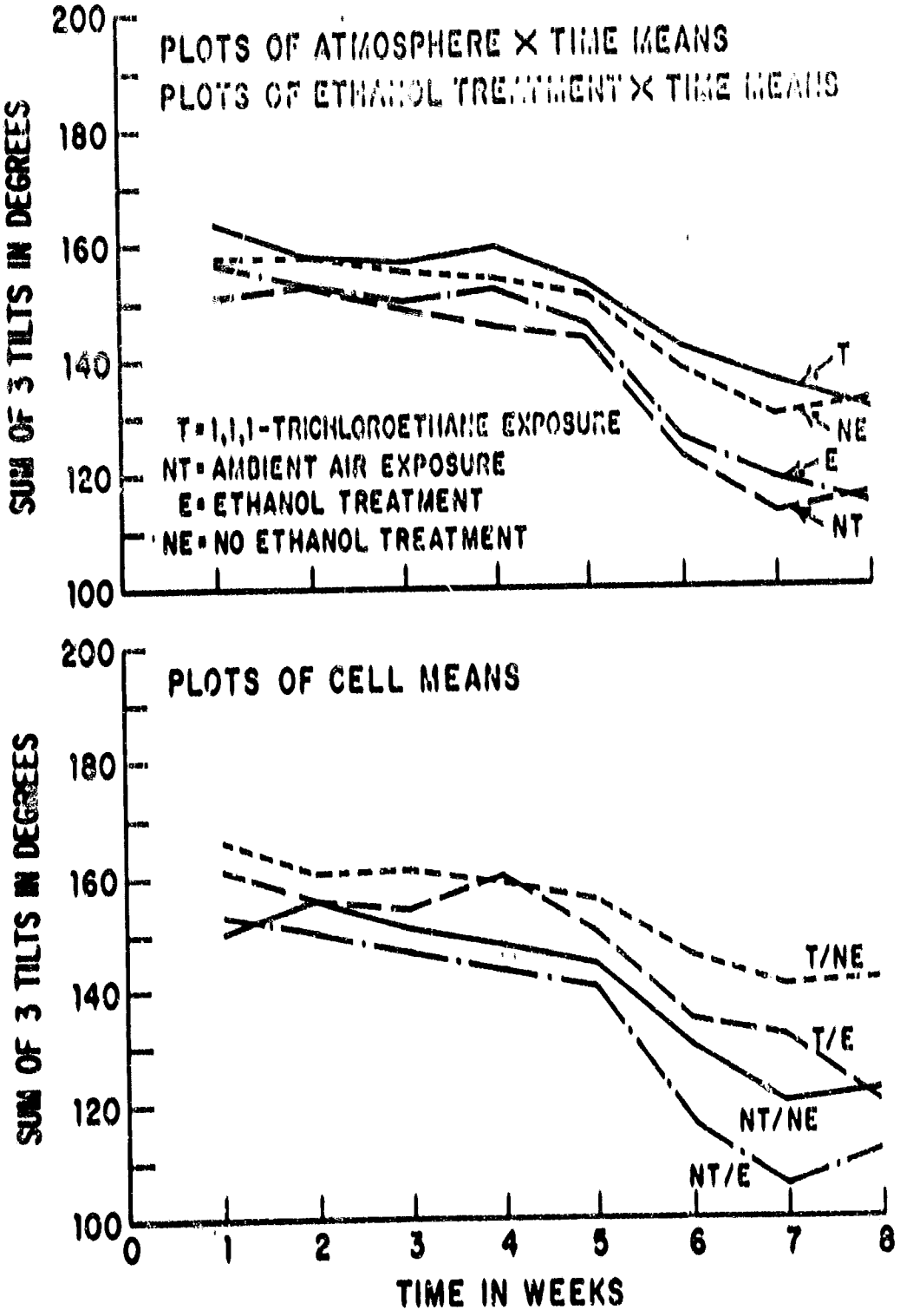
TABLE 6C: ETHANOL TREATMENT × TIME MEANS

TREATMENT	WEEK								OVERALL TREAT MEANS
	1	2	3	4	5	6	7	8	
NO ETHANOL	158.0	158.4	155.6	153.9	151.0	136.0	130.4	132.0	147.2
ETHANOL	157.3	153.1	150.3	152.0	145.5	126.2	119.1	115.3	139.8

TABLE 6D: OVERALL TIME MEANS

WEEK							
1	2	3	4	5	6	7	8
157.7	155.7	153.1	152.9	148.2	132.1	124.7	123.7

FIG 8 TILTING PLANE TEST



of repeated tilting plane testing of animals. Most studies are concerned with the results of a one time set of trials or a series of trials over a 24 hour period (58,59.60,61,62). The fact that the degenerative trend occurs with each group suggests a common denominator or dominators. It is, therefore, suggested that the degenerative trend could be due to the increased weight gains of the subjects or increased fatigue from the repeated swimming sessions or both. Since the average rat from each group increased his weight by nearly 20% over the 8 week period, it seems possible that such an increase in weight could make holding onto the rising plane by the rat an increasingly difficult task. It also seems possible that the stress of swimming to exhaustion once weekly could, over the weeks, cause an overall physical debilitating effect.

Significant atmosphere x time and ethanol treatment x time interactions were detected at the ($P < .001$) level in each case. In the first instance, the animals in the 1,1,1-trico atmosphere tended to perform increasingly better than those in the control ambient atmosphere. The trend is illustrated well in the top of Figure 8 and in Table 5B. In the second instance, the performance of the animals receiving ethanol became increasingly poorer than the performance of the animals not receiving ethanol.

The trend is illustrated well in Figure 8 and in Table 8C. Furthermore, the opposite effects of ethanol and 1,1,1-trich appear to be additive. This can be seen in the lower half of Figure 8. It can be noted that the animals receiving both ethanol and 1,1,1-trich have a similar trend to those under complete control conditions.

No statistically significant coordination, dexterity, and equilibrium type performance changes have been noted in past studies involving experimental exposure to 1,1,1-trich in the TLV range (41,42). Table 1 (p. 10) shows that in humans equilibrium aberrations were only seen at a level of exposure approximately 3 times greater than the level investigated in this study. It is, therefore, apparent from these previous studies that one would expect to see no statistically significant changes between individuals exposed to the ambient air and those exposed to 1,1,1-trich, and if changes were noted they would most likely be degenerative. Contrary to this assumption, the tilting plane results of this study demonstrate a surprising stimulatory effect from the 1,1,1-trich exposure. Such an outcome has not been previously reported in either human or animal investigations.

The fact that animals not receiving ethanol performed better than those receiving ethanol was not surprising. Arvola et al (58) demonstrated in a series of tilting

plane tests in rats that the regression of performance to alcohol dose did not deviate significantly from linearity. In his study a correlation was found between impairment of performance and blood alcohol concentration in rats given as little ethanol as 0.0 g/kg body weight per os. It was also reported in the study that rats given ethanol orally at a dose of 2.7 g/kg body weight performed on the tilting plane an average of 94% as well as when the animals were not treated with ethanol. It was shown in Figure 6 (p.60) that approximately equal blood alcohol values were obtained when ethanol was given as either 1.5 g/kg body weight ip or 2.5 g/kg body weight per os, therefore, ethanol given at a dose of 2.7 g/kg body weight per os would produce just slightly greater blood alcohol levels. The overall treatment mean difference between rats treated with ethanol and rats not treated with ethanol was 95%, thus reflecting comparable data between the 2 studies.

SWIMMING STUDY

The exposure of the test subjects to swimming first occurred during the preliminary testing period. During this period the rats were forced to swim for a predetermined period of time which was progressively lengthened from week to week. Upon their first few

exposures to the water the rats exhibited rather inefficient swimming habits characterized by wasted excessive motion of both front and rear limbs. In most cases the rats, probably as a consequence of inexperience and fright, struggled in the water for a period of 3 to 5 minutes before they relaxed and began to swim in a more efficient manner. The struggling, following placement of the rats into the water, was seen throughout the preliminary testing period, but was noted to be of a shorter duration as the weeks of swimming progressed. At the final swimming session, 12 minutes of forced swimming, the animals demonstrated efficient swimming within 2 minutes of entering the water. Similar improvements in swimming performance have been reported by several investigators (70,76,77). In each of these studies it was suggested that learning and adaptation to the stress were the main contributory factors in the reported improved swimming performance.

During the course of the 8 week experimental period the rats demonstrated ever decreasing time periods of inefficient swimming. At the same time the animals exhibited a new sequence of swimming traits not noted during the preliminary swimming sessions. These traits were most likely induced by the introduction of weights attached about their chest. The sequence of events,

which was demonstrated by all of the rats during the experimental period, is described as follows. As each animal was slowly lowered into the water by means of his tail (Figure 5C, p. 50), he would make an all out effort to keep as much of his body out of the water as possible. Each animal accomplished the feat by rapid but inefficient paddling of both front and rear limbs. After 1 to 2 minutes of vigorous struggling each rat would relax and swim more efficiently by means of rhythmic paddling of the rear limbs. After a variable period of time, each test subject became visibly exhausted as noted by an overall lowering of the body in the water and irregular paddling of the rear limbs. Shortly afterwards the rat would begin to swim just beneath the surface of the water, periodically surfacing by means of strong paddling of the rear limbs. Most rats were able to continue swimming in this manner for several minutes. The final stage of swimming consisted of the rat resting motionless while allowing himself to drop to the tank bottom, and then vigorously paddling to the surface of the water every 5 to 10 seconds by means of both front and rear limbs. The rats were able to repeat the procedure several times but usually the stage was brief, lasting less than 1 minute. Each rat was finally removed from

the water after remaining submerged for a period of 15 seconds.

Although the animals were completely exhausted after each swimming session, no debilitating effects were apparent during the 8 week period. Overall, the 8 swimming sessions were relatively successful with the exception of 1 animal drowning during the third week session.

The swimming test demonstrated results statistically similar to that noted for the tilting plane test, although not as obvious. There was firstly, an indication of a significant atmosphere x time interaction ($P < .05$). The pattern, however, is a little different from that seen for the tilting plane test. There does not appear to be much of a gradually increasing difference between the responses of animals exposed to 1,1,1-trico and the responses of those maintained in ambient air. Rather, the difference appears to be quite large as early as the end of the second week (Table 7B, p. 82 and top of Figure 9, p. 83). Statistically, the apparent stimulatory effect of the 1,1,1-trico exposure is much more visibly shown in the data from the swimming test versus the tilting plane test. In the tilting plane test the difference between the animals exposed to 1,1,1-trico and those maintained in ambient air in terms of overall performance is approximately 10%, whereas the difference detected

TABLE 7 SWIMMING TIME MEANS (SECONDS) FROM SWIMMING TEST

TABLE 7A: CELL MEANS

ATMOSPHERE	TREATMENT	WEEK 1	2	3	4	5	6	7	8	N
NO TRICO	NO ETHANOL	270.2	264.5	270.9	253.7	285.1	252.7	254.5	227.7	10
NO TRICO	ETHANOL	233.3	256.6	257.8	193.4	198.4	156.1	147.8	119.9	9
TRICO	NO ETHANOL	297.7	337.1	401.4	336.4	330.6	299.6	379.7	323.9	12
TRICO	ETHANOL	295.8	460.3	466.7	329.4	296.0	361.4	327.0	227.1	9

TABLE 7B: ATMOSPHERE × TIME MEANS

ATMOSPHERE	WEEK 1	2	3	4	5	6	7	8	OVERALL ATMOS MEANS
NO TRICO	255.0	261.6	264.3	223.6	241.8	204.4	201.1	173.8	228.3
TRICO	296.7	308.7	434.0	332.9	317.2	320.0	353.3	275.5	341.1

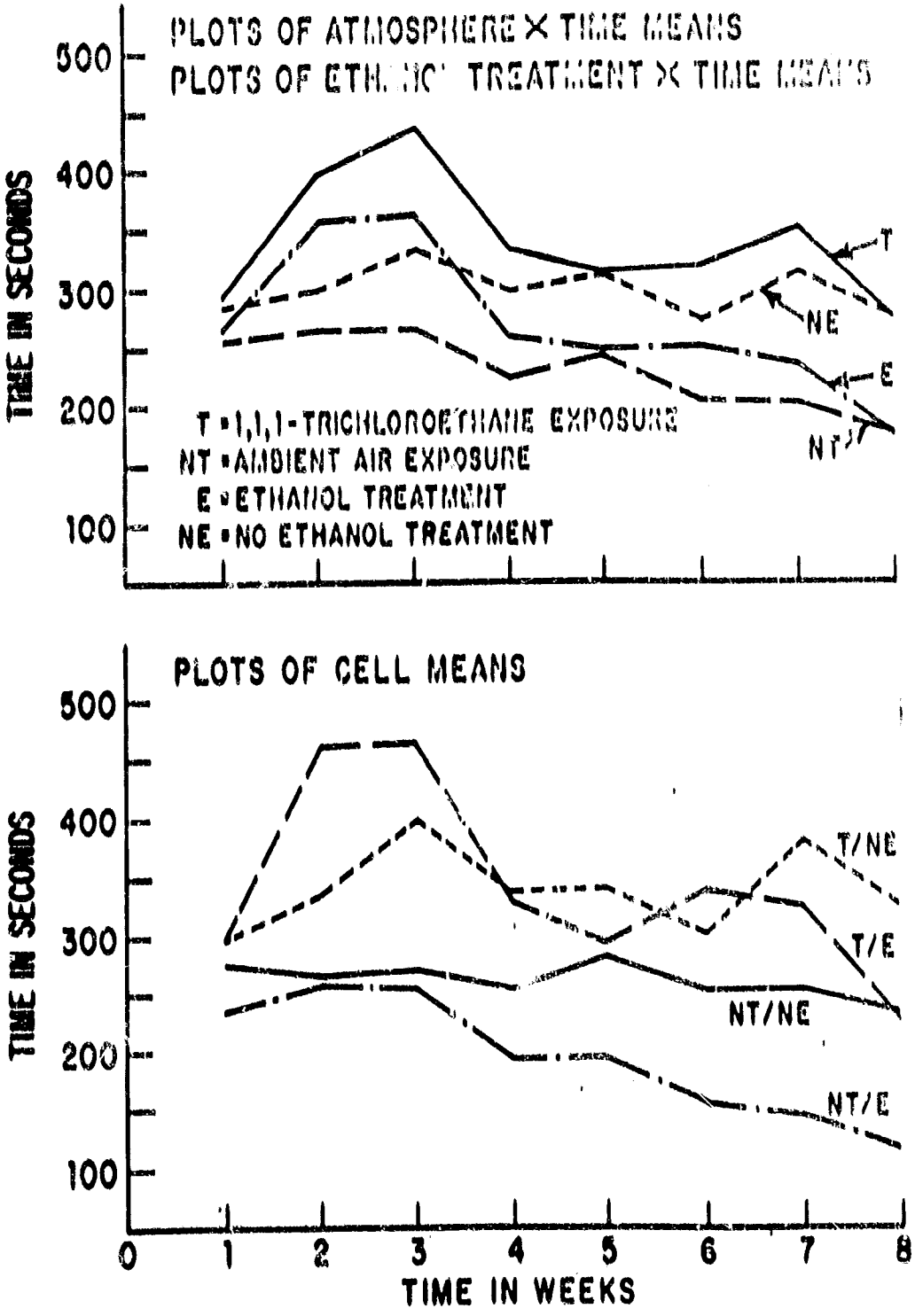
TABLE 7C: ETHANOL TREATMENT × TIME MEANS

TREATMENT	WEEK 1	2	3	4	5	6	7	8	OVERALL TREAT MEANS
NO ETHANOL	287.9	300.8	336.2	295.1	311.8	276.1	317.1	275.8	300.1
ETHANOL	264.6	359.4	362.2	261.4	247.1	248.8	237.4	173.5	269.3

TABLE 7D: OVERALL TIME MEANS

WEEK 1	2	3	4	5	6	7	8
276.2	330.1	349.2	278.3	279.5	262.5	277.2	224.7

FIG 9 SWIMMING TEST



between the 2 groups tested by swimming to endurance is approximately 50%.

A significant ethanol treatment x time interaction was also detected ($P < .005$). The result is very similar to that found for the tilting plane test. That is, it would appear that toward the end of the experiment the swimming time of the animals receiving ethanol became increasingly poorer than that of the animals not receiving ethanol (Table 7C and top of Figure 9). The result, however, is not perfectly clear, as can be seen from the bottom of Figure 9. It can be seen that even though the pattern of the nonethanol versus ethanol curves is obvious for these animals in the control atmosphere, it is not so obvious for those animals that were in the 1,1,1-trico atmosphere. There is a lot of variation in the latter case. There are 2 suggested explanations which together or independently may be responsible for the fluctuating results.

It is suggested that the superior performance noted in rats treated with ethanol during the first 2 weeks of the study was due in part to the high level of ethanol administered during this time period. An oral dose of 5 g/kg body weight of ethanol has been previously shown to provide a much higher blood alcohol level, and in turn greater intoxication, than ethanol administered ip at

1.5 g/kg body weight (Figure 6, p. 60). This fact was also evident when viewing in retrospect the swimming traits of the rats during the 2 time periods." The rats treated with ethanol during the first 2 weeks of the study appeared to be so intoxicated that they struggled very little when first placed in the water, and thus conserved energy. During the last 6 weeks of the study when the rats received ethanol via the ip route they were visibly less intoxicated and struggled much more than during the earlier swim sessions. The rats not receiving ethanol performed superior to the rats treated with ethanol after the third week, thus correlating well with the recorded observations.

Secondly, it has been reported that the swimming test does not provide as reproducible data as the tilting plane test. The investigators demonstrated that a linear regression curve could be drawn from the tilting plane but not from the swimming time results (58). The raw data correlate well with the findings of the above mentioned study. The overall range of extremes for the swimming test data is approximately 4 fold greater than for the tilting plane data. The range of extremes for an individual rat during the course of the study is approximately 10 fold greater in swimming test versus the tilting plane test. The fluctuations noted beyond the third week of the study

could, therefore, be explained by the much greater standard deviations inherited with the swimming test.

Significant overall time differences were also detected ($P < .001$) but are probably not of much consequence in view of the above results.

SERUM GLUTAMIC PYRUVIC TRANSAMINASE STUDY

The pathologic phase of the investigation included both clinical laboratory and necropsy evaluation. The serum glutamic pyruvic transaminase (SGPT) procedure was selected as the *in vitro* liver function test of choice to complement the post mortem liver weight determinations and the gross and histologic necropsy findings.

SGPT determinations were made on blood samples from each animal both before and after the experimental period. A preliminary statistical analysis was performed on the preexperimental data to test for inherent group differences. Such differences could affect the interpretation of other results since it is generally assumed that all groups were the same under "control" conditions. In this case, no significant differences were detected at the 5% significance level. It can be noted from Table 8 (p. 87) that different numbers were present in each group. This was unavoidable due to the loss of animals, especially ethanol treated subjects, during the course

TABLE 8

Pre and post experiment SGPT determinations*

TABLE 8A: Cell means

Atmosphere	Treatment	Pre Exp	Post Exp	Number
No 1,1,1-Tricc	No Ethanol	36.5	36.7	10
No 1,1,1-Tricc	Ethanol	35.7	47.2	6
1,1,1-Tricc	No Ethanol	32.3	39.2	9
1,1,1-Tricc	Ethanol	37.7	48.5	6

TABLE 8B: Atmosphere x time means

Atmosphere	Pre Exp	Post Exp	Overall Atmos Mean
No 1,1,1-Tricc	36.1	41.9	39.0
1,1,1-Tricc	35.0	43.9	39.4

TABLE 8C: Ethanol treatment x time means

Treatment	Pre Exp	Post Exp	Overall Treat Mean
No Ethanol	34.4	38.0	36.2
Ethanol	36.7	47.8	42.2

TABLE 8D: Overall time means

	Pre Exp	Post Exp
Overall Time Mean	35.5	42.0

*All SGPT values are expressed in Sigma-Frankel units.

of the study. There were also several blood samples collected from the animals which hemolyzed and thus had to be discarded.

An analysis of the complete data set yielded the following results. No atmosphere x time interraction was noted (Table 8B, p. 87). Several investigators evaluating the SGPT levels of animals exposed to near lethal levels of 1,1,1-trico have reported no statistically significant increase in SGPT values (36,37,85). It is, therefore, not surprising that no significant changes in SGPT levels were noted at the comparatively low 1,1,1-trico exposure levels utilized in this study.

Although there was only a slight indication of an ethanol treatment x time interaction ($P < .1$), it appears from an inspection of the means in Table 8C (p. 87) that an interaction is truly present. In this particular case, since the preexperiment time actually represents a "control" condition for all subjects, the interaction should actually reflect a simple ethanol effect. Specific testing verifies this. That is, there is no significant change from pre to post under the nonethanol condition, but there is a significant SGPT increase due to treatment with ethanol ($P < .005$). This result is plausible but somewhat surprising. Although Wrobleski and LaDue (78) consider SGPT the best of the hepatotoxic tests, it still

is a relatively insensitive test except in cases of moderate to severe liver damage. Lukasik et al (86) found that of 27 patients of whom 12 were chronic alcoholics, only a few showed increased serum enzyme activities suggestive of liver damage after administration of ethanol. Among 110 alcoholics studied while in a treatment service, SGPT levels were elevated in several cases, but the increased serum enzyme activity was not correlated with the physical state of the patient. The distribution of the values also showed considerable overlap with that of a control group. These findings fail to support the concept of a consistent toxic effect of alcohol on the liver, and also show that serum enzyme tests have little diagnostic value for detection of alcoholic liver disease (87). In contrast, it has been reported that in rats an oral dose of ethanol as low as 1 g/kg body weight produced an increase in SGPT values (21). In view of these conflicting investigations, it is difficult to ascertain if SGPT determinations provide an acceptable indicator for liver damage in general or if the value of the test is restricted to liver function determinations in only the rat. Because of inadequate studies in this area this problem remains unsolved at the present time. The present investigations, however, would at least support the point of view that SGPT

determinations are of some experimental value as an indicator of liver damage in rats treated with ethanol.

One of the important considerations of this study was to evaluate the possible synergistic effect of ethanol and 1,1,1-trico exposures in producing liver damage. Table 8C demonstrates that the increased SGPT values were a consequence of the ethanol treatment only and thus no potentiation of liver damage, as detected by the SGPT test, occurred as a result of the combination of exposures.

LIVER WEIGHT STUDY

After completion of the experiment, each animal was euthanatized and the liver weight determined. Statistical analysis has been performed on 2 variables: actual liver weight and liver weight expressed as a relative percentage of total body weight. The results were similar in the 2 cases. The overall average liver weight of rats exposed to 1,1,1-trico was significantly higher than that of the control atmosphere rats, ($P < .001$) in both cases. Specific comparisons at each treatment level supported this result. That is, the 1,1,1-trico versus ambient air difference was seen whether the animals were receiving ethanol or not. On the other hand, there was no strong indication from the analyses that ethanol

affected the liver weights of the animals. Table 9 (p. 92) reflects the data for actual liver weight and liver weight relative to body weight. It should be pointed out that odd sample sizes exist in this post mortem study because of the losses previously described.

The finding that the livers of animals exposed to the 1,1,1-trico atmosphere were significantly heavier than the livers of animals in the control atmosphere is an outcome which is not consistent with previous studies. Torkelson et al (30) found that after repeated 7 hour exposures of male rats to 500ppm 1,1,1-trico 5 days per week for 6 months, no significant liver weight increases were noted between exposed subject and controls. The average liver weight value of 2.43 g/100g body weight for the control animals and 2.38 g/100g body weight for exposed animals in the above study matches closely the mean control value of 2.30 g/100g body weight obtained in this investigation. In the present investigation, however, the overall mean value of subjects exposed to 1,1,1-trico was 3.02 g/100g body weight, 26.4% greater than the mean value of rats in the control group. The present study parallels the Torkelson et al (30) study closely in terms of experimental design with the exception of the periods of exposure. However, it is the periods of exposure which may explain the difference in results.

TABLE 9

Means for liver weights

TABLE 9A: Actual liver weights (grams)

Atmosphere	No Ethanol	Ethanol	Overall Atmos Mean
No 1,1,1-Trico	10.60	11.43	11.01
1,1,1-Trico	15.74	14.32	15.03
Overall Treat Mean	13.17	12.88	

TABLE 9B: Relative percentage*

Atmosphere	No Ethanol	Ethanol	Overall Treat Mean
No 1,1,1-Trico	2.24	2.54	2.39
1,1,1-Trico	3.08	2.96	3.02
Overall Treat Mean	2.66	2.75	

Sample sizes were as follows:

No 1,1,1-trico and no ethanol = 10 observations

No 1,1,1-trico and ethanol = 9 observations

1,1,1-Trico and no ethanol = 12 observations

1,1,1-Trico and ethanol = 9 observations

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*Relative % = Liver weight/body weight x 100

Although the Torkelson et al (30) study was 6 months in duration, the exposure was not continuous and overall resulted in less total exposure time to the agent than was obtained in the 8 week period of the present study. The suggestion is made that whereas the present study was truly chronic in nature, the Torkelson et al (30) study allowed the liver of each subject exposed to the 1,1,1-trico vapors adequate time between exposures to remove lipid material built up during periods of exposure.

Adams et al (29) suggested that the effects of 1,1,1-trico upon the liver weights of subjects were dependent primarily upon the vapor concentration of the agent. He reported a liver weight increase of 20.9% in rats given a single 7 hour exposure to 12,000ppm 1,1,1-trico, but no significant increase in the liver weights of rats subjected to repeated 7 hour exposures of 5,000ppm or less. The present study, although demonstrating a liver weight increase comparable to that noted above, differs in that the liver weight gains appear to be dependent upon the duration of exposure rather than vapor concentration.

The finding in this investigation that the chronic ethanol intake had no statistically significant effect on liver weight is as surprising as the significant 1,1,1-trico effect previously discussed. Lieber et al (49)

found that when rats were given ethanol for 24 days at a level equal to 36% of their daily total caloric intake, their livers increased in weight 20% over controls. Mallov (26), demonstrated that when ethanol was given daily in the food of rats at the approximate level of 6 g/kg body weight, fatty livers developed in the rats within 30 days. The rats chronically intoxicated with alcohol accumulated liver lipids in excess of the amount found in the corresponding controls fed isocalorically by an average of 36%, a significant increase ($P < 0.01$). The negative ethanol effect in the present study is difficult to explain in view of numerous reports pointing to a direct relationship between ethanol intake and resulting fatty liver infiltration. It is suggested in the present study that the dietary component was perhaps the important factor in protecting the liver. Lieber *et al* (49) demonstrated that with a low-fat diet much less lipid accumulated in the liver than with a high-fat diet, although the same quantity of alcohol was given. In the present study the entire caloric intake, other than by ethanol intake, was provided by a low-fat standard laboratory chow ration.

GROSS AND HISTOPATHOLOGY

Following euthanasia of the test subject, completion of blood collection and liver weighing procedures, the lungs, kidneys and liver of each animal were placed in buffered 10% formalin solution for fixation. Tissue sections of each of the above mentioned organs were prepared and stained with hematoxylin-eosin. Each liver section from each rat was carefully examined for the appearance of excessive lipid infiltration, and frozen sections stained with Oil Red O were prepared from selected liver sections. The results of this section of the paper have been divided into a discussion of the individual organs; the lungs, the kidneys, and the liver.

The selection of the rat as a test subject proved to be a good choice throughout the study with the exception of the pathologic investigation of the lungs. It was hoped that because the animals were less than 6 months of age at termination of the project they would be relatively free of the inevitable chronic respiratory disease (CRD) complex of rats. However, every animal examined, even those rats which had died during the experimentation period, exhibited histopathologic lesions consistent with varying degrees of CRD. In all of the lung sections examined there was mild to severe perivascular and peribronchiolar cuffing by lymphocytes of

small to medium sized vessels and bronchi. There was thickening of alveolar septa with macrophages frequently present within the septal walls and a few lungs exhibited areas of atelectasis. Not infrequently there was enlargement of the lymphoid nodules in the hilus and focal invasion of the lamina propria of the mucosa of some of the large bronchi. Unfortunately, the evaluation of the lung tissue was made more difficult by the fact that blood had been aspirated by the animals following decapitation, resulting in pooling of blood in bronchi, bronchioles and alveolar spaces.

The lung sections from each animal were evaluated subjectively with the intention of grading the extent of CRD damage as either a mild lesion or a moderate lesion. Table 10 below reflects the compiled histopathologic data for the lung sections.

TABLE 10

Lung histopathologic data

Group	Atmos/Treat	Mild CRD	Moderate CRD
1	1,1,1-Trico/Ethanol	----	9/9
2	1,1,1-Trico/No Ethanol	----	12/12
3	Ambient Air/No Ethanol	3/10	7/10
4	Ambient Air/Ethanol	6/8	2/8

Although no statistical approach was applied to the data it is apparent on superficial evaluation of the figures that a 1,1,1-trico effect may exist. It can be noted that under ambient air conditions 9 of 18 (50%) of the CRD lesions were considered mild whereas under the influence of the 1,1,1-trico atmosphere all of the CRD lesions, 21 of 21 (100%), were of at least a moderately severe nature. The data, therefore, implies that although all rats had CRD type lesions, the rats in the 1,1,1-trico atmosphere developed a more severe state of CRD involvement than their counterparts in an ambient air environment.

Lung involvement, consisting primarily of marked congestion and edema, has been reported in fatal human exposures and acute laboratory animal studies (26,45,46, 47). Torkelson et al (30) reported that there was a higher than normal incidence of interstitial inflammation of the lungs in guinea pigs repeatedly exposed for short periods of time to 2000ppm of 1,1,1-trico. Prendergast et al (31) reported the death of 2 of 15 rats and 1 of 3 rabbits exposed continuously for 90 days to 135ppm 1,1,1-trico. Necropsy and subsequent histopathologic examination of the animals revealed lung congestion and pneumonitis which in the opinion of the investigator may have been severe enough to have caused the deaths.

Because similar but less severe lung involvement was present in the controls it was determined that no positive conclusion could be drawn as to whether the deaths were associated with the exposure.

It is difficult to compare the pathology of the lungs described in the present study versus the earlier studies because of the vast dissimilarity in the dose and exposure time to the 1,1,1-trico vapors. However, it is interesting that the present study and the Prendergast et al (31) study, the only low level 1,1,1-trich continuous exposure studies conducted to date, noted results suggesting that the agent may play a contributory role in enhancing the severity of lesions and rapidity of onset of existing lung conditions such as the CRD complex of rats.

The role of ethanol in terms of lung lesions appears to be negligible. There were, however, gross and histopathologic lung findings associated indirectly with the ethanol treatment. One rat in Group 1 and 1 rat in Group 4 had adhesions involving the caudal lobe of the lungs and the diaphragm. The etiology of these lesions can most likely be traced to the first 2 weeks of the experimental period when ethanol was administered with a stainless steel stomach tube. Four animals examined after sudden deaths during the per os dosing period demonstrated

adhesions involving the esophagus, trachea, lungs and diaphragm with periesophageal and peritracheal inflammatory reactions and fibrosis noted on histopathologic examination. Although no esophageal rupture was present in these 2 rats when examined at the termination of the study it is likely that the etiology of the lesions was the same.

No remarkable gross lesions were noted on examination of the intact kidneys of the rats. A few lesions, however, were noted upon microscopic examination of the tissue sections. The table below reflects the results of the microscopic examination in terms of the presence or absence of demonstrable lesions.

TABLE 11

Kidney histopathologic data

Group	Atmos/Treat	Normal Kidney	Abnormal Kidney
1	1,1,1-Tricc/Ethanol	8/9	1/9
2	1,1,1-Tricc/No Ethanol	8/12	4/12
3	Ambient Air/No Ethanol	8/10	2/10
4	Ambient Air/Ethanol	5/8	3/8

Once again no statistical approach was applied to the data; however, on superficial observation of the data it

is apparent that no distinct pattern of pathologic involvement exists. All 4 groups demonstrated some type of renal damage and no particular treatment, ethanol administration or 1,1,1-trico exposure, appears to have influenced the results. The kidney lesions observed for each group were as follows: One rat in Group 1 demonstrated multiple focal areas of necrosis of the proximal convoluted tubular epithelium with some cellular casts in the tubular lumina. There were also numerous areas where a cellular recuperative process was evident. Two rats in Group 2 possessed similar lesions to those described in the rat from Group 1. Two other rats in this group had kidney lesions consistent with a pyelonephritis, as evident by the presence of numerous neutrophils in the pelvis, the pelvic epithelium and the adjacent interstitial tissue of the medulla. One rat in Group 3 had the same type of pyelonephritis as previously described and the second rat in this group had areas of focal necrosis similar to the lesions previously described. All 3 of the rats in Group 4 had renal lesions consisting of areas of focal necrosis as previously described.

The random distribution of lesions in individuals of all 4 groups is consistent with the literature concerning kidney pathology under the influence of 1,1,1-trico and ethanol treatment. The literature contains

very little information which would inoriminate 1,1,1-trico as a nephrotoxic agent. Plaa and Larson (30) found that in mice neither necrosis nor even swelling of the proximal convoluted tubules could be induced by near lethal intraperitoneal injections of 1,1,1-trico. Klaassen and Plaa (36,37) found that after near lethal intraperitoneal injections of 1,1,1-trico in mice and dogs the only demonstrable kidney lesions were a small number of calcified tubules in the medulla.

Ethanol intake has likewise been considered to produce little or no kidney damage. Orten et al (88) reported the only kidney damage associated with alcohol intake in a study utilizing rats. He described enlarged kidneys, damage to the epithelial cells of the renal proximal tubules and hematuria in rats receiving 20% or 40% alcohol as their sole source of fluid. It is likely, however, that dehydration was the actual etiology of the lesion since a 20% or 40% alcohol solution far exceeds the level, 8.6% alcohol solution, at which rats will reject the solution (89).

Two methods were utilized in the histopathologic examination of the livers. The livers were first evaluated for the presence or absence of necrosis. Then the livers were evaluated in a subjective manner for the quantity of lipid infiltration demonstrable on

hematoxylin-eosin and Oil Red O stained sections. Table 12 (p. 103) reflects the compiled data recorded after careful examination of the rat livers.

The first evaluation, presence or absence of necrotic lesions, was based strictly on a quantitative criteria. If a lesion or lesions of any gradation were observed on histopathologic examination the specific liver was reported as positive. Morphologically the typical positive lesion consisted of a small area or areas of hepatic necrosis with focal aggregations of inflammatory cells, particularly around bile ducts. None of the reported lesions were large enough to be noted on gross observation of the intact or freshly cut liver.

The data from Table 12 (p. 103) represents an experimental result which is difficult to explain. It is quite remarkable that hepatic lesions were seen in those rats exposed to 1,1,1-trico or treated with ethanol but not in rats receiving the combination. It is unlikely that the combination of treatments provides a more benign situation than the individual variables. It is more likely, instead that the outcome was a consequence of either probability or some other variable not defined. This suggestion is reinforced by the outcome of the "control" group, group 3. Since approximately an equal incidence of lesions were present between a nontreated

TABLE 12

Liver histopathologic data

Group	Atmos/Treat	Necrosis Present	H&E Stain* Lipids Min	H&E Stain* Lipids Mild	Oil Red O** Lipids Mild
1	1,1,1-Tricc/Ethanol	0/9	8/9	1/9	1/4
2	1,1,1-Tricc/No Eth	7/12	9/12	3/12	2/4
3	Ambient Air/No Eth	5/10	6/10	4/10	3/4
4	Ambient Air/Ethanol	3/8	3/8	1/8	0/4

*The placement of a given liver section into the minimal or mild lipid infiltration classification was based strictly upon a subjective evaluation of the number and size of vacuoles in the section.

**Frozen sections were prepared of those livers recorded as having mild lipid infiltration. When less than 4 sections were classified during H&E slide examination as having mild lipid infiltration, frozen sections were prepared of those H&E sections which appeared to have the largest amount of lipid infiltration among those sections classified as demonstrating minimal lipid infiltration.

group and treated groups the observations probably represented an expected result rather than a result influenced by the ethanol treatment or 1,1,1-trico exposure.

Previous investigations concerned with the hepatotoxic nature of ethanol or 1,1,1-trico support the assumption that the distribution of necrotic lesions among the groups was by chance and not necessarily associated with either variable. No investigator to date has reported a definite relationship between ethanol intake and liver necrosis, and only 3 incidental accounts of liver necrosis have been reported as a direct consequence of 1,1,1-trico exposure (29,36,37). Klaassen and Plaa (36) reported that a slight necrosis of the liver occurred when mice were exposed to near lethal levels of 1,1,1-trico. A descriptive account of the area of the liver involved and the type of necrosis was not provided. In a later study in dogs, Klaassen and Plaa (37) reported that although no liver necrosis occurred with 1,1,1-trico exposures, there was a moderate neutrophilia infiltration in the sinusoids and portal areas. Adams et al (29) reported a few cases of hemorrhagic necrosis in the central areas of the livers of rats exposed to 12,000ppm of 1,1,1-trico for 7 hours. In all of these accounts,

however, little significance was attributed to the hepatotoxic findings.

The observations made concerning the degree of lipid infiltration in the livers of the rats from the various groups provided still another controversial finding. It would appear from the data from Table 12 (p. 103) that the likelihood of lipid infiltration into the liver was greater in the control group than in either the ethanol treatment or 1,1,1-trico exposure groups. In view of previous investigations this present finding is an outcome which is exactly opposite to the expected result.

Several investigators have contended that chronic ethanol consumption contributes to a fatty liver under various dietary conditions and Lieber and associates (49,50) have concluded that chronic ethanol administration can induce fatty livers in both rats and humans even when the diet contains ample amounts of protein and lipotropic factors (25,26,90).

Likewise, 1,1,1-trico has been implicated in fatty changes of the liver. Klaassen and Plaa (36) reported an enlargement of hepatocytes with cellular lipid infiltration and vacuolation when mice were exposed to near lethal levels of the agent. Adams et al (29) reported the appearnce of numerous small clear vacuoles throughout the cytoplasm of the hepatic cells of rats exposed to

12,000ppm of 1,1,1-trico for 7 hours and similar central fatty degeneration in the livers of guinea pigs exposed to 5,000ppm of 1,1,1-trico for 7 hours.

CONCLUSION

A review of the literature has shown that both ethanol consumption and 1,1,1-trico exposure have been associated with industrial accidents, resulting injuries, and deaths. The literature also reveals that a combination of ethanol intake and exposure to various chlorinated hydrocarbons has resulted in increased likelihood and severity of pathologic lesions. Although no investigator to date has reported a possible synergistic effect between 1,1,1-trico exposure and ethanol treatment, the reason for such negative findings may be due to the fact that the bulk of such investigations have been short term studies involving acute exposures. It has, therefore, been suggested in this study that a possible synergistic relationship may exist between these 2 agents if they were investigated on a basis of chronic exposure. Furthermore, the levels of ethanol intoxication and 1,1,1-trico exposure investigated in this study are quite comparable to the realistic levels noted under various industrial situations.

To determine the effect of chronic ethanol treatment and low level 1,1,1-trico exposure, and the possibility of a synergistic effect with the combinations, 3 areas of interest were considered in the study. The first area of interest was consideration of the effect of the

treatments upon the weights of growing rats over a 56 day period. The second was consideration of the effect of the exposures upon endurance, coordination, and the equilibrium capacities of the rats as determined by the psychophysiologic tests, tilting plane test and swimming to endurance test. The final area of interest was consideration of the direct effect of the exposures upon the bodies of the rats as determined by SGPT determinations, liver weight comparisons, and gross and microscopic examination of various organs.

Statistically significant 1,1,1-trico x time ($P < .001$) and ethanol x time ($P < .025$) interactions were detected in the weight gain study. There was, however, a great deal of fluctuation in the pattern of the 1,1,1-trico x time curves thus nullifying the significance of the results. On the other hand, the ethanol x time curves remained essentially parallel from week to week during the course of their fall below the "control" curves. This finding confirmed earlier reports which had concluded that ethanol intake in some manner depresses food intake or utilization resulting in poor weight gains. No synergistic effect was noted.

Similar results were obtained in a comparison of the results of the 2 psychophysiologic tests. In both tests a statistically significant decline in performance was

demonstrated as a consequence of ethanol treatment. This degenerative pattern was consistent with data from previous chronic ethanol studies. In both studies there was also a statistically significant increase in test results as a consequence of an apparent stimulatory reaction to the 1,1,1-trico exposure. No previous studies have investigated the agent adequately under conditions comparable to those utilized in this study, thus it is suggested from the data of this study that continuous exposure to 1,1,1-trico in the TLV range may result in definite increased capabilities in performing certain psychomotor tasks. No synergistic response was note since the stimulatory effect of the 1,1,1-trico exposure was offset by the depressing effect of the ethanol treatment.

The SGPT study demonstrated no atmosphere x time interaction but a slight ($P < .1$) ethanol x time interaction. The ethanol effect was made more meaningful when the postethanol SGPT values were compared to all preexperimental SGPT values. In this case a significant SGPT increase ($P < .005$) was apparent from the ethanol treatment. The significance of the ethanol effect is debatable in view of the conflicting reports in existing literature. In addition, the sensitivity of the SGPT test has been questioned in cases of minimal hepatic damage. In view of the data collected in this study,

however, it was concluded that the SGPT determinations were of some experimental value as an indicator of liver damage in rats treated with ethanol. No synergistic response was noted in this study.

The liver weight study revealed a statistically significant ($P < .001$) increase in liver weights in animals exposed to the 1,1,1-trico. Previous investigators have not demonstrated this result, however, none of these investigations involved a continuous exposure as implemented in this study. No statistical effect on liver weight was attributed to ethanol intake. Previous studies conflict with this negative finding, but it has been suggested by the investigator that inadequate diets possibly contributed to the reported positive findings of earlier studies. No synergistic effect was noted in this study.

Gross and histopathologic examination of the lungs, kidneys and liver of each rat provided interesting but again conflicting results. The data from examination of pathology of the lungs suggests that 1,1,1-trico may play a contributory role in enhancing the severity and speed of onset of certain lung conditions. The kidneys grossly and microscopically presented an unremarkable picture which was consistent between various groups and which was consistent with the findings of earlier studies.

The compiled data from the 2 approaches to the liver, necrosis determination and lipid infiltration evaluation, presented a most controversial set of conclusions. Necrosis was present in the livers of all the groups with the exception of the rats exposed to ethanol treatment and 1,1,1-trico exposure. Lipid infiltration into the livers occurred in all groups but there was a higher incidence in the controls than in the treated rats. The literature does not support either of these observations. On the contrary, it tends to support the view that the presence of necrotic lesions and lipid infiltration is more likely to be related to treatment with either variable. No conclusion may be drawn from this histopathologic study but it does suggest that the observations could reflect inherent inaccuracies of subjective evaluations and the probability of errors associated with small sample sizes. No synergistic effect was apparent in this study.

In summary, it can be concluded that under the conditions of this investigation there was no indication of any synergistic responses resulting from a combination of ethanol treatment and 1,1,1-trico exposure. There were, however, several conclusions drawn from the data from this study which would seem to warrant further investigation with the agent 1,1,1-trico.

Firstly, the apparent stimulatory effect of the 1,1,1-trico exposure upon psychomotor performance is an outcome not previously reported. It appears that continuous 1,1,1-trico inhalation produces a sequence of results similar to that seen with some intravenous barbituates. That is, there appears to be an excitement or stimulatory stage at low absorption levels, narcosis at slightly higher levels, and anesthesia at a still higher level of absorption. Additional studies in this area should include a duplication of the present study, expanded psychophysiological testing, and utilization of other research animals to verify the extent of stimulatory reaction.

Secondly, in view of conflicting results concerning liver weight gains as a consequence of 1,1,1-trico exposure or ethanol treatment, a series of tests should be performed with consideration of the diet as an additional variable. The lipid content and lipotropic factor content of diets appears from earlier studies to be so instrumental in dictating the presence or absence of lipid infiltration into the liver that it is not feasible to make definite conclusions from existing data.

Finally, it seems justified, on the basis of the present study, to expand research into the area of

1,1,1-trico exposure and accompanying pathologic lesions of the lungs. It has been demonstrated in this study that as in previous studies there is no indication of 1,1,1-trico exposure inducing lung lesions. However, the present study data suggested a relationship of the 1,1,1-trico exposure predisposing an individual to more severe complications and rapidity of the pathogenesis of existing lung lesions. Further investigations should be initiated to consider the effects of low level chronic 1,1,1-trico exposure of test subjects who possess such lung conditions as asthma, tuberculosis, emphysema, etc. This study would be of importance to individuals concerned with occupational medicine since it is highly likely that somewhere among the ranks of vapor degreasing operators there are at least a few individuals with chronic respiratory disease.

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VITA

DeWayne H. Walker is the son of Mr. and Mrs. Darrell W. Walker. He has one sister, Mrs. Roxanne C. Bridges. He was born in Moline, Illinois on November 16, 1942, and was reared in Minneapolis, Minnesota. His domicile is Lake Orion, Michigan, the current home of his parents.

He graduated from John Marshall High School, Minneapolis, Minnesota in 1960. He first entered the University of Minnesota the same year in the field of chemical engineering, later becoming a zoology and history major prior to entering the preveterinary medicine curriculum in the same university in 1962. In 1966 he received a Bachelor of Science degree and in 1968 the degree of Doctor of Veterinary Medicine from the University of Minnesota.

In July 1968 he entered active duty with the United States Air Force. Between September 1968 and July 1971 he served as Chief of Veterinary Services, RAF Chicksands, England. Since July 1971 he has been assigned to Texas A&M University and the USAF School of Aerospace Medicine, Brooks AFB, Texas, to pursue a residency and Master of Science degree in Laboratory Animal Medicine.

The typist for this thesis was Mrs. Joyce G. Braxton, San Antonio, Texas.

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NINETY DAY PERCUTANEOUS ABSORPTION STUDY IN RABBITS WITH DOW CHEMICAL COMPANY - CHLOROTHENE DOW CHEMICAL COMPANY - METHYLENE CHLORIDE DOW CHEMICAL COMPANY - ISOPROPYL ALCOHOL PREPARED BY DOW CHEM CO

Chemical Name (300 per name)	25	CAS No. (10)	24
CHLOROTHENE		71-55-6	

A.K 5/21/84

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NINETY DAY PERCUTANEOUS ABSORPTION STUDY IN RABBITS

WITH

DOW CHEMICAL COMPANY - CHLOROTHENE

DOW CHEMICAL COMPANY - METHYLENE CHLORIDE

DOW CHEMICAL COMPANY - ISOPROPYL ALCOHOL

INDUSTRIAL BIOLOGY RESEARCH
AND TESTING LABORATORIES, INCORPORATED

MORRIS V. SHELANSKI, M.D., C.M.
Director

RECEIVED

NINETY DAY PERCUTANEOUS ADSORPTION STUDY IN RABBITS

WITH

DOW CHEMICAL COMPANY - CHLOROTHENE

DOW CHEMICAL COMPANY - METHYLENE CHLORIDE

Purpose: To study the systemic toxicity of two Dow Chemical Company products - Chlorothene and Methylene Chloride when each was applied percutaneously to rabbits over a ninety day period.

Materials:
1. Dow Chemical Company - Chlorothene
2. Dow Chemical Company - Methylene Chloride
3. Isopropyl Alcohol as a control material

Procedure: Sixty young adult male albino rabbits weighing approximately 2 to 3 kilos were observed for a two week period to assure normalcy. The rabbits were then divided into fifteen groups of four rabbits each for purposes of dosing.

The following dosage levels were used in this study:

Group I - Control - handled but without chemical treatment

Group II - 15 mg/Kg. Chlorothene daily

Group III - 50 mg/Kg. Chlorothene daily

Group IV - 100 mg/Kg. Chlorothene daily

Group V - 200 mg/Kg. Chlorothene daily

Group VI - 500 mg/Kg. Chlorothene daily

Group VII - Control - handled but without chemical treatment

Group VIII - 15 mg/Kg. Methylene Chloride daily

Ninety Day Percutaneous Absorption Study in Rabbits With
Dow Chemical Co. - Chlorothene & Methylene Chloride

Procedure: (Continued from Page 1)

Group IX - 50 mg/Kg. Methylene Chloride daily

Group X - 100 mg/Kg. Methylene Chloride daily

Group XI - 200 mg/Kg. Methylene Chloride daily

Group XII - 500 mg/Kg. Methylene Chloride daily

Group XIII - 15 mg/Kg. Isopropyl Alcohol daily - handled and chemically treated control

Group XIV - 100 mg/Kg. Isopropyl Alcohol daily - handled and chemically treated control

Group XV - 500 mg/Kg. Isopropyl Alcohol daily - handled and chemically treated control

All sixty animals were restrained in stocks during the eight hour dosing period. The stocks were situated in exhaust hoods leaving only the heads of the animals exposed to the external atmosphere, to prevent inhalation of fumes from the experimental materials. The total daily doses were divided into four equal parts which were administered directly to the shaved skin of the animals at 10:00 A. M., 12:00 Noon, 2:00 P. M., and 4:00 P. M. respectively. At the end of the daily dosing period the skin was wiped dry and the animals were returned to their cages and fed colony diet ad lib. The dosages were administered on the basis of a five day week.

All animals were weighed at the beginning of the study and weekly thereafter. Total dosages for each animal were adjusted accordingly.

Two animals in each group were studied with intact skin and two animals in each group were studied with abraded skin. The abrasions were renewed weekly.

Hematologic studies, including hemoglobin, red blood cell count, white blood cell count, icterus index and differential were performed on all animals at the beginning of the study and at the end of thirty days, sixty days and ninety days.

Ninety Day Percutaneous Absorption Study in Rabbits With
Dow Chemical Co. - Chloroethane & Methylene Chloride

Procedure: (Continued from Page 2)

At the end of thirty days, one animal in each group was sacrificed for gross and microscopic study of the tissues. The tissues studied histologically included the skin from the local area of contact, brain, heart, lung, liver, kidney, spleen, stomach, intestine, gonad and thyroid. Organ weights were taken of the brain, lung, heart, liver, stomach, kidney, spleen, gonad and thyroid.

At the end of ninety days again, one animal in each group was sacrificed for gross and microscopic study of the tissues.

The surviving animals are being maintained for an additional thirty day rest period for the purpose of observing possible latent effects.

Results: See attached charts.

1. Hematology

All changes remained within normal limits. No consistant trend was noted in any aspect of the blood picture.

2. Weight Gain

There was no significant difference in the weight gain between the experimental and the control animals during the ninety day dosing period.

3. General Appearance and Behaviour

There was no difference in general appearance and behaviour of experimental and control animals during the ninety day dosing period. There was no difference in the food intake of the experimental and control animals during the ninety day dosing period.

Ninety Day Percutaneous Absorption Study In Rabbits With
Dow Chemical Co. - Chlorothene & Methylene Chloride

Results: (Continued from Page 3)

4. Pathology

a. Local Effects

All experimental groups showed similar local effects on the skin. These consisted of cellular infiltration and slight focal epidermal hyperplasia. No sensitization occurred with any of the materials.

b. General Effects

No consistant pathology was observed in either the experimental or the control groups.

Conclusions: Chlorothene

The application of Chlorothene in daily doses from 15 mg/Kg. to 500 mg/Kg. to the intact or abraded skin of young adult male albino rabbits produces no effects of local or systemic toxicity which would gainsay its safety of use even for periods as long as ninety days.

Methylene Chloride

The application of Methylene Chloride in daily doses from 15 mg/Kg. to 500 mg/Kg. to the intact or abraded skin of young adult male albino rabbits produces no effects of local or systemic toxicity which would gainsay its safety of use even for periods as long as ninety days.

INDUSTRIAL BIOLOGY RESEARCH
AND TESTING LABORATORIES, INC.

MORRIS V. SHELANSKI, M.D., C.M.
Director

MVS/ld
Enc.

AVERAGE HEMATOLOGICAL VALUES ([illegible]) FROM [illegible]
[illegible]

[illegible]	No.	Daily Dose (mg./kg.)	RBC X10^6	WBC X10^3	Hb. g./100 cc.	Differential (%)				
						U	L	M	E	B
[illegible]	1	-	4.51	8.49	9.9	49	49	2	1	0
[illegible]	2	15	4.67	11.04	10.6	48	46	4	0.5	1
	3	50	4.81	7.63	10.6	48	47	3	1	1
	4	100	4.32	8.71	9.6	47	50	2	0	0.5
	5	200	4.44	8.87	10.3	49.5	46	4	0.5	1
	6	500	4.33	12.33	9.8	49	48	3	1	1
[illegible]	7	-	4.84	8.47	10.5	48	51	1	0	0.5
Methylene Chloride	8	15	4.06	7.21	9.1	47	49	4	0	1
	9	50	4.93	8.30	11.2	48	49	2	1	1
	10	100	4.67	7.70	9.8	50	46	3	0.3	1
	11	200	4.81	6.33	11.0	49	49	1	0	1
	12	500	5.43	7.53	12.4	48	49	2	0.2	1
[illegible]	13	15	4.16	7.69	9.7	45	49	3	1	2
	14	100	4.04	8.82	8.9	51	47	1	0	1
	15	500	4.95	7.25	10.8	47	46	4	1.5	1

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[illegible]

Group	Group No.	Daily Dose (mg./kg.)	Average Body Weight (g.) Initial	30-day	60-day	Weight Gain (g.)
Control	1	-	2370	2170	2510	140
Chloroform	2	15	1970	[illegible]	[illegible]	[illegible]
"	3	50	2190	2500	[illegible]	700
"	4	100	2180	2560	2910	[illegible]
"	5	200	2550	2520	[illegible]	[illegible]
"	6	500	[illegible]	[illegible]	[illegible]	[illegible]
Control	7	-	[illegible]	[illegible]	[illegible]	[illegible]
Methylene Chloride	8	15	[illegible]	[illegible]	[illegible]	[illegible]
	9	50	[illegible]	2660	[illegible]	[illegible]
"	10	100	[illegible]	[illegible]	[illegible]	[illegible]
"	11	200	1915	2070	2390	[illegible]
"	12	500	[illegible]	[illegible]	[illegible]	[illegible]
Isopropyl Alcohol	13	15	[illegible]	[illegible]	[illegible]	[illegible]
"	14	100	[illegible]	[illegible]	2780	[illegible]
"	15	500	2110	[illegible]	[illegible]	[illegible]

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AVERAGE HEMATOLOGICAL VALUES (AFTER 90 DAYS) [illegible]
[illegible]

[illegible]	No.	Daily Dose (mg./kg.)	RBC $\times 10^6$	WBC $\times 10^3$	Hb. g./100 cc.	Differential (%) N	L	[illegible]	[illegible]	B
[illegible]	1	-	450	10.41	9.9	49	48	1.5	1.5	0
[illegible]	2	15	4.74	9.84	10.8	49	48	2	0	0
	3	50	4.89	9.72	10.7	51	48	1	1	0
	4	100	5.01	9.69	11.2	49	49	1	1	0
	5	200	4.55	9.86	10.7	49	49	1.5	0.5	0
	6	500	4.67	10.61	10.4	50	49	1	0	0
[illegible]	7	-	5.11	9.85	10.6	49.5	49.5	1	0	0
Methylene Chloride	8	15	4.50	9.54	10.4	49	48	2	1	0
	9	50	4.64	9.11	11.7	47	50	2	1	0.3
	10	100	4.83	8.13	10.8	50	49	1	0.3	0.3
	11	200	4.72	6.05	10.9	43.5	55.0	1.5	0	0
	12	500	4.65	7.36	11.0	46	52	2	1	0
[illegible]	13	15	5.17	8.76	11.6	49	48	2	0.5	0.5
	14	100	4.04	8.68	12.5	48	50	1	0	0.5
	15	500	5.27	8.77	11.7	50	48	1	0.2	0

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[illegible]

[illegible] (g./100 [illegible])

[illegible]

[illegible]	[illegible]	[illegible] (mg./kg.)	Body Wt. kg.	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]										
[illegible]	1		1.99	0.41	0.25	4.52	0.75	[illegible]	[illegible]	[illegible]
[illegible]	7		3.12	0.37	0.21	3.59	[illegible]	[illegible]	[illegible]	[illegible]
	Average		2.91	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]										
[illegible]	2	15	2.30	0.35	0.25	3.85	0.63	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	50	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	100	2.41	0.41	[illegible]	5.51	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	5	[illegible]	2.55	0.35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	6	500	1.91	0.35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Methylene Chloride										
[illegible]	8	15	2.33	0.39	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	50	3.05	0.32	[illegible]	3.85	[illegible]	[illegible]	[illegible]	[illegible]
	10	100	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	11	200	2.03	0.35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	12	500	2.66	0.35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]										
[illegible]	13	15	2.73	0.36	[illegible]	5.29	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	100	[illegible]	[illegible]	[illegible]	5.50	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	500	2.11	[illegible]	[illegible]	3.91	[illegible]	[illegible]	[illegible]	[illegible]

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[illegible] - [illegible]

Organ Weight Summary (g./100 g. Body Wt.)

90 Day Exposure

Rabbit No.	Group No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Control										
1	1		2.14	0.40	0.22	7.04	1.03	0.05	0.10	0.[illegible]
2	7		2.21	0.52	0.25	8.41	1.00	0.07	0.06	0.[illegible]
	Average		2.18	0.46	0.27	7.73	1.0[illegible]	0.05	0.0[illegible]	0.[illegible]
Chloroform										
2	2	15	2.49	0.36	0.30	4.61	0.90	0.06	0.20	0.41
1	3	50	2.68	0.42	0.34	5.48	0.75	0.07	0.19	0.33
4	4	100	2.64	0.38	0.28	4.20	0.[illegible]	0.07	0.21	0.36
	5	200	2.62	0.39	0.32	4.91	0.[illegible]	0.07	0.25	0.[illegible]
	6	500	2.56	0.45	0.30	5.38	0.[illegible]	0.11	0.22	0.34
Methylene Chloride										
1	3	15	2.44	0.48	0.27	4.10	0.75	0.05	0.18	0.39
2	9	50	2.93	0.30	0.27	3.61	0.65	0.03	0.25	0.33
3	10	100	2.47	0.46	0.30	5.26	0.90	0.11	0.12	0.[illegible]
4	11	200	2.25	0.39	0.26	4.65	0.[illegible]	0.11	0.11	0.42
5	12	500	2.33	0.40	0.27	5.13	0.85	0.08	0.24	0.[illegible]
Isopropyl Alcohol										
3	13	15	2.50	0.39	0.30	3.56	0.93	0.04	0.24	0.34
4	14	100	2.53	0.36	0.24	3.82	0.78	0.04	0.21	0.[illegible]
5	15	500	3.13	0.35	0.29	5.66	0.79	0.[illegible]	0.17	0.[illegible]

000011

[illegible] - [illegible]

Organ Weight Summary (g./100 g. Body Wt.)

90 Day Exposure + 30 Day Rest Period

Rabbit No.	Organ No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Control										
3	1		2.82	0.3[illegible]	0.27	4.35	0.63	0.0[illegible]	0.17	0.30
[illegible]	7		3.74	0.33	0.24	4.46	0.69	0.04	0.19	0.[illegible]
3	7		3.28	0.43	0.28	4.26	0.65	0.05	0.19	0.[illegible]
	Average		3.28	3.67	0.2[illegible]	4.36	0.[illegible]	0.[illegible]	0.1[illegible]	0.2[illegible]
Chlorothrum										
[illegible]	2	15	3.87	0.29	0.25	4.52	0.76	0.04	0.25	0.2[illegible]
[illegible]	2	15	3.48	0.31	0.26	4.80	0.68	0.05	0.26	0.[illegible]
	Average		3.68	0.30	0.2[illegible]	4.6[illegible]	0.7[illegible]	0.0[illegible]	0.25	0.[illegible]
[illegible]	3	50	3.20	0.34	0.24	5.49	1.00	0.47	0.25	0.34
[illegible]	3	50	3.22	0.40	0.29	5.48	0.85	0.62	0.20	0.32
	Average		3.21	0.37	0.2[illegible]	5.49	0.9[illegible]	0.5[illegible]	0.25	0.[illegible]
[illegible]	4	100	3.48	0.32	0.26	5.20	0.66	0.07	0.13	0.26
3	4	100	3.28	0.38	0.27	5.27	0.74	0.05	0.18	0.30
	Average		3.3[illegible]	0.35	0.2[illegible]	5.23	0.70	0.0[illegible]	0.16	0.2[illegible]
[illegible]	5	200	3.02	0.32	0.23	4.81	0.88	0.03	0.18	0.30
[illegible]	5	500	3.16	0.2[illegible]	0.22	4.25	0.8[illegible]	0.0[illegible]	0.19	0.32
3	6	500	3.06	0.3[illegible]	0.2[illegible]	4.3[illegible]	0.7[illegible]	0.[illegible]	0.2[illegible]	0.[illegible]
	Average		3.11	0.29	0.23	4.27	0.7[illegible]	0.[illegible]	0.2[illegible]	0.[illegible]

000012

[illegible] - [illegible]
Organ Weight Summary (g./100 g. Body Wt.)
90 Day Exposure + 30 Day Rest Period

Rabbit No.	Group No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Methylene Chloride										
2	3	15	4.59	0.24	0.20	3.55	0.60	0.03	0.17	0.22
3	8	15	4.22	0.26	0.19	3.81	0.56	0.03	0.18	0.23
	Average		4.41	0.25	0.20	3.88	0.58	0.03	0.15	0.25
1	4	50	3.03	0.30	0.19	3.96	0.70	0.04	0.15	0.29
4	9	50	3.51	0.33	0.25	5.56	0.75	0.07	0.25	0.28
	Average		3.27	0.31	0.22	4.70	0.73	0.05	0.20	0.29
3	10	100	3.36	0.32	0.22	4.60	0.76	0.04	0.18	0.23
4	10	100	4.24	0.26	0.21	3.99	0.60	0.03	0.18	0.24
	Average		3.80	0.29	0.22	4.30	0.68	0.04	0.18	0.25
1	11	200	2.87	0.32	0.25	4.06	0.72	0.05	0.20	0.32
1	12	500	3.40	0.31	0.26	3.74	0.64	0.04	0.23	0.28
4	12	500	2.83	0.35	0.24	4.19	0.71	0.05	0.20	0.35
	Average		3.12	0.33	0.25	3.97	0.68	0.05	0.21	0.32
Isopropyl Alcohol										
1	13	15	3.84	0.38	0.25	3.90	0.61	0.04	0.22	0.26
2	14	100	3.25	0.30	0.21	4.17	0.73	[illegible]	[illegible]	[illegible]
3	14	100	3.44	0.36	0.27	3.98	0.70	0.04	[illegible]	[illegible]
	Average		3.35	0.33	0.24	4.05	0.71	[illegible]	[illegible]	[illegible]
3	15	500	3.16	0.32	0.25	4.45	0.85	0.04	0.19	0.30
4	15	500	3.08	0.31	0.24	4.56	0.66	0.04	0.25	0.32
	Average		3.12	0.31	0.25	4.52	0.73	0.04	0.22	0.31

000013

[illegible] SKIP CONTACT - [illegible]

Organ Weight Summary (g./100 g. Body Wt.)

90 Day Exposure + 30 Day Rest Period

Rabbit No.	Group No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Control										
3	1		2.82	0.34	0.27	4.[illegible]	0.63	0.0[illegible]	0.17	0.30
1	7		3.74	0.33	0.24	4.[illegible]	0.69	0.0[illegible]	0.19	0.2[illegible]
3	7		3.28	0.43	0.28	4.26	0.65	0.03	0.19	0.33
	Average		3.36	3.67	0.26	4.36	0.66	0.04	0.18	0.2[illegible]
Chlorothane										
3	2	15	3.87	0.29	0.25	4.52	0.76	0.04	0.25	0.24
[illegible]	2	15	3.48	0.31	0.25	4.80	0.68	0.05	0.26	0.23
	Average		3.68	0.30	0.26	4.66	0.72	0.04	0.25	0.25
3	3	50	3.20	0.34	0.24	5.49	1.00	0.47	0.25	0.34
4	3	50	3.22	0.40	0.29	5.48	0.86	0.62	0.20	0.32
	Average		3.21	0.37	0.27	5.49	0.93	0.55	0.23	0.33
2	4	100	3.48	0.32	0.26	5.20	0.66	0.07	0.13	0.26
3	4	100	3.28	0.38	0.27	5.27	0.74	0.05	0.18	0.30
	Average		3.38	0.35	0.26	5.23	0.70	0.06	0.16	0.28
4	5	200	3.02	0.32	0.23	4.81	0.88	0.03	0.18	0.30
2	6	500	3.16	0.28	0.22	4.23	0.82	0.05	0.19	0.32
3	6	500	3.06	0.31	0.24	4.31	0.71	0.0[illegible]	0.21	0.3[illegible]
	Average		3.11	0.29	0.23	4.27	0.73	0.05	0.20	0.32

REPEATED SKIN CONTACT - [illegible]
Organ Weight Summary (g./100 g. Body Wt.)
90 Day Exposure + 30 Day Rest Period

Rabbit No.	Group No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Methylene Chloride										
2	8	15	4.59	0.24	0.20	3.95	0.60	0.03	0.17	0.22
3	8	15	4.22	0.26	0.19	3.81	0.56	0.03	0.18	0.23
	Average		4.41	0.25	0.20	3.88	0.58	0.03	0.18	0.23
1	4	50	3.03	0.30	0.19	3.96	0.70	0.04	0.15	0.29
4	9	50	3.51	0.33	0.25	5.56	0.75	0.07	0.25	0.28
	Average		3.27	0.31	0.22	4.76	0.73	0.05	0.20	0.29
3	10	100	3.36	0.32	0.22	4.60	0.76	0.04	0.16	0.23
4	10	100	4.24	0.26	0.21	3.99	0.60	0.03	0.18	0.24
	Average		3.80	0.29	0.22	4.30	0.68	0.04	0.18	0.25
1	11	200	2.87	0.32	0.25	4.05	0.72	0.05	0.20	0.32
1	12	500	3.40	0.31	0.26	3.74	0.64	0.04	0.23	0.28
4	12	500	2.83	0.35	0.24	4.19	0.71	0.05	0.20	0.35
	Average		3.12	0.33	0.25	3.97	0.68	0.05	0.21	0.32
Isopropyl Alcohol										
1	13	15	3.84	0.38	0.25	3.91	0.61	0.04	0.22	0.26
2	14	100	3.26	0.30	0.21	4.17	0.73	[illegible]	[illegible]	[illegible]
3	14	100	3.44	0.36	0.27	3.98	0.70	[illegible]	[illegible]	[illegible]
	Average		3.35	0.33	0.24	[illegible]	0.71	[illegible]	[illegible]	[illegible]
3	15	500	3.16	0.32	0.25	4.45	0.85	0.04	0.19	0.30
4	15	500	3.08	0.31	0.24	4.56	0.60	0.04	0.25	0.32
	Average		3.12	0.31	0.25	4.52	[illegible]	0.04	0.22	0.31

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METHYL CHLOROFORM (1,1,1-TRICHLOROETHANE):

A COMPARISON OF THE FATE OF INHALED

METHYL CHLOROFORM FOLLOWING SINGLE OR

REPEATED EXPOSURE IN RATS AND MICE

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6

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Methyl Chloroform (1,1,1-Trichloroethane): A Comparison of the Fate of Inhaled Methyl Chloroform Following Single or Repeated Exposure in Rats and Mice

By

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April 10, 1981

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ABSTRACT

Male Fischer 344 rats and B6C3F1 mice were exposed by inhalation to 1500 ppm of methyl chloroform (MC) 6 hours/day, 5 days/week for approximately 16 months. On the last day of repeated exposure ^{14}C-labeled MC was used. The fate of the ^{14}C-MC in the repeatedly exposed animals was compared to a group of rats and mice which had been exposed concurrently for 16 months to chamber air (age-matched controls) prior to receiving the single 6 hour exposure to 1500 ppm of ^{14}C-MC. The routes of excretion and tissue concentration of ^{14}C activity were similar between the singly and repeatedly exposed rats and mice. The major route of elimination of MC was exhalation of the parent chemical in the expired air and constituted approximately 97% of the total recovered radioactivity in rats and 92-94% in mice. The remaining radioactivity (~3-9%) was recovered as metabolized MC in the expired air ($^{14}CO_2$) and as nonvolatile radioactivity in the urine, feces, carcass and cage wash. Mice were found to eliminate MC more rapidly via the pulmonary route and to biotransform approximately 6-fold more MC on a body weight basis than rats. Repeated exposure to MC did not significantly affect the disposition of MC compared to the singly exposed rats and mice. Thus even after long-term repeated exposure to MC, its low order of toxicity appears to remain related to its limited biotransformation. Comparison of these results to those obtained in young-adult rats and mice indicates that alterations in the pharmacokinetics of ^{14}C-MC occur with age but not prior repeated exposure to MC.

INTRODUCTION

In a study recently completed in our laboratory, the pharmacokinetics of inhaled ^{14}C-methyl chloroform (MC) were investigated in Fischer 344 rats and B6C3F1 mice following a single 6 hour exposure (Schumann et al., 1981). Consistent with previous reports in rats and man (Nako et al., 1960; Stewart et al., 1969; Ikeda and Ohtsuji, 1972; Eben and Kimmerle, 1974; and Monster et al., 1979) the primary route of excretion of MC was found to be the elimination of the parent chemical in the expired air. The metabolism of MC, shown to be a saturable process, occured to only a limited extent thus reinforcing the view that the low order of toxicity of MC may be related to its limited biotransformation.

Most of the pharmacokinetic and metabolic studies conducted on MC have concentrated on the fate of this chemical after one or at best several exposures. However many toxicologic studies designed for assessing the potential human health hazard of a chemical involve long-term repeated exposure to the chemical. Although not routinely evaluated, it is important to consider whether the disposition of the test chemical in the body is altered during the prolonged exposure interval.

Thus, it was the objective of the present study to determine whether the fate of MC is indeed altered with repeated exposure. This was accomplished by exposing rats and mice to nonlabeled MC for approximately 16 months and, on the last exposure, to ^{14}C-MC. The fate of ^{14}C-MC was then followed for 72 hours and compared to age-matched control animals exposed concurrently to chamber air for 16 months prior to receiving the single ^{14}C-MC exposure.

METHODS

Animals. The animals used in the present study consisted of predesignated groups of male Fischer 344 rats and B6C3F1 mice included in the chronic toxicity and oncogenicity study of the MC formulation CHLOROTHENE® VG which is currently being conducted in our laboratory (McKenna et al., 1979). The animals were purchased from the Charles River Breeding Laboratories, Inc. (Wilmington, MA) at 5-6 weeks of age and allowed to acclimate to our laboratory for an additional 4 weeks prior to initiation of the study. All of the animals were uniquely identified with a metal ear tag and housed in rooms designed to maintain a temperature of ~70°F, humidity of ~50% and a 12 hour lighting cycle. During the repeated exposure segment of this study, food (Purina Laboratory Chow, Ralston Purina Co.) was removed prior to each exposure but water was provided ad libitum via an automatic watering system. Both food and water were withheld during exposure to ^{14}C-MC. The animals were assigned to experimental groups by appropriate computer randomization routines.

Materials. A commercial formulation of methyl chloroform (CHLOROTHENE® VG) was used throughout the study (Lot No. TAO 4259-1). The purity of the formulation was found to be within product specifications by gas chromatographic analysis and contained 94% MC by volume. The balance of the formulation was comprised of stabilizers, with ~0.2% attributable to aliphatic hydrocarbon impurities. ^{14}C-Methyl chloroform (2-^{14}C) was purchased from the New England Nuclear Corp., Boston, MA (Lot No. 1119-293; 2.51 mCi/mmol). Prior to each inhalation exposure to ^{14}C-MC, the isotope was further purified by collecting the vapor effluent corresponding to MC from a gas chromatographic column (thermal conductivity detection). The radiochemical purity of this fraction has been verified as >99% by mass spectrometry/radio-gas chromatography with a Finnigan 3200 mass spectrometer (chemical ionization mode) interfaced with a Panax beta detector (Hermann, 1980).

Inhalation Exposure. Rats and mice were exposed to either chamber air (0 ppm) or 1500 ppm of MC 6 hours/day, 5 days/week for approximately 16 months. The rats designated for the balance/metabolism and tissue concentration studies received 330 exposures/477 days and 341 exposures/492 days, respectively, while the mice designated for identical studies

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required [illegible] exposures, [illegible] During the [illegible]
[illegible] were housed and exposed to [illegible] inhalation chamber
operated under dynamic air flow conditions. MC vapor was generated
by returning the liquid at a calculated rate into a heated air stream
flowing through a glass, [illegible]
The vapor was then swept with filtered air into the main chamber air flow
of ~2000 liters per minute. The analytical concentration of MC vapor
in the chamber was determined at least once per hour by infrared
spectrometry at an analytical wavelength of [illegible].

One day following their last exposure to either 0 or 1500 ppm of MC, the animals were exposed to 1500 ppm of ^{14}C-MC for 6 hours in a 30 liter glass chamber under dynamic air flow conditions ([illegible] l/min). For exposure to ^{14}C-MC, a 100 liter Saran bag containing 64,000 ppm of MC was prepared. The purified ^{14}C-MC vapor was then injected directly into the bag and the concentrated ^{14}C-MC vapor metered from the bag into the chamber air flow with a Masterflex pump (Cole-Parmer Instrument Co., Chicago, IL). The analytical concentration of MC in the chamber atmosphere was assessed at 30 min intervals by gas chromatography (Varian, Model 2400) using flame ionization detection. Samples of the chamber atmosphere were pumped into a one ml gas sampling loop and directly injected onto a 6' x 1/8" O.D. stainless-steel column packed with 3% SP 1000 on Supelcoport (Supelco Inc., Bellefonte, PA) (column 100°C, helium carrier gas at 30 ml/min.). At hourly intervals the ^{14}C activity in the chamber was determined by bubbling a 1-ml aliquot of the chamber atmosphere into a vial containing a mixture of Concifluor (Mallinckrodt Chemical Company), 2-methoxyethanol:toluene (6:11:183) and counting with a liquid scintillation spectrometer. Throughout the study, radioactivity was quantitated on either a Mark III (Searle) or LS-9000 (Beckman) liquid scintillation spectrometer with automatic quench correction.

The inhalation chamber used for exposure to ^{14}C-MC was operated in a laboratory fume hood to prevent contamination of the work environment. All radioactive waste was disposed of according to standard regulations.

Experimental Procedures. After each of the last 1-2 exposures to unlabeled MC, four rats or mice per group (0 or 1500 ppm) were acclimated in glass Roth-type metabolism cages (one animal/cage) designed for separate collection

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Following inhalation of 1500 ppm of ^{14}C-MC, radioactivity was detected in the expired air as ^{14}C-MC and $^{14}CO_2$, and in the urine, feces and carcasses of the rats and mice (Tables 2 and 3). The major route of excretion of MC was the elimination of the unmetabolized chemical in the expired air. Prior repeated exposure to 1500 ppm of MC did not significantly alter the quantity of MC excreted via the pulmonary route compared to those singly exposed for either species. Elimination of MC in the expired air constituted approximately 97% of the total body burden in rats and 92-94% in mice. As shown in Table 4, MC was eliminated more rapidly in the expired air of mice than rats with little difference between the repeated and singly exposed animals.

The remaining radioactivity recovered during the 72 hour collection interval (approx. 3-7%) was detected as metabolized MC in the expired air ($^{14}CO_2$) and as nonvolatile radioactivity in the urine, feces, carcass and cage wash (Tables 2 and 3). No significant differences were observed between those repeatedly or singly exposed rats or mice in the quantity of MC (umole equivalents) recovered via the various routes. MC was nearly completely eliminated by mice within 24 hours after termination of the exposure to ^{14}C-MC as indicated by the fact that >96% of the total body burden was excreted during this time interval. Approximately 80% of the total body burden was excreted during this time interval in rats (data not shown).

The mean end-exposure body burden of ^{14}C activity expressed as umole equivalents of MC per kg of body weight, while approximately 2-fold greater in mice, was not found to differ significantly between the singly and repeatedly exposed rats (1547-1484) or mice (3759-3117) (Tables 2 and 3). Similarly, no significant group differences for either species

were observed in the quantity of MC metabolized per kg of body weight. It was observed however that the mice biotransformed approximately 5-fold more MC per kg of body weight than did the rats.

Immediately upon termination of the exposure to ^{14}C-MC, the highest tissue concentration of ^{14}C activity was found in the fat (Table 5). The concentration of ^{14}C activity in the liver and kidneys of both species immediately after exposure was found to be only approximately 10-20% of that found in the fat. Prior repeated exposure to MC did not significantly alter the concentration of ^{14}C activity in any tissue of the repeatedly exposed rats or mice compared to those singly exposed. At 72 hours after the termination of exposure to ^{14}C-MC, radioactivity was not detected in the fat of mice or the liver of rats (Table 5). Radioactivity was only marginally detectable in the remaining tissues at 72 hours.

[illegible]

[illegible] the [illegible] degree of metabolism of MC which does occur, the mean values [illegible] a tendency toward a greater extent of metabolism for [illegible] exposed; however, the magnitude of this effect relative [illegible] exposed was not statistically significant. Thus, long-term repeated exposure of rats and mice to 1500 ppm of MC had little discernible impact on the overall fate of ^{14}C-MC.

A critical factor to consider in evaluating potential differences in the fate of a chemical following single versus repeated exposure is the age of the animal. For this reason, age-matched animals were used for single exposure in this study. Table 6 shows a comparison of the present results with those obtained in young-adult male Fischer 344 rats and B6C3F1 mice following a single 6 hour exposure to 1500 ppm of ^{14}C-MC (Schumann et al., 1981). From these data it is readily apparent that the age of the animal had more influence on the disposition of ^{14}C-MC than repeated exposure. For rats, an age-related 1.3-fold increase in the body burden and 1.6-fold increase in the amount of MC metabolized were observed in the aged versus young-adult rats (both parameters normalized by body weight). The magnitude of these age-related differences was even greater in mice as indicated by a 2-3 fold increase in the body burden and a 5-6 fold greater extent of metabolism of MC in the aged relative to the young-adult mice.

The increased body burden and extent of metabolism of MC in the older animals may be a function of the greater fat content of the older rodents which would be expected to act as a deep compartment for MC. This would be consistent with the slower rate of clearance of MC in the expired air of the aged rats and mice in the present study compared to that observed in the young-adult animals (Schumann et al., 1981). Presumably the slower rate of clearance of MC would result in more time available for its biotransformation. The 5-fold greater extent of metabolism of MC per kg of body weight in mice relative to rats in the

present study suggests that the impact of these events may be greater in [illegible] young adult mice have previously been shown to metabolize MC to [illegible] a 2-fold greater extent than rats [illegible] The greater extent of metabolism of MC by mice is consistent with their known greater rate of oxidative metabolism relative to rats (Schmidt-Nielsen, [illegible]). It cannot however be discounted at this time that the inherent capability of the animals for biotransforming MC may have simply increased with age.

Carlson [illegible] has reported that exposure of rats to 11,600 ppm of MC vapors for 2 hours failed to result in hepatotoxicity as evaluated by assessing serum levels of SGPT, SGOT, and glucose-6-phosphatase 22 hours after the termination of exposure. However, pretreatment of the rats with phenobarbital resulted in a significant increase in all three determinations. If as hypothesized from Carlson's work the hepatotoxicity of MC is due to a metabolite(s), it is toxicologically significant that repeated exposure to MC did not result in an induction of its own metabolism. Also, the limited extent of metabolism of MC even after long-term repeated exposure is consistent with its low order of hepatotoxicity.

Several studies have addressed the potential of MC to enhance hepatic biotransformation. Fuller et al. (1970) and Lal and Shah (1970) reported an apparent enhancement of the hepatic mixed-function oxidase (MFO) system in rodents exposed to high concentrations of MC vapor for a prolonged interval ($\geq$ 3000 ppm, 24 hours). In contrast, MC was reported to be essentially without effect in enhancing the hepatic MFO system of mice orally exposed to 1650 mg/kg/day of MC for 7 days (Platt and Cockrill, 1969). In a report more closely related to the present study, Eben and Kimmerle (1974) hypothesized that MC enhanced its own biotransformation in rats exposed to 5200 ppm of MC for 14 weeks. This conclusion was based on an increasing quantity of urinary trichloroethanol excreted per 24 hours throughout the first 10 weeks of exposure which then fell to a somewhat lower level. It is not clear from their data however whether this effect was due to a stimulation of the hepatic MFO system or more closely related to the increasing age of the rats as suggested by the present study. It thus appears that if MC does have any inherent capability to stimulate the hepatic MFO system, and hence its own biotransformation, it does so only weakly requiring exposure concentrations above those used in the present study and greatly above those to which man would likely be exposed.

In conclusion, the results of the present study have shown that repeated exposure of rats and mice to [illegible] ppm for approximately 6 months did not significantly alter the rate of metabolism, the extent of metabolism or the tissue concentration of radioactivity when compared to age-matched singly exposed animals. Comparison of these results to those obtained in young-adult rats and mice exposed for a single 6 hour interval to ^{14}C-MC revealed that the age of the animal had more influence on the pharmacokinetics of this chemical than prior repeated exposure.

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The authors wish to express their appreciation to the following members of our laboratory: Dr. M. J. McKenna, Linda L. Calhoun and Terry S. Gushow for conducting the chronic inhalation phase of this study. We are also grateful to Emile A. Hermann for conducting the radiochemical purity analysis of ^{14}C-methyl chloroform and Elizabeth J. Hackney for her technical assistance.

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[illegible] the Study Director of the dates listed below. The report accurately reflects the data generated in accordance with the regulations and standard operating procedures of the laboratory. All data and the records are located at the submitting laboratory.

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TABLE 1

Summary of Experimental Conditions During the 8 Hour Inhalation of 1500 ppm of ^{14}C-Methyl Chloroform in Repeated and Unrepeated Fischer 344 Rats and [illegible] Mice[a]

	(^{14}C) Methyl Chloroform			
Species	Target Concentration (ppm)	Analytical[b] Chamber Concentration (ppm)	Specific[c] Activity (dpm/μmole)	Experimental Description
Mice	1500	1530 ± 27	7670 ± 335	Balance/metabolism ^{14}C tissue concentration
Rats	1500	1508 ± 24	9307 ± 615	Balance/metabolism
		1499 ± 54	4634 ± 160	^{14}C tissue concentration

[a]The rats and mice had been chronically exposed to 0 or 1500 ppm of methyl chloroform for approximately 16 months prior to the single 8 hour exposure to 1500 ppm of ^{14}C-methyl chloroform.

[b]Mean ± S.D. as determined at 30 minute intervals.

[c]Mean ± S.D. as determined at 60 minute intervals.

TABLE 2

Recovery of Radioactivity for 72 Hours After a 6 Hour Inhalation Exposure to 1500 ppm of ^{14}C-Methyl Chloroform in Repeated and Single Exposed Fischer 344 Rats[a]

	Recovery			
	Single Exposure		Repeated Exposure	
	Smole Eq of Methyl Chloroform	Percentage[b]	Smole Eq of Methyl Chloroform	Percentage[b]
Expired Air Methyl Chloroform	725.49 ± 89.04	97.5 ± 0.3	679.96 ± 64.43	96.0 ± 0.5
CO_2	5.36 ± 0.71	0.7 ± 0.1	8.15 ± 3.96	1.2 ± 0.6
Urine	12.27 ± 1.14	1.7 ± 0.2	12.55 ± 0.85	1.8 ± 0.2
Feces	0.73 ± 0.25	0.1 ± 0.0	0.95 ± 0.22	0.1 ± 0.0
Carcass	0.34 ± 0.29	<0.1	0.12 ± 0.18[c]	<0.1
Cage Wash	0.43 ± 0.21	<0.1	0.22 ± 0.08	<0.1
Body Burden -				
Smole Eq/rat	744 ± 90		702 ± 44	
Smole Eq/kg body weight	1547 ± 49		1444 ± 74	
Smole Eq metabolized[d]	18.0 ± 2.3	2.6 ± 0.3[e]	22.2 ± 2.3	3.2 ± 0.6[e]
Smole Eq metabolized/kg body weight	39.5 ± 5.0		47.4 ± 6.0	

[a]The rats received 330 exposures to 0 (chamber air) or 1500 ppm methyl chloroform over a 477 day period prior to a single 6 hour exposure to 1500 ppm of ^{14}C-methyl chloroform. Each value represents the mean ± SD of data from four rats.

[b]Percentage of the total recovered radioactivity

[c]Detectable for only 2 of 4 rats

[d]Radioactivity recovered by all routes less expired ^{14}C-methyl chloroform.

[e]Represents the percentage of the body burden recovered as metabolized methyl chloroform.

TABLE 3

Recovery of Radioactivity for 72 hours After a 6 hour Inhalation Exposure to 1500 ppm of ^{14}C-Methyl Chloroform in Repeated and Single Exposed B6C3F1 Mice[a]

	Recovery			
	Single Exposure		Repeated Exposure	
	nmole Eq of Methyl Chloroform	Percentage[b]	nmole Eq of Methyl Chloroform	Percentage[b]
Expired Air Methyl Chloroform	130.93±19.62	94.3 ± 0.7	114.24±42.62	91.5 ± 3.2
CO_2	3.80± 0.73	2.6 ± 0.6	4.33± 0.23	3.8 ± 1.4
Urine	3.44± 0.94	2.3 ± 0.6	4.04± 1.18	3.6 ± 1.7
Feces	0.49± 0.18[c]	0.3 ± 0.1	0.52± 0.24	0.5 ± 0.1
Carcass	0.23± 0.08	0.2 ± 0.1	0.18± 0.09	0.1 ± 0.0
Cage Wash	0.86± 0.38	0.4 ± 0.3	0.83± 0.26	0.5 ± 0.4
Body Burden •				
nmole Eq/mouse	140 ±10		134 ±42	
nmole Eq/kg body weight	3759 ±371		3117 ±901	
nmole Eq metabolized[d]	8.39±0.30	5.7 ± 0.7[e]	9.57±0.80	8.6 ± 3.2[e]
nmole Eq metabolized/ kg body weight	212 ±17		245 ±23	

[a]The mice received 329 exposures to 0 (chamber air) or 1500 ppm methyl chloroform over a 470 day period prior to a single 6 hour exposure to 1500 ppm of ^{14}C-methyl chloroform. Each value represents the mean ± SD of data from four mice.

[b]Percentage of the total recovered radioactivity

[c]Detectable for only 3 of 4 mice

[d]Radioactivity recovered by all routes less expired ^{14}C-methyl chloroform

[e]Represents the percentage of the body burden recovered as metabolized methyl chloroform.

TABLE 4

Clearance of ^{14}C-Methyl Chloroform in the Expired Air of Repeatedly and Singly Exposed Fischer 344 Rats and B6C3F1 Mice During the First 36 Hours After Exposure to 1500 ppm ^{14}C-Methyl Chloroform

Species and Exposure[a]	% of Body Burden as Expired ^{14}C-Methyl Chloroform[b]				
	0-1 hr	1-6 hr	6-12 hr	12-24 hr	24-36 hr
Mouse					
Single	66.3±4.6	18.0±2.1	7.8±2.6	1.3±0.7	0.13±0.08
Repeated	69.6±9.6	15.1±7.2	5.3±4.3	1.4±1.2	0.16±0.14
Rat					
Single	29.6±3.7	17.7±0.9	19.1±0.3	17.8±1.4	8.8 ±1.2
Repeated	28.7±1.8	18.9±1.6	18.9±0.7	17.1±1.9	8.8 ±0.7

[a]The mice received 325 exposures to 0 or 1500 ppm methyl chloroform over a 370 day period and the rats 330 exposures/477 days prior to a single 6 hour exposure to 1500 ppm ^{14}C-methyl chloroform.

[b]The data are expressed as the mean ± SD (n=4).

TABLE 5

Concentration of ^{14}C Activity in Various Tissues Immediately (0 hr) or 72 hours After a 6 hour Inhalation exposure to 1500 ppm of ^{14}C-Methyl Chloroform (MC) in Repeated and Singly Exposed Fischer 344 Rats and B6C3F1 Mice[a]

	nmole equivalents of MC/g tissue[b]			
	Mice		Rats	
Tissue and Time of Sacrifice	Single Exposure	Repeated Exposure	Single Exposure	Repeated Exposure
0 Hr				
Liver	1704±302	1409±415	606±75	641±83
Kidney	877±186	817±214	480±146	490±120
Fat	10,603±1528	8709±2269	5385±1424	4591±1226
72 Hr				
Liver	50.5±9.0[c] (3)	45.2±16.8 (3)	ND[d]	ND
Kidney	47.3±14.4 (3)	36.5±4.9 (2)	30.0 (1)	76.6 (1)
Fat	ND	ND	191±113	144±1.9 (2)

[a]Two mice received 345 exposures to 0 or 1500 ppm of methyl chloroform over 470 days and the rats 330 exposures/472 days (72 hr) and 341 exposures/492 days (0 hr) prior to a single 6 hour exposure to 1900 ppm of ^{14}C-methyl chloroform.

[b]Each value represents the mean ± SD of data from four animals.

[c]One, two, or three determinations had values below the detection limit. The number in parenthesis represents the number of values within the detection limit.

[d]Not detectable.

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TABLE 9

Summary of the Disposition of ^{14}C-Methyl Chloroform (MC) in Repeatedly Exposed Versus Singly Exposed Age-Matched and Young-Adult Fischer 344 Rats and BoCAFI Mice

Body Burden	1500 ppm ^{14}C-MC for 6 hours Exposure Age: Single[a] (2.5-3.5 mo)	Single[b] (18 mo)	Repeated[b] (19 mo)
Rats			
μmole Eq MC/kg body wt.	1177 ± 21	1547 ± 49	1484 ± 74
μmole Eq MC metabolized/kg body weight	24.6 ± 1.2	39.5 ± 5.0	47.4 ± 6.0
Mice			
μmole Eq MC/kg body wt.	1416 ± 471	3759 ± 373	3117 ± 901
μmole Eq metabolized/kg body weight	42.1 ± 8.6	212 ± 17	245 ± 23

[a]Taken from Schumann et al. (1981). The mice and rats received a single 6 hour exposure to 1500 ppm of ^{14}C-MC at 2.5 and 3.5 months of age, respectively.

[b]See the legend for Tables 2 and 3 for experimental details.

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INHALATION OF HALOGENATED HYDROCARBONS - METHODS FOR INTRATRACHEAL ADMINISTRATION

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November, 1977

RELEASED

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ABSTRACT

Preliminary work was done in our laboratory with halogenated hydrocarbons (methyl chloroform, methylene chloride, [illegible] to develop a [illegible] for intratracheal administration of halocarbons. Even these limited studies have shown that administration of small quantities of these compounds is extremely hazardous. This work, in rats, indicates the toxicity of these halocarbons to be greater if aspirated than if ingested.

Further work in this area would facilitate development of proper halocarbon labelling and aid in establishing recommendations to physicians in treating cases of halocarbon ingestion.

INGESTION OF HALOGENATED HYDROCARBONS - [illegible]
FOR INTRATRACHEAL ADMINISTRATION

INTRODUCTION

In the case of accidental ingestion of hydrocarbons, the labelling information given for treatment varies widely from one compound to the next. It may indicate a specific course of action or give no indication what so ever. When a course of action is given it may be either to induce vomiting, not to induce vomiting or consult a physician who will treat symptomatically. Physicians do not agree on a course of action to be taken with hydrocarbon ingestion. They tend to use personal discretion in choosing a treatment. For the most part, information on the treatment of ingestion of certain hydrocarbons is not available.

Aliphatic hydrocarbons, such as naptha, hexane, and petroleum distillates, have labelling information which indicates that vomiting should not be induced. Vomiting would increase the risk of aspirating the hydrocarbon into the lungs. Aspiration of a hydrocarbon, as documented with kerosene, can cause pneumonitis (Wolfsdorf

and Kundig, 1972). Research by Bratton and Hauber (1974) has demonstrated that petroleum distillates show a lower risk of toxic effects if left in the stomach than if they are aspirated.

Present labelling instructions for many halogenated hydrocarbons direct that vomiting be induced. This may be worse than doing nothing at all. That is why more work should be done in this area to determine proper labelling information. This would also give physicians a clearer indication of treatment for victims of halocarbon ingestion. Preliminary animal work was done to devise a method for intratracheal administration of halogenated hydrocarbons so that further studies will determine whether these compounds have a greater toxic effect in the stomach or in the trachea.

METHODS

To develop a method for studying the toxicity halocarbons, Sprague-Dawley or Harlan rats (200 to 300 g) were anesthetized, then administered a chlorinated hydrocarbon by one of two routes - gastric or intratracheal. In gastric administration a single dose of a compound

is given through a #10 feeding needle. The intratracheal administration involves exposing the trachea surgically and injecting the compound into the trachea with a 20% needle, as described by Bratton and Haddow (1975).

compounds were used in this study.

and methyl chloroform, which contained 500 ppm butylene oxide as a stabilizer were obtained from Dow. Reagent grade methylene chloride was purchased from Burdick and Jackson.

One group of rats were anesthetized with 1 mg/kg sodium pentobarbital (Robinson).

Another group of rats was anesthetized lightly with methoxyflurane. The surgical opening and injection of the compound took place while the animal was light and beginning to recover from anesthesia.

Yet another group was anesthetized with methoxyflurane but the procedure was varied by cannulating the trachea. The compound was administered through the cannula after the rat had fully recovered from anesthesia (~3 hr. later) for one group and before regaining consciousness in another group.

The sedative-muscle relaxant, xylazine (GNR[illegible] purchased from Haver-Lockhart), was administered [illegible] mg/kg to one group of rats .

After administration, rats were observed for [illegible] minutes to an hour for breathing patterns and the recovery of the righting reflex. In some cases only one animal was tested at a single dose.

The results of intratracheal tests are summarized in Table I. Each compound was administered to give upper and lower limits. At the highest concentration the animal usually dies, the lower dose is tolerated for the duration of the test. Death in this experiment was observed to be caused by CNS depression and a cessation of respiration. Animals which survived dosing showed a small degree of central nervous system (CNS) depression, if any.

Due to deep and prolonged anesthesia, and in the case of the gastric administration, sodium pentobarbital appears to synergize with the compounds to increase CNS depression.

Table I

Results of Intratracheal Administration

Compound	Tube Cannulation		
	Anesthesia With Methoxyflurane	Anesthesia w/Methoxyflurane and Cannulation	[illegible]
Methylene Chloride	0.1 ml/kg Died	0.1 ml/kg Died	[illegible]
	0.2 ml/kg Lived	0.05 ml/kg Lived	[illegible]
Methyl Chloroform	0.4 ml/kg Lived	0.2 ml/kg Died	[illegible]
	0.2 ml/kg Lived	0.03 ml/kg Lived	[illegible]

Methoxyflurane anesthesia with cannulation seems to be a poor route of administration. By the time the animal has begun to recover from anesthesia the cannula has already started to plug with mucus. Cannulation was abandoned; the results would indicate that rats injected directly into the trachea under methoxyflurane anesthesia are least compromised by the anesthesia.

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The rats treated with Rylazine were not sedated enough to expose the trachea for injection. This was true even after the recommended dose was doubled.

RESULTS

This study was primarily concerned with intratracheal testing since values from oral dosing in rodents could be obtained from the literature. Oral dosing of rats was done at least once under each type of anesthesia given. All rats given gastric doses survived except in the case where a rat was given sodium pentobarbital and 1 mg/kg methylene chloride. It appears that the two compounds synergized to depress CNS activity, thereby, stopping respiration.

LD_{50} values in rodents

from values cited in the Merck Index (1976)

Methylene Chloride - 1.6 ml/kg

Methyl Chloroform - 7.7 ml/kg

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Doc Title 23

PREDICTING THE FLUX OF ORGANICS ACROSS THE AIR WATER INTERFACE

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6

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15 PAGES IN FULL REPORT

AUTHOR(S): W. Brock Neely

AUTHOR(S) SIGNATURE(S): W. Brock Neely

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This report is: [ ] INTERIM [X] FINAL and mainly: [X] NEW [ ] REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS: (Include in this space references to data books, and to earlier related reports, patents and publications.)

Two methods have appeared in the literature recently for measuring the flux of organics from water to air (1,2). The predictive ability of these two methods was compared with some actual laboratory data. The analysis indicates that the method of Liss and Slater(2) is superior and can be used with a fair degree of confidence in making these kinds of estimations. The experimental data that is required is a knowledge of the vapor pressure and water solubility.

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INTRODUCTION

There are many times when a knowledge of the flux of an organic chemical between air and water is valuable. For example, in estimating the movement of halocarbons in the biosphere it is necessary to know the rate at which the material moves between the atmosphere and the hydrosphere(3). In a different situation such as the spill of a chemical into a river, a knowledge of the rate of evaporation is fundamental in estimating the resulting concentration of the chemical as it moves downstream(4). Another example, discussed by Moyer(5) is the loss of organics from a tertiary treatment plant which is dependant on both degradation and evaporation mechanisms.

Two techniques for calculating this rate have recently appeared in the literature(1,2) and for convenience they will be identified in this report as A and B. It is the purpose of this report to compare these methods with some laboratory data.

METHOD A (MACKAY AND WOLKOFF) REF. 1

This method has been adequately discussed both in the original article and in a report by Moyer(5). The resulting equation for expressing the rate constant is shown in equation 1.

$$k = \left[\frac{E}{G}\frac{P_{is}}{C^{w}_{is}}\frac{M_i}{M_{is}}\frac{10^6}{P^{w}_{is}}\right] hr^{-1} \qquad (1)$$

where G = grams of water containing the chemical
E = grams of water evaporating/hour
P_{is} = vapor pressure of pure material
P^{w}_{is} = vapor pressure of water
C^{w}_{is} = maximum solubility of organic (ppm)
M_i = molecular weight of organic
k = rate constant for evaporation

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as compared to the field data where Schooley(6) evaluated the exchange constants. If conditions in both places had been identical, the slope would be 1 with an intercept of 0.

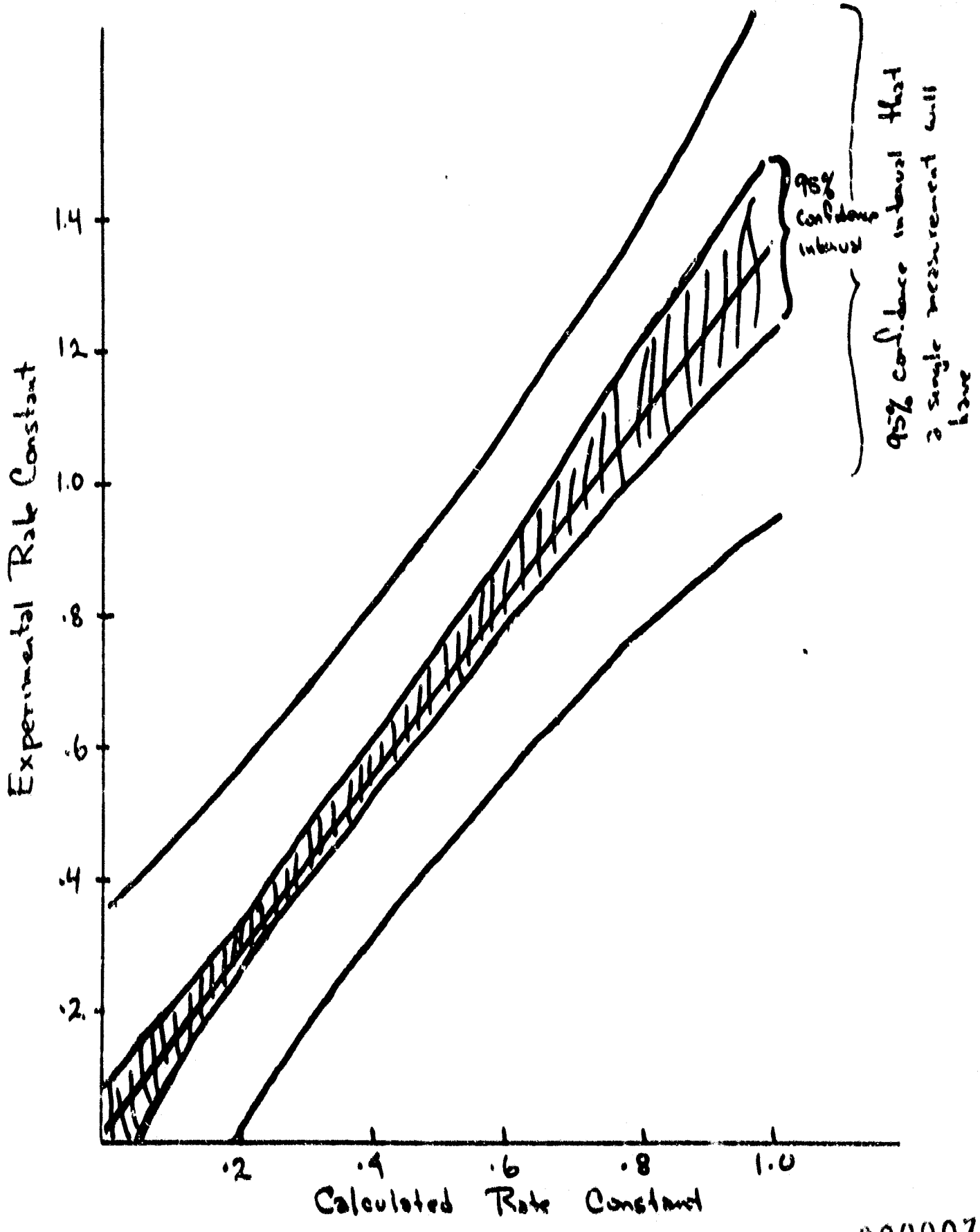
A distinct advantage of the Liss and Slater(2) technique is that it generates rate constants for the flux of a chemical in both directions across the air water interface. If this estimation continues to be validated through experimental results, it will provide a convenient method for determining these types of rate constants.

TABLE IV

SET OF CHEMICALS TO TEST PREDICTABILITY OF EQUATION 13

	Chemical	Mol. Wt.	Sol. ppm measured	V.P. mmHg at 25°C	% Loss Exp.	% Loss Est.
1.	Bromobenzene	159	446	4.1	100	94
2.	Acrolein	56	215,000	56.1	0	0
3.	Chloroform	119.5	7,100	265	100	90
4.	1,2-Dichloroethane	99	865	105	100	93
5.	Trichloroethylene	131.4	300	800	100	90
6.	4-t-butylcatechol	166	2,000	0.0020	0	0
7.	4-Chlorophenol	129	32,400	0.09	0	0
8.	2,4-Dichlorophenol	163	4,000	0.36	0	0
9.	Benzene	78	1,790	95.2	100	100
10.	α-Methylstyrene	118	560	3.13	100	89
11.	Styrene	104	320	6.12	100	90

FIGURE 2



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2. The standard error for a predicted value of an <u>individual</u> observation will have a broader range and is given by equation 16.

$$\hat{Y}_k \pm 2.68\left[1 + \frac{1}{n} + \frac{(X_k - \bar{X})^2}{\Sigma(X_i - \bar{X})^2}\right]^{\frac{1}{2}} \qquad (16)$$

where 2.68 is the 95% confidence value for the t statistic with 11 degrees of freedom.

These estimates were evaluated for the regression line and are shown in Figure 2.

In order to test the predictive capability of equation 14 it was necessary to go back to the data assembled by Moyer(5). This time the criteria used to make the selection were:

1. Solubility of 100 ppm or greater were still eliminated from further consideration.

2. A loss of 0 or 100% in four hours was included.

This last criteria necessitated placing a maximum and a minimum value on the theoretical rate constant. In other words if the calculated rate constant was equal to or greater than 0.55 than 100% loss in four hours could be expected. If the rate constant was equal to or less than 0.17, then 0% loss in four hours could be expected.

The selected chemicals along with the calculations are shown in Table IV. For the most part there is fairly good agreement between experimental and calculated result.

DISCUSSION

The method of Liss and Slater(2) does a good job of predicting rate of evaporation in laboratory experiments. The slope in equation 13 represents the difference in the laboratory conditions

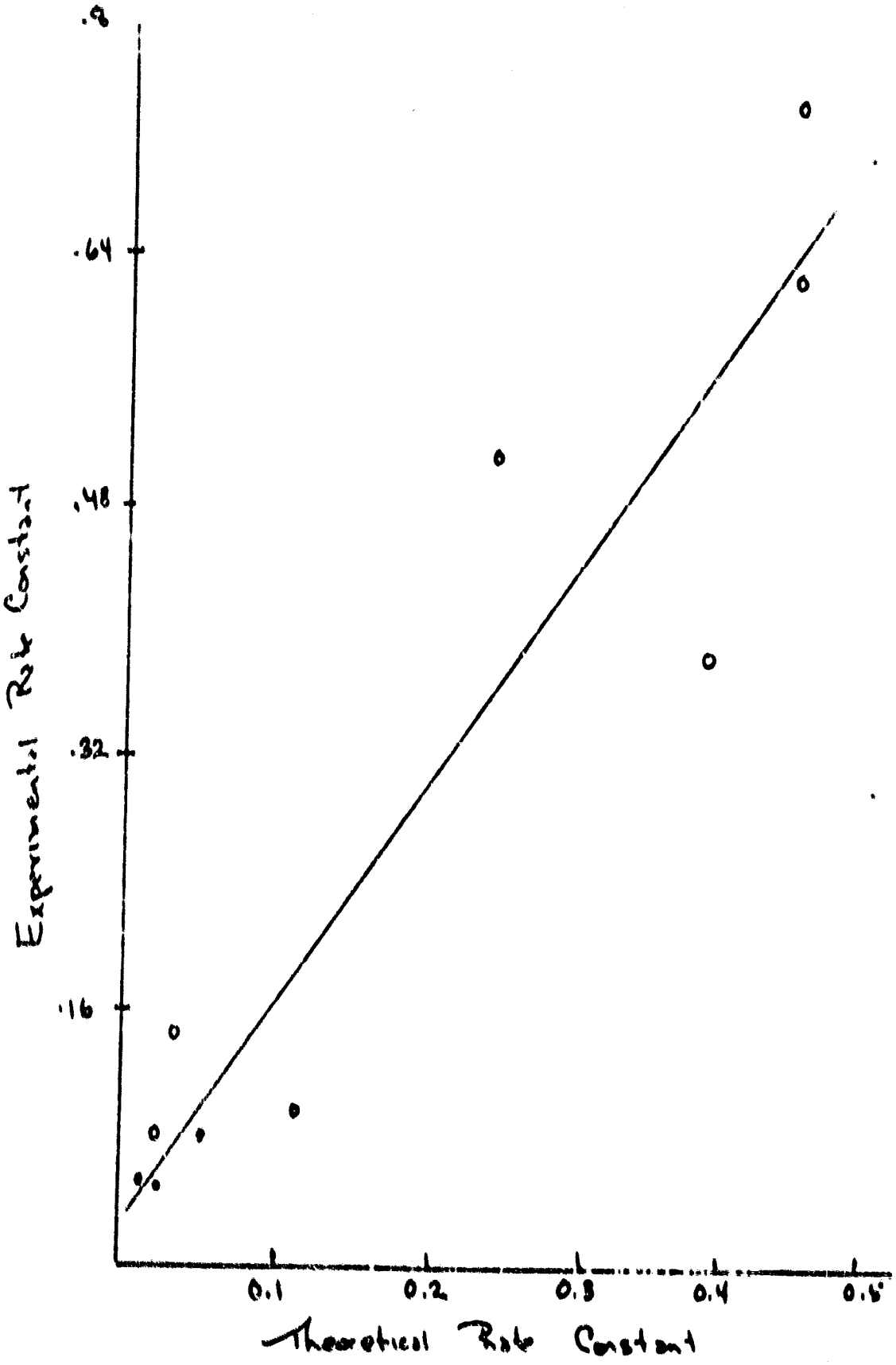
.8
.64
.48
.32
.16
Experimental Rate Constant
0.1
0.2
0.3
0.4
0.5
Theoretical Rate Constant

FIGURE 1

Equation 13 or Method B is by far the best description of the experimental data. Examination of the residual table (the amount by which the estimated k_e deviates from the experimental k_e) indicated one chemical that could be considered an outlier. This was 4-t-butyl acetophenone which had a much higher actual rate of evaporation than could be explained by equation 13. This would indicate one of two things: i) there is something wrong with either the reported solubility and vapor pressure or the experimentally reported loss. ii) there is something wrong with the model. Until we have more data there is no way of making a decision on which alternative is right. In the meantime this chemical was eliminated from the data set and the regression analysis repeated yielding equation 14. This is plotted in Figure 1.

$$k_e = 1.37\ B + 0.03 \qquad (14)$$
Standard error = 0.09
% variation explained 88.7

Performing the regression on this data using A as the independant variable did not significantly improve the statistics from equation 12. The % variation explained went from 33% to 39%.

Before using equation 13 for predicting laboratory rate constants a measure of the variation to be expected in individual predictions must be determined. These ranges were calculated by means of the equations shown before and taken from Draper and Smith(10).

1. The standard error for the predicted <u>mean</u> value of k_e for a given B is given by equation 15.

$$\text{est s.e.}\ (\bar{Y}_k) = s\left\{\frac{1}{n} + \frac{(X_k - \bar{X})^2}{\sum(X_i - \bar{X})^2}\right\}^{\frac{1}{2}} \qquad (15)$$

where $\bar{Y}_k$ is the predicted mean value for the rate constant evaluated at point k

s = standard error of estimate for the regression
n = number of data points used to establish the equation
X_k = theoretical rate constant at point k
$\bar{X}$ = average value of X's

Theoretical Calculation

i) Method A - The experimental values for E and G in equation 1 are 0.27 gms/hour and 1000 gms respectively. Again, using 15.5" for the depth the expression in equation 11 can be used to evaluate the rate constant

$$k = \left[\frac{0.27 \times 10^6 \times 15.5}{1000 \times 12}\right]\left[\frac{P_{is} - M_i}{C_{is} - P_w}\right] \qquad (11)$$

The results of this calculation are listed in Table III under Method A.

ii) Method B - Henry's constant is evaluated with equation 7 using a value of 0.001205 gms/cc for the density of air. By substituting this calculated value of H into equations 2 and 3 an estimation of the rate constant for both directions, namely air to water and water to air, may be made. For this calculation only the rate constant for water to air will be evaluated. This again is corrected for depth and the results are shown in Table III under Method B.

Statistics

The experimental rate constant (k_e) was treated as the dependant variable and regressed against the independant variable A or B. The results of these calculations are shown in the regression equations (12-14) along with the relevant statistics

$$k_e = 0.011\ A + 0.178 \qquad (12)$$
Standard error = 0.22
% variation explained 33.3

$$k_e = 1.21\ B + 0.039 \qquad (13)$$
Standard error = 0.15
% variation explained 69.5

TABLE III

COMPARISON OF THEORETICALLY DERIVED RATE CONSTANTS WITH THE EXPERIMENTAL VALUE OF THE CHEMICALS LISTED IN TABLE II

	Experimental		Theoretical	
Chemical*	% loss	k(ft/hour)	A(ft/hr)	B(ft/hr)
1	20	0.072	9.7×10^{-4}	.022
2	4	0.02	6.6×10^{-5}	1.6×10^{-4}
3	90	0.74	8.34	0.445
4	25	0.092	.129	0.110
5	86	0.63	45.4	0.442
6	80	0.519	0.413	0.236
7	21	0.076	0.019	0.053
8	70	0.38	10.93	0.38
9	15	0.052	0.0078	0.0219
10	75	0.45	0.0019	0.0042
11	12	0.04	0.005	0.013
12	36	0.14	0.0135	0.032

*Numbers refer to chemicals listed in Table II.

TABLE II

KEY CHEMICAL AND PHYSICAL PROPERTIES OF THE DATA SET USED IN THIS STUDY

No.	Chemical	Mol. Wt.	Vap. Press. mm Hg ↓	Solubility ppm
1	1-Br,3-NO_2 Benzene	170	0.07	10,000
2	3-Br,1-propanol	139	0.1	170,000
3	Methyl bromide	95	1400	13,000
4	Acetylene tetrabromide	344	0.3	650
5	Ethyl bromide	109	460	900
6	Ethylene dibromide	188	11.6	4,300
7	Epichlorohydrin	92.5	17.3	65,800
8	1,1,1-Trichloroethane	133.4	130.6	1,300
9	Triethylamine	101	7.0	73,000
10	4-t-butyl acetophenone	176	0.01	750
11	4-t-butyl phenol	150	0.046	1,000
12	Nitrobenzene	123	0.27	2,000

↓These measurements were made at 25°C.

of solution containing 100 ppm of the organic and noting the change in chemical oxygen demand after four hours. The data on a number of compounds were collected in the report by Moyer(5). In order to compare the theoretical calculation with the experimental rate a selection of the data was made based on the following criteria:

1. Experimental data which had 0 or 100% removal in four hours was eliminated. This was done since determining a rate constant would not be very meaningful on this set.

2. Compounds whose solubility was less than 100 ppm were also deleted from the test set. This was necessary since the experiments of Alexander used 100 ppm as the initial concentration regardless of solubility.

3. Compounds where the vapor pressure or solubility were questionable were also eliminated from consideration.

This screening operation reduced the data set from 83 to 12. The chemicals along with the key chemical and physical properties are shown in Table II.

DETERMINATION OF RATE CONSTANTS AND STATISTICAL EVALUATION OF THE RESULTS

Experimentally derived rate constant - The result in Alexander's report(9) is reported as % loss in four hours. For purposes of this calculation it was assumed that the rate of evaporation followed a simple first order reaction. With this assumption and knowing that the depth of the aeration cylinder was 15.5 inches a value for the rate constant in ft/hour was determined for each of the chemicals in Table II by using equation 10.

$$k = \frac{1}{4}\left(\ln \frac{C_o}{(C_o - \text{loss in 4 hours})}\right)\frac{15.5}{12} \qquad (10)$$

The results of this calculation are shown in Table III.

TABLE I

COMPARISON OF CALCULATED VS

EXPERIMENTAL VALUE FOR HENRY'S CONSTANT

Chemical	Henry's Constant Exp.[1]	Calculated
Methyl chloride	0.30	0.36
Methylene chloride	0.12	0.13
Chloroform	0.12	0.12
Carbon tetrachloride	0.91 1.08[2]	0.99
Ethylene dichloride	0.04	0.04
1,1,1-Trichloroethane	1.41	1.51
Vinyl chloride	50	16[4]
Trichloroethylene	0.36	0.39
Perchloroethylene	0.82	0.87
Fluorotrichloromethane	33.3[3] 9[2]	4.7

[1] All values except as indicated from Reference 7 were taken.

[2] Reference 2

[3] This value appears to be in error.

[4] The solubility data for vinyl chloride is questionable.

$$\frac{\text{weight of chemical}}{\text{weight of air}} = \frac{P}{P_t} \times \frac{M}{M_a} \qquad (5)$$

where P_t = partial pressure of air
M = molecular weight of organic
M_a = molecular weight of air ~29

Substituting the expression for p from (4) into (5) yields (6).

$$(\text{gms/cc})\text{air} = \frac{X}{X_s} \frac{P_v}{P_t} \frac{M}{29} \text{ density of air} \qquad (6)$$

$$\text{or } H = \frac{(\text{gms/cc})\text{air}}{(\text{gms/cc})\text{water}} = \frac{P_v}{P_t} \frac{M}{29} \frac{\text{density of air}}{X_s} \qquad (7)$$

For soluble materials: $p = X \frac{M}{M_{H_2O}} P_v$

Again, substituting this expression for p into (5) yields (8)

$$(\text{gms/cc})\text{air} = \frac{X}{M} \frac{18}{29} \frac{P_v}{P_t} M \qquad (8)$$

which on rearranging gives (9).

$$H = \frac{(\text{gms/cc})\text{air}}{(\text{gms/cc})\text{water}} = \frac{18}{29} \frac{P_v}{P_t} \text{density of air} \qquad (9)$$

Using these sets of equations Henry's constant was evaluated for several materials and compared with values reported in the literature. This comparison is shown in Table I and indicates that a reasonable estimate can be made with this procedure. To a first approximation these equations are obeyed provided the mole fraction of the solute is less than 0.02(8).

TEST DATA

The "Pollutional Evaluation of Compounds" edited by H. Alexander of Environmental Science(9) formed the basic data set by which these two methods could be evaluated. Essentially, the volatility measurements were made by gently aerating a liter

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METHOD B (LISS AND SLATER) REF. 2

This method was originally developed to measure the flux of various gases across the air-sea interface. It essentially calculates the rate constant for the movement of a gas in either direction across this interface and is based on the assumption that the exchanging gas obey's Henry's law (this assumption is also true for Method A). Two equations (2 and 3) resulted from this derivation and are shown below.

$$K(\text{liquid}) = \frac{H\, k_g\, k_l}{H\, k_g + k_l} \qquad (2)$$

$$K(\text{air}) = \frac{k_g\, k_l}{k_l + H\, k_g} \qquad (3)$$

where K's are in units of (distance/time)
H is Henry's constant
(conc in gas phase)/(conc in liquid)
k_g and k_l are exchange constants

The two exchange constants were estimated from field data taken in the ocean by Schooley(6). These had a mean value of 3000 cm hr^{-1} for $k_g(H_2O)$ and 20 cm hr^{-1} for $k_l(CO_2)$. In order to apply these constants to other materials, they should be multiplied by the ratio of the square roots of the molecular weights of H_2O and CO_2 with the gas in question.

The other parameter that needs to be evaluated is a number for Henry's constant. For slightly soluble materials the following relation holds in water.

$$p = \frac{X}{X_s} P_v \qquad (4)$$

where p = partial pressure of the organic
P_v = vapor pressure of pure organic
X = weight fraction of organic in water
X_s = solubility of organic in water

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Chemical Name (300 per name)	25	CAS No. (10)	24
1,1,1-TRICHLOROETHANE		71-55-6	

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TITLE: Interphase Transfer Processes. II.* Evaporation Rates of Chloro Methanes, Ethanes, Ethylenes, Propanes, and Propylenes from Dilute Aqueous Solutions. Comparisons with Theoretical Predictions

PAGES IN FULL REPORT: 68

AUTHOR(S): Wendell L. Dilling

AUTHOR'S SIGNATURE(S): Wendell L. Dilling

REVIEWER'S SIGNATURE: R. J. Moolenaar

This report is: [ ] INTERIM [X] FINAL and mainly: [X] NEW [ ] REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS: (Include in this space references to data books, and to earlier related reports, patents and publications.)

The evaporation rates of 27 of the title compounds from dilute (~1 ppm) aqueous solutions were analyzed using experimental data obtained previously (W. L. Dilling, N. B. Tefertiller, and G. J. Kallos, Environ. Sci. Technol., 9, 833 (1975),

Most of the rates followed first order kinetics for the first 2-5 half-lives. Under the experimental conditions (200 rpm stirring (shallow pitch propeller), ~25°C, still air (<0.2 mph), solution depth 6.5 cm.), the half-lives ranged from 17 minutes for 1,1,1-trichloroethane to 56 minutes for 1,2,3-trichloropropane. The experimental half-lives correlated well with those calculated by the method of Mackay and Leinonen (Environ. Sci. Technol., 9, 1178 (1975)) and Liss and Slater (Nature, 247, 181 (1974)), especially for series of compounds which were run simultaneously in the same solution. The experimental half-lives did not correlate with those calculated by the method of Mackay and Wolkoff (Environ. Sci. Technol., 7, 611 (1973)).

For compounds with partition coefficients (C_{air}/C_{water}) > ~0.1, the evaporation half-life is nearly independent of the partition coefficient, and thus of the solubility and vapor pressure, according to the Mackay, Leinonen, Liss and Slater theory. The theoretical half-life is essentially a function of the square root of the molecular weight.

An extensive tabulation of literature values for the solubilities in water, vapor pressures, and partition coefficients for 30 of the title compounds is included.

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Interphase Transfer Processes

II. Evaporation Rates of Chloro Methanes, Ethanes, Ethylenes, Propanes, and Propylenes from Dilute Aqueous Solutions. Comparisons with Theoretical Predictions

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The evaporation rates of 27 of the title compounds from dilute (~1 ppm) aqueous solutions were determined in order to test two theoretical models. Most of the rates followed first order kinetics for the first 2-5 half-lives. Under the experimental conditions [200 rpm stirring (shallow pitch propeller), ~25°C, still air (< 0.2 mph), solution depth 6.5 cm], the half-lives ranged from 17 minutes for 1,1,1-trichloroethane to 36 minutes for 1,2,3-trichloropropane. The experimental half-lives correlated reasonably well with those calculated by the method of Mackay and Leinonen and Liss and Slater, especially for series of compounds which were run

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[1]For part I of this series see reference (1).

simultaneously in the same solution. The experimental half-lives did not correlate with those calculated by the method of Mackay and Wolkoff.

Two theoretical models for predicting the rate of evaporation of slightly soluble materials from aqueous solution have been published recently (2-4). In a continuation of our work on the evaporation of low molecular weight chlorohydrocarbons from dilute aqueous solution (1) we have measured the evaporation rates of 27 chlorohydrocarbons from water in order to test these two theoretical models. Preliminary work (1) had indicated that the earlier model (2) was inadequate since neither absolute nor relative predicted rates were in agreement with the experimental rates. In the present work the evaporation rates of all of the chloro methanes, ethanes, and ethylenes, and a few of the chloro propanes and propylenes were measured in order to carry out a more extensive test.

Experimental

The same hollow fiber-mass spectrometric procedure as reported previously (1) was used to determine the evaporation rates. Two to five compounds were run simultaneously in the same solution. The initial concentration of each component was

1.0 ppm (weight basis). Typical data are shown in Figures 1 and 2. The zero-peak height was correlated with concentration by extrapolation of the decay portion of the curves in Figures 1 and 2 to zero time. This extrapolated concentration at zero time was taken as 1.0 ppm. Successive half-lives were determined from the decay portion of the curves and are reported in Table I. The rising portion of the curve for the higher molecular weight compounds, e.g., CCl_3CCl_3 (Figure 2), is due to the time required for diffusion of the compound through the silicone rubber hollow fiber.

Figs. 1 & 2→

Table I→

Results and Discussion

The measured half-lives for evaporation, $t^{\circ}_{1/2}$, of the chlorohydrocarbons from dilute (1 ppm) aqueous solutions are shown in Table I. Under the experimental conditons which included 200 rpm stirring of the solution with a shallow-pitch propeller stirrer, ~25°, still air (< 0.2 mph air currents), and a solution depth of 6.5 cm, the half-lives ranged from 17 minutes for CH_3CCl_3 to 56 minutes for $CH_2ClCHClCH_2Cl$. Most of the rates followed first order kinetics as shown by the constancy of the first 2-3 half-lives.

The more recent theoretical model by Mackay and Leinonen (3) employed liquid and vapor phase mass transfer coefficients for exchange between the ocean and the atmosphere reported by Liss and Slater (4). Various parameters involved in this treatment

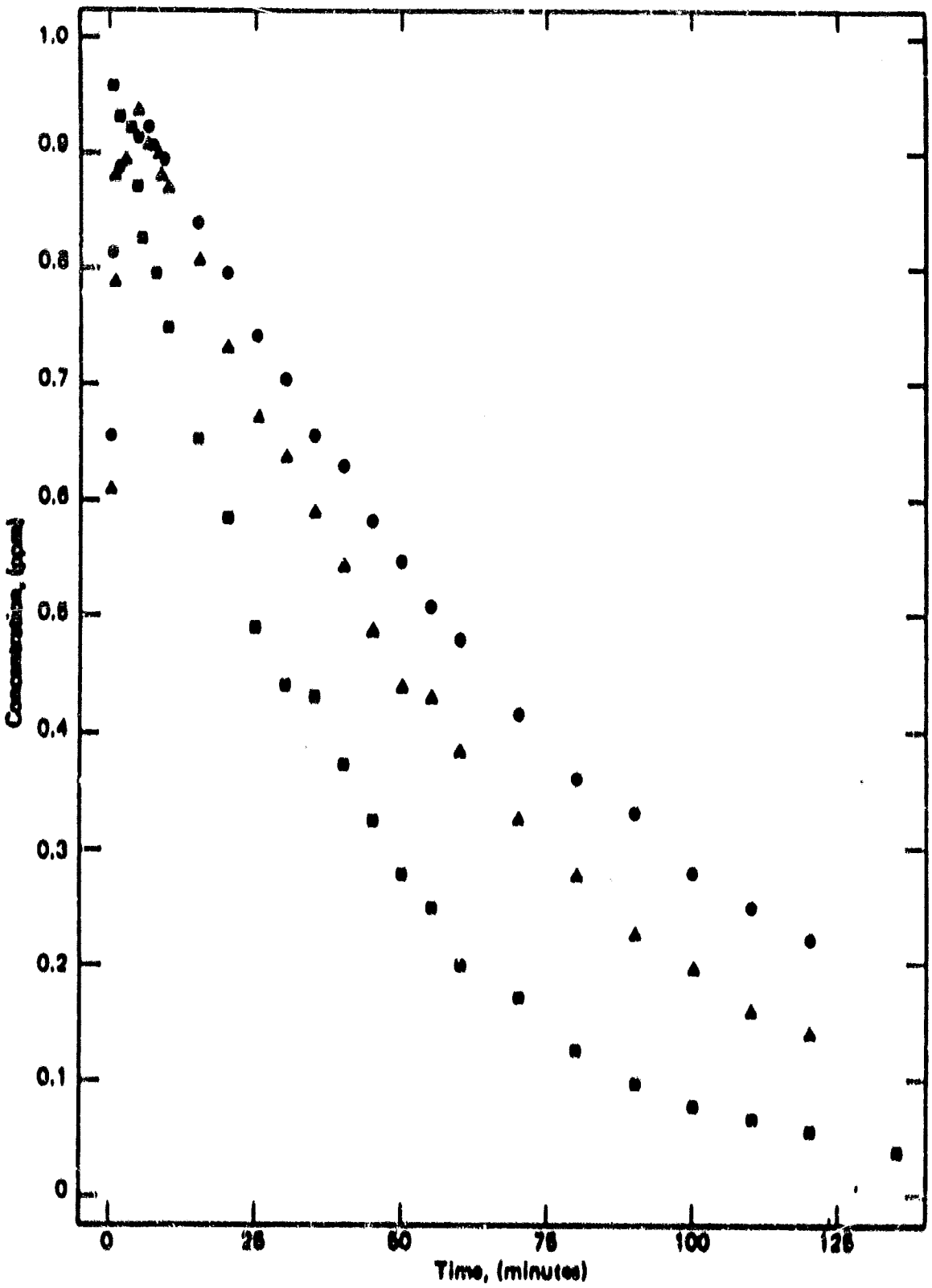

Figure 1. Evaporation rates of $CH_2=CHCH_2Cl$ (■), CH_3CCCl_3 (▲), and $CHCl_2CHCl_2$ (●) from water

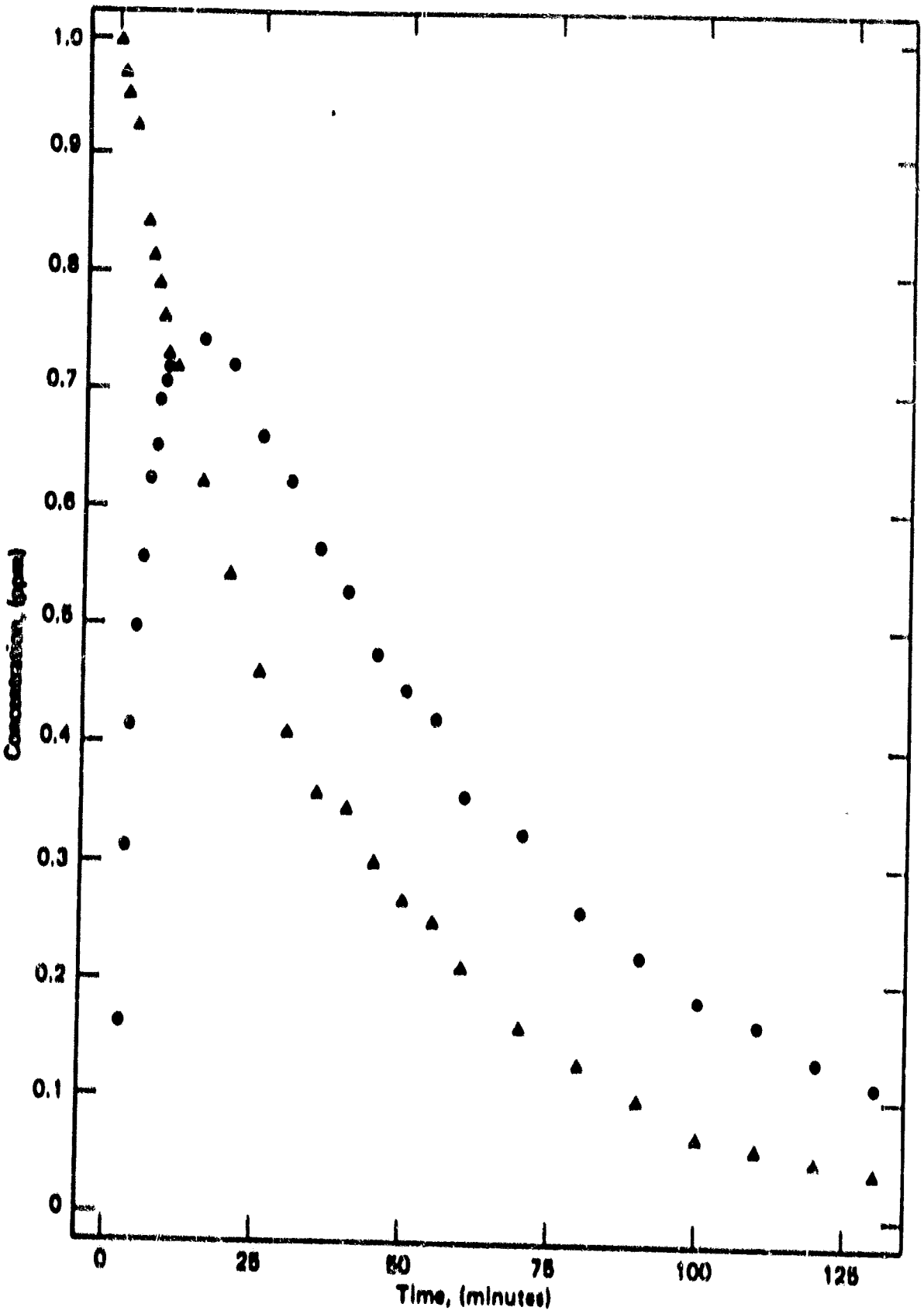

Figure 2. Evaporation rates of $CH_2=CCl_2$ (▲) and CCl_3CCl_3 (●) from water

Table 1. Calculated and Experimental Evaporative Parameters and Rates of Chlorofluorocarbons from Water[a]

No.	Compound	Solubility in water, ppm[b]	Vapor pressure, mm Hg	Partition coefficient, calcd.	Partition coefficient, found	Overall liquid exchange constant, cm min^{-1}	Percent mass in liquid	Approx. initial mass, g	Successive experimental evaporation half-lives,[c] min	Evaporation half-lives, min, calcd. 20 °C	Evaporation half-lives, min, calcd. 25 °C	Evaporation half-lives, min, experimental
1	CF_2Cl_2	[illegible]	[illegible]	0.20		0.000	91.4	1.00	19.0, 20.0, 21.0	0.000	16.0	21.0
		[illegible]	[illegible]	0.21	0.20							
2	CF_2ClCF_2Cl	[illegible]	[illegible]	0.12	0.11	0.000	90.0	0.00	21.0, 20.0, 20.0, 21.0 20.0	0.00	20.0	20.0
								0.00	19.0, 19.0, 21.0, 20.0, 20.0, 20.0			20.0
								0.00	19.0, 20.0, 20.0, 20.0, 20.0			20.0
		[illegible]	[illegible]	0.010		0.000	76.0	0.00	20.0, 20.0, 21.0 20.0		20.0	20.0
3	$CHCl_3$	[illegible]	[illegible]	0.10	0.11	0.000	93.0	0.00	20.0, 20.0, 20.0, 20.0 20.0	0.00	20.0	20.0
								0.00	19.0, 20.0, 20.0, 20.0 20.0			20.0
								0.00	20.0, 20.0, 20.0, 20.0 20.0			20.0
		[illegible]	[illegible]	0.000		0.000	81.0	0.00	20.0, 20.0, 20.0, 21.0 20.0		21.0	20.0
4	CH_2Cl_2	[illegible]	[illegible]	1.0	0.01	0.000	99.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.00	20.0	20.0
5	CH_2ClCH_2Cl	[illegible]	[illegible]	0.00		0.000	97.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.00	20.0	21.0
6	CH_2ClCH_3	[illegible]	[illegible]	0.00		0.000	98.0	0.00	20.0, 20.0, 20.0, 20.0	0.00	21.0	20.0
7	$CH_2ClCH_2CH_2Cl$	[illegible]	[illegible]	0.000	0.0000	0.000	98.0	0.00	20.0, 20.0, 20.0, 20.0	0.0	20.0	20.0
8	$CH_2ClCH_2CH_3$	[illegible]	[illegible]	1.0		0.000	99.0	0.01	20.0, 20.0, 20.0, 20.0, 20.0	0.00	20.0	20.0
								0.00	20.0, 20.0, 20.0, 20.0, 20.0			20.0
								0.00	20.0, 20.0, 20.0, 20.0, 20.0			20.0
		[illegible]	[illegible]	1.00	1.00							
		[illegible]	[illegible]	0.200		0.000	97.0	0.00	20.0, 20.0, 20.0, 20.0		20.0	20.0
9	$CH_2ClCH_2CH_3$	[illegible]	[illegible]	0.000		0.000	98.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.0	20.0	20.0
10	CH_3ClCH_3	[illegible]	[illegible]	0.00		0.000	98.0	0.00	20.0, 20.0, 20.0	0.00	20.0	20.0
11	CH_2ClCH_2Cl	[illegible]	[illegible]	0.010		0.000	97.0	0.00	20.0, 20.0, 20.0	0	20.0	20.0
12	CH_2ClCH_2Cl	[illegible]	[illegible]	0.10		0.000	99.0	0.00	20.0, 20.0, 20.0	0.0	20.0	20.0
13	CH_2ClCH_3	[illegible]	[illegible]	0.00		0.00	93	0.00	20.0, 20.0, 20.0, 20.0	0	20	20.0
14	CH_3CH_2Cl	[illegible]	[illegible]	0.00	[illegible]	0.000	99.00	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.0000	20.0	20.0
		[illegible]	[illegible]	0.00		0.000	99.0			0.000	20.0	
15	CH_3CH_2Cl	[illegible]	[illegible]	0.0		0.000	99.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.000	20.0	20.0
		[illegible]	[illegible]	0.00	0.00							
16	[illegible]	[illegible]	[illegible]	0.01		0.000	99.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.00	20.0	20.0
17	[illegible]	[illegible]	[illegible]	0.00		0.000	99.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.00	20.0	20.0
18	$CH_3CH_2CH_2Cl$	[illegible]	[illegible]	0.00		0.000	99.0	0.00	20.0, 20.0, 20.0, 20.0, 20.0	0.00	20.0	20.0
								0.00	20.0, 20.0, 20.0, 20.0			20.0
								0.00	20.0, 20.0, 20.0, 20.0			20.0

		1100[X,B']	95[X,B']	0.40[A]	0.365[X,A]							
		1000[C,B']	33[C,X]	0.17[C]		0.161[C]	94.1[C]	0.93	30.9,31.4,35.3[X]		34.8[C]	31.3[X]
19	CCl_3-CCl_3[A]	140[B']	10.4[X]	1.2	0.50[A]	0.170	99.1	0.92	34.5,38.3,34.8, 36.7	0.28	36.5	37.1
								0.92	33.2,35.5,37.3, 34.6			35.4
								0.92	30.6,19.4,26.2, 30.9			30.2
		120[X,B']	14.3[X,X]	1.0[A]	0.02[X,A]							
		110[C,B']	4.0[C,X]	0.35[C]		0.161[C]	97.1[C]	0.96	36.0,38.1[X]		37.0[C]	37.[illegible]
20	$CH_2ClCHClCH_2Cl$	1000[X']	3.1[B']	0.013		0.191	95.3	0.93	53.3,58.9	18	44.7	56.1
21	$CH_2ClCCl_2CH_2Cl$	[B']						0.89	47.7,50.4			49.4
22	CH_2=$CHCH_2Cl$	3370[X']	361[X']	0.44		0.347	97.7	0.96	26.5,28.4,24.9	0.529	18.2	25.4
23	CH_2=$CClCH_3$	[B']						0.99	29.7,33.9,35.4			33.1
24	$CHCl$=$CHCH_3$	[B']						0.95	16.0,17.9,20.1 20.1,16.9			17.9
25	CH_2=$CClCH_2Cl$	2150[X']	93[X']	0.19		0.196	93.4	0.94	23.3,19.8,20.9, 20.3	1.4	23.0	23.1
26	cis-$CHCl$=$CHCH_2Cl$	3760[X']	63[X']	0.095		0.189	98.1			1.4	33.4	
								0.91[B']	30.4,31.4,25.1			29.[illegible][C']
27	trans-$CHCl$=$CHCH_2Cl$	3000[X']	34[X']	0.075		0.186	97.4			3.3	34.6	
28	$CHCl$=$CClCH_2Cl$	[B']						0.70	46.2,48.1			45.1

[A]All data for 25° unless specified otherwise. [B]Initial concentration used for determining the first half-life. [C]The first decimal place is somewhat uncertain. [D]Average of data in refs 8 and 6. [E]The vapor pressure of the solute in equilibrium with the solution for the cited solubility was 760 mm. [F]At 20°. [G]Datum from ref 1. [H]Data from refs 7 and 8. [I]Results from three separate experimental runs are given. [J]Datum from ref 9. [K]By interpolation of data in ref 10. [L]Datum from ref 11. Vapor phase was nitrogen; liquid phase was Mississippi River water. [M]At 1.5°. [N]By interpolation of data in ref 9. [O]At 1-2°. [P]Datum from ref 13. [Q]Average of values obtained by interpolation of data in refs 7-9. [R]Average of values obtained by interpolation of data in refs 7-9 and 13. [S]Value reported in refs 14 and 15 at 20° and 17.5°. [T]The vapor pressure of the solute in equilibrium with the solution for the cited solubility was presumably >760 mm. [U]See also ref 16. [V]Average of values obtained by interpolation of data in refs 9 and 17. [W]Average of values obtained by interpolation of data in refs 8 and 18. [X]Average of values obtained by extrapolation of data in refs 7-9, 14, and 17 using average temperature dependence in refs 14 and 17. [Y]Average of values obtained by interpolation of data in refs 7-9 and 18. [Z]Average of values obtained by extrapolation of data in refs 7, 8, 13, and 20 using temperature dependence in ref 17. [A']Average of values obtained by interpolation of data in refs 7-9, 18, and 21. [B']Average of data in refs 7-9, 18, and 21. [C']Average of values obtained by interpolation of data in refs 9, 18, and 21. [D']Average of values obtained by interpolation and extrapolation of data in refs 13, 14, and 17. [E']By interpolation of data in ref 17. [F']By interpolation of data in ref 18. [G']Average of values obtained by interpolation of data in refs 13, 14, and 17. [H']Average of values obtained by extrapolation of data in refs 13 and 17 using temperature dependence in ref 13. [I']Average of values obtained by extrapolation of data in refs 23 and 23 using temperature dependence in ref 23. [J']Value reported in refs 7 and 8 at 18°. [K']Datum from ref 24. [L']Value reported in refs 7-9 at 20°. [M']Average of values obtained by interpolation of data in refs 7,8, and 18. [N']Average of values obtained by extrapolation of data in refs 7, 8, 13, and 14 using temperature dependence in ref 13. [O']Average of data in refs 7-9 and 18. [P']Average of values obtained by extrapolation of data in refs 7, 8, 13, and 23 using temperature dependence in ref 23. [Q']Average of values obtained by extrapolation of data in refs 7, 8, 13, and 23 using temperature dependence in ref 13. [R']Average of values in refs 19, 25, and 26 at 20° and 30°. [S']Average of values obtained by interpolation of data in refs 14 and 25. [T']No solubility data available. [U']Average of values obtained by interpolation and extrapolation of data in refs 19 and 27-29 using temperature dependence in ref 29. [V']Data from refs 27 and 28. [W']By extrapolation of data in ref 29. [X']Average of values obtained by interpolation and extrapolation of data in refs 19 and 26. [Y']Value reported in ref 31 at 20°. [Z']Datum from ref 19. [A"]A mixture of cis and trans isomers was used.

are shown in Table I. The partition coefficient, H_c, or Henry's law constant, can be calculated from equation (1), where C_{air}

$$H_c = \frac{C_{air}}{C_{water}} = \frac{16.04\ PM}{TS} \qquad (1)$$

and C_{water} are the equilibrium concentrations in the air and water phases respectively, P is the vapor pressure of the pure solute in mm of Hg, M is the gram molecular weight of the solute, T is the temperature in °K, and S is the solubility of the solute in water in mg $l.^{-1}$ (~ppm). Values for S and P were obtained from published data and other sources, and are noted in Table I. The agreement in values of P among various workers is quite good, but there are wide discrepancies in values of S for some of the compounds, e.g., the value for $CH_2{=}CHCl$ (see Table I). For purposes of calculation we chose values of S on the basis of agreement among various workers or the most recent data. Experimentally determined values of the partition coefficient, H_c, are in reasonably good agreement with the calculated values in most cases. The overall liquid exchange constant, K_l, in cm min^{-1} was calculated by equation (2), where k_l and k_g are the exchange constants

$$K_l = \frac{H_c k_l k_g}{H_c k_g + k_l} = \frac{221.1}{\left(\frac{1.042}{H_c} + 100.0\right) M^{1/2}} \qquad (2)$$

for the liquid and gas phases respectively (4). The resistance in the liquid phase, Ωr_l, is given by equation (3), where R_l is

$$\%r_1 = \frac{100r_1}{R_1} = \frac{10^4}{100.0 + \frac{1.042}{\eta_c}} \qquad (3)$$

the total resistance expressed on a liquid phase basis. The solute evaporation half-life, $t^L_{1/2}$, in minutes was calculated by equation (4) where d is the solution depth (3).

$$t^L_{1/2} = \frac{0.6931d}{R_1} \qquad (4)$$

A correlation of the half-lives for evaporation, $t^L_{1/2}$, calculated by equation (4) with the experimental values, $t^o_{1/2}$, is shown in Figure 3. The reasonably close agreement between the calculated and experimental half-lives is somewhat fortuitous since the calculated values depend on the exchange constants, k_l and k_g (4), while the experimental values depend on the stirring rate, air currents, and other factors (1). A more significant test of the model is a comparison of the relative values of the two sets of data. For compounds which were run in the same solution, an approximately linear relationship with an intercept near the origin was observed in most cases (Figure 3). The variation among different runs was apparently due to some uncontrolled variables in the experiments (1).

Fig. 3→

One experiment was run at 1-2° (Table I). The increased half-lives at the lower temperature are predicted by equations (3) and (4). The increase in the calculated half-lives is

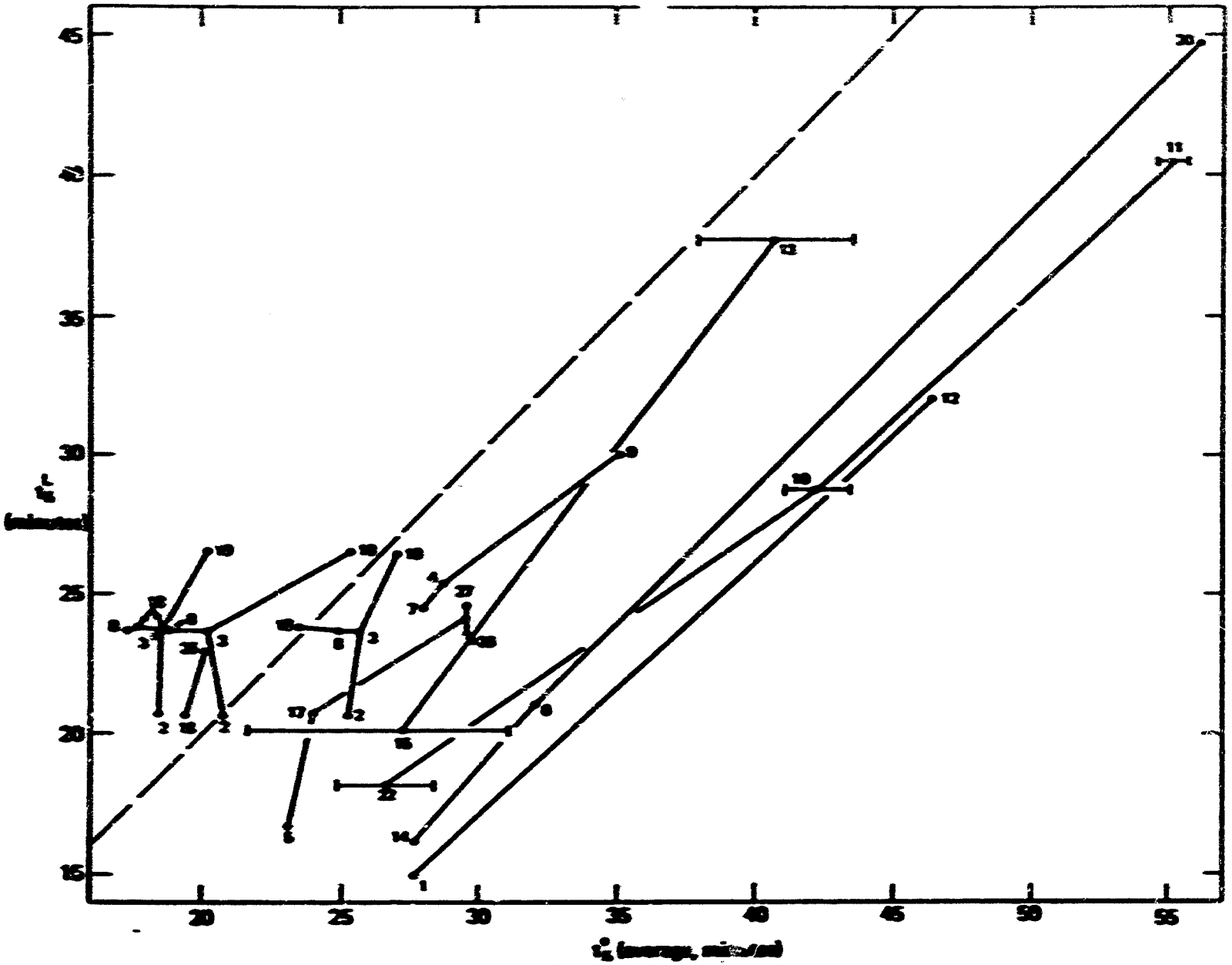

Figure 1. Correlation of evaporation half-lives, $t^{L}_{\frac{1}{2}}$, calculated by equation (4) with experimental half-lives, $t^{e}_{\frac{1}{2}}$. Numbers refer to compound numbers in Table I. Compounds which were run in the same solution are indicated by the lines which connect data points. Error bars for compounds 10, 11, 13, 15, and 22 are for total variation of experimental half-lives. Other error bars are [illegible] for clarity. Dashed line is the 1:1 correlation line.

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due to a decrease in H_c with the decrease in temperature. However, on lowering the temperature the experimental half-life increased more than the calculated half-life did, possibly because k_l and k_g were assumed to be independent of temperature (3).

One interesting feature of equation (2) involves the effect of changes in H_c on the exchange constant, K_l, and thus on the half-life, $t^L_{1/2}$. For values of H_c greater than about 0.1 (> 90% liquid phase resistance) there is relatively little change in K_l with major changes in H_c. For example the 45-fold variation in the two values of H_c for CH_2=CHCl in Table I leads to only a 1% change in K_l and $t^L_{1/2}$. Thus for compounds with $H_c > \sim 0.1$, i.e. most but not all of the chlorohydrocarbons in this study, the calculated half-life, $t^L_{1/2}$, is almost directly proportional to the square root of the molecular weight and is nearly independent of H_c.

The earlier theoretical model by Mackay and Wolkoff (2) leads to equation (5) where $t^M_{1/2}$ is the solute evaporation

$$t^M_{1/2} = \frac{0.01797\ GP_wS}{EPM} \qquad (5)$$

half-life in minutes, G is the weight of water in grams, P_w is the partial vapor pressure of water in mm of Hg, and E is the weight of water in grams which evaporates per day. In our experiments at ~25°, G=200, P_w=23.76, and E=20. Calculated values of $t^M_{1/2}$ are listed in Table I. A correlation of the

calculated half-lives, $t^{M}_{1/2}$, with the experimental values, $t^{e}_{1/2}$, is shown in Figure 4. One notes, as observed previously (1), that neither the absolute nor relative half-lives are predicted correctly by equation (5). The possible reasons for this failure of equation (5) have been discussed previously (1,3).

Fig. 4 →

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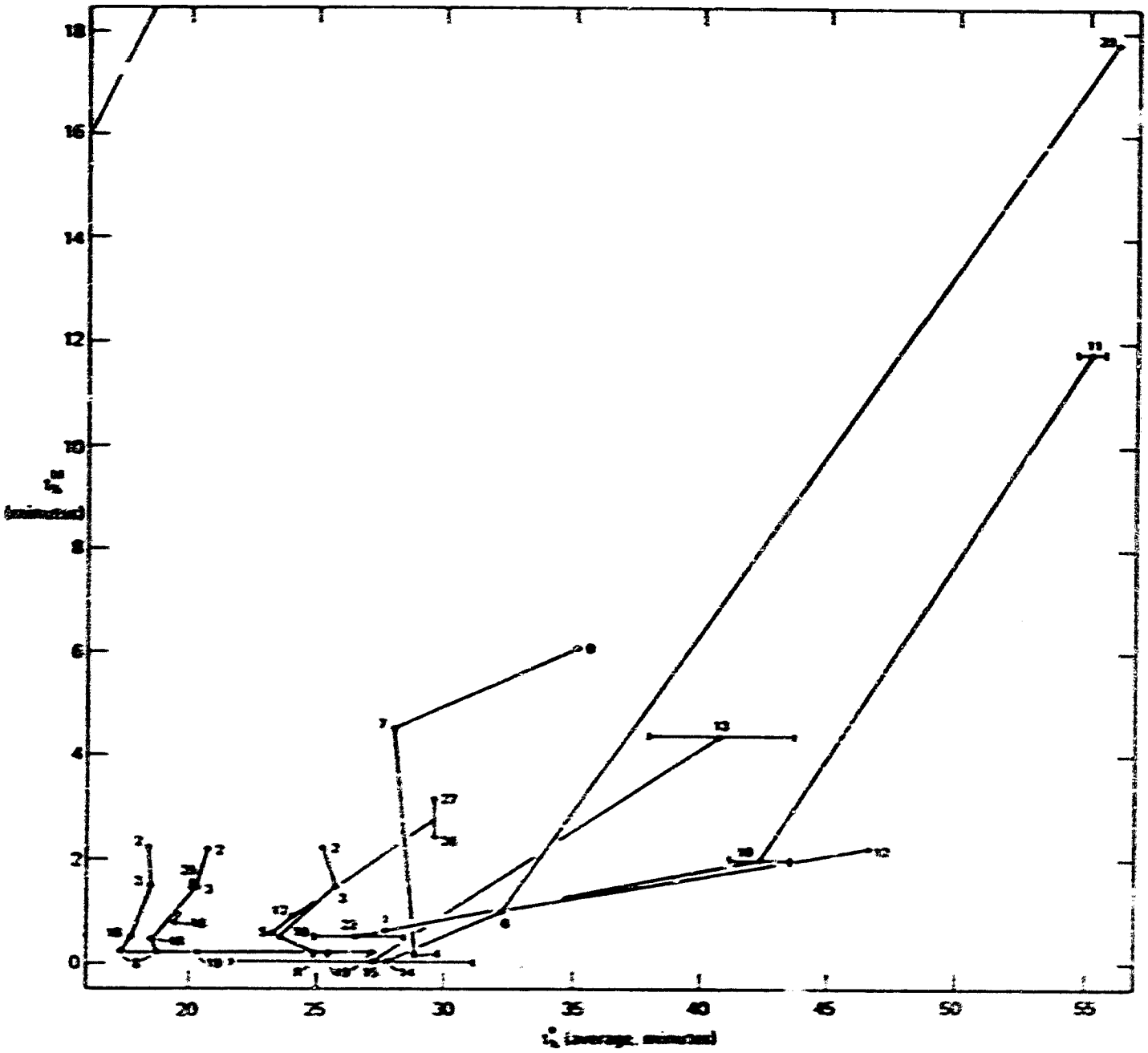

Figure 4. Correlation of evaporation half-lives, $t_{\frac{1}{2}}^{M}$, calculated by equation (5) with experimental half-lives, $t_{\frac{1}{2}}^{e}$. See also other notes in caption for Figure 3.

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Appendix

The evaporation half-lives, $t^{L}_{1/2}$, calculated by equation (4) as a fraction of H_c for molecular weights of 10, 100, and 1000 are shown in Figure 5. In general, for compounds which have $H_c \geq 0.1$ and which were run simultaneously in the same solution, the higher the molecular weight (M), the greater was the experimental half-life for evaporation ($t^{e}_{1/2}$). Unfortunately there are insufficient data for a reliable test of the linearity of the expression, $t^{e}_{1/2}$ = constant x $M^{1/2}$.

The literature data on the solubilities in water and vapor pressures for the chlorohydrocarbons discussed in this report are presented in Tables I.-LV. The absence of tables for solubilities of some compounds indicates that no data were found. In the tables on solubilities in water, the column with the heading RCl pressure refers to the pressure of the chlorohydrocarbon in question in equilibrium with the aqueous solution. The solubility (S) and vapor pressure (P) data for many of the compounds in Tables II-LV are plotted (S *vs.* T, log P *vs.* 1/T) in data books ES-WLD-37, -41, and -42 (1976).

Additional experimentally determined partition coefficients (Henry's law constants), H_c, are listed in Table LVI along with the values already presented in Table I.

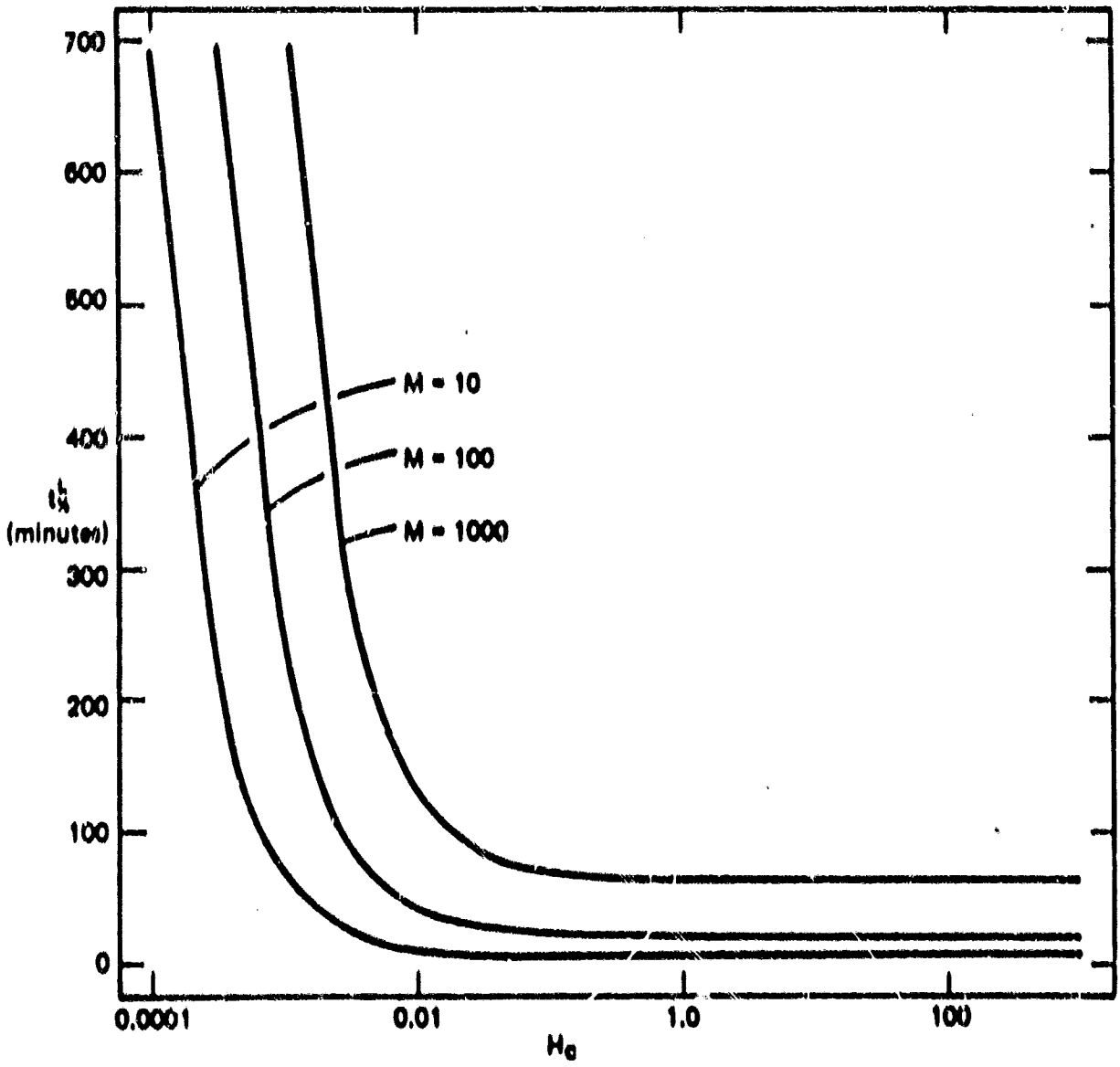

Figure 2. Calculated evaporation half-lives as a function of H_a for various molecular weights.

Table II. Methyl Chloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
30	7300	760	12
25	7180	760	32
25	~5400	760	6
25	5400	760	5
25	4800	[a]	9
25	4770	760	33
20	7990	760	32
20	7250	760	7,8
20	6300	760	5
20	4600	760	35
0	7600	760	35

[a] Not reported, presumably atmospheric, ~760 mm.

Table III. Methyl Chloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
25	~4170	12
20	3756	7,8
20	3600	19
-24.2	760	7,8
-38	400	19
-50	210	19

Table IV. Methylene Chloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
30	19000	a	9,17
25	20000	a	20
25	17800	760	32
25	13200	760	8
25	13200	a	7
25	13000	a	12
25	10300	438	32
25	7640	438	8
20	19600	a	9
20	13000	a	19
10	20800	a	9
0	23100	a	9

[a] Not reported, presumably autogenous.

Table V. Methylene Chloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
51.67	1139.3	10
40.1	760	7,8
39.8	760	20
37.78	709.0	10
35	600	9
30	511.4	9
23.89	419.4	10
20	362.4	7,8
20	348.9	9
20	340	20
10.00	233.8	10
10	229.7	9
0	147.4	9
- 3.89	121.5	10

Table VI. Chloroform Solubility in Water

Temperature, °C	Solubility, ppm	HCl pressure, mm Hg	Reference
30	7700	a	9
30	7650	a	17
30	~7500	a	12
25	7800	a	12
25	3640	760	32
25	923	192	32
20	8200	a	7,8
20	~8160	a	12
20	8150	a	9
20	7900	a	19
10	8870	a	9
0	10510	a	9

[a]Not reported, presumably autogenous.

Table VII. Chloroform Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
61.3	760	7,8
40	366.4	9
30	246.0	9
25	~190	12
20	159.6	9
20	150.5	7,8
10	100.5	9
0	61.0	9

Table VIII. Carbon Tetrachloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
30	814	a	17
25	800	a	9,12
25	540	760	32
25	93	a	23
25	81	113	32
20	800	a	19
20	785	a	7,8

[a]Not reported, presumably autogenous.

Table IX. Carbon Tetrachloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
76.8	760	7,8
56.160	387.970	13
40	210.9	9
36.930	189.100	13
30	143.0	34
30	139.6	9
25	~115	12
25	114.5	34
25	114.175	13
20	91.0	34
20	90.0	7,8
20	89.55	9
19.88	90.600	13
10	55.65	9
0	32.9	34

Table X. Ethyl Chloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
20	5700	a	34
17.5	5700	a	15
0	4500	a	12
0	4450	760	35
0	4450	a	9,17

[a]Not reported, presumably atmospheric, ~760 mm.

Table XI. Ethyl Chloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	1932	16
30	1414	18
25	~1180	12
25	1150	36
20	1003	18
13.2	760	19
10	702	18
0	472	19
-20	189	19
-79.9	5	19

Table XII. 1,1-Dichloroethane Solubility in Water

Temperature °C	Solubility, ppm	RCl pressure, mm Hg	Reference
50	5160	a	17
35	4800	a	17
20	5500	a	9
20	5470	a	19
20	5030	a	17
0	5900	a	17

[a]Not reported, presumably autogenous.

Table XIII. 1,1-Dichloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	415	18
30	278	18
30	277.2	9
20	183	18
20	182.1	9
10	116	18
10	115.3	9
0	71.0	18

Table XIV. 1,2-Dichloroethane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
56	10190	a	17
40	9600	a	34
35	8870	a	17
30	8500	a	17
30	7940	760	32
30	1080	103	32
25	8300	a	12
20	8800	a	7,8
20	8615	a	9
20	8420	a	17
20	8000	a	34
10	8300	a	34
0	9000	a	34
0	8650	a	17

[a]Not reported, presumably autogenous.

Table XV. 1,2-Dichloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
83.6	760	7,8
40	163	18
40	160	9
30	106	18
30	100	9
25	81.2	36
25	~78	12
20	67.0	18
20	64	9
20	63.9	7,8
10	40.5	18
10	40	9
0	23.8	18

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Table XVI. 1,1,1-Trichloroethane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
50	1260	a	17
37	950	a	19
35	1260	a	17
25	700	a	20
20	4400	a	9
20	1320	a	17
20	480	a	7,8
0	1590	a	17

[a]Not reported, presumably autogenous.

Table XVII. 1,1,1-Trichloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
74.1	760	7,8
74	760	20
40	240	9
40	238	16
31.29	162.31	21
30	157	18
30	150	9
26.47	132.00	21
25	130.6	37
25.00	123.70	21
22.79	112.79	21
20	101	18
20	100	9
20	98.66	21
20	96.0	7,8
10	63	18
10	62	9
0	38.0	18
0	37	9

Table XVIII. 1,1,2-Trichloroethane Solubility in Water

Temperature, °C	Solubility, ppm	HCl pressure, mm Hg	Reference
55	5290	a	17
35	4600	a	17
25	4400	a	12
20	4500	a	9,34
20	4340	a	17
0	4640	a	17

[a]Not reported, presumably autogenous.

Table XIX. 1,1,2-Trichloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
113.5	760	9
40	52.0	18
30	32.0	18
25	~22	12
20	18.8	18
20	16.7	9
10	10.7	18
0	5.77	19

Table XX. 1,1,1,2-Tetrachloroethane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
50	1250	a	17
35	1150	a	17
20	1090	a	17
0	1200	a	17

[a]Not reported, presumably autogenous.

Table XXI. 1,1,1,2-Tetrachloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	30.0	18
30	18.1	18
20	10.6	18
10	5.98	18
0	3.15	18

000040

Table XXII. 1,1,2,2-Tetrachloroethane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
55-56	3350	a	17
25	3200	a	34
25	2900	a	12
20	2870	a	17

[a]Not reported, presumably autogenous.

Table XXIII. 1,1,2,2-Tetrachloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
100	189	19
70	62	9
60	40	9
45	19.5	9
40	15.0	18
32	10.0	9
30	8.65	16
25	6.9	12
20	4.85	18
10	2.62	18
0	1.32	18

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Table XXIV. Pentachloroethane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	500 (480)[a]	b	12
20	500	b	34
20	470	b	17
20	470[a]	b	12

[a]From graph. [b]Not reported, presumably autogenous.

Table XXV. Pentachloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
100	128	19
80	59	9
60	26	9
40	10.2	18
30	5.95	18
25	~4.1	12
20	3.5	9
20	3.33	18
10	1.80	18
1	1	9
0	0.9	18

Table XXVI. Hexachloroethane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
~25	12.8	a	23
22.3	50 (?)	a	17
~22	10.7	a	23
22	2.95[b]	a	22

[a]Not reported, presumably autogenous. [b]±5%.

Table XXVII. Hexachloroethane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
150	261	19
100	38	19
60	4.10	9
50	2.16	18
40	1.60	18
40	1.00	9
30	0.48	18
25	~0.6	12
20	0.22	9
20	0.21	18
15	0.15	9

Table XXVIII. Vinyl Chloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	2700	760	24
25	1100	760	35
25	1100	a	9
10	60	760	7,8
0.2	7490	760	24

[a]Not reported, presumably atmospheric, ~760 mm.

Table XXIX. Vinyl Chloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	4600	18
30	3470	18
25	2936.25	20
20	2580	18
20	2320	7,8
10	1880	18
0	1330	18
-13.9	760	7,8
-20	580	18

Table XXX. Vinylidene Chloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	<3000	a	38
20	5500	a	19
20	400	a	7-9

[a]Not reported, presumably autogenous.

Table XXXI. Vinylidene Chloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	1016	10
40	1015	18
31.9	760	7,8
31.8	760	9
30	721	10
30	720	9,18
25.00	600.32	38
20	498	10
20	498.5	7,8
20	495	9,18
10	334	10
10	330	18
0	215	9
0	212	18
-20	77.0	18

Table XXXII. cis-Dichloroethylene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	3500	a	9,12
10	400	a	34

[a]Not reported, presumably autogenous.

Table XXXIII. cis-Dichloroethylene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	385	18
40	350	9
30	256	18
30	250	9
25	~216	12
20	180	9
20	165	18
10	110	9
10	104	18
0	65	9
0	62.1	18

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Table XXXIV. trans-Dichloroethylene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	6300	a	9,12
10	300	a	34

[a]Not reported, presumably autogenous.

Table XXXV. trans-Dichloroethylene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
40	585	10
40	575	9
30	410	9
30	390	10
25	~325	12
20	265	9
20	260	10
10	185	9
10	165	10
0	113	9
0	103	10

Table XXXVI. Trichloroethylene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	1100		
25	1000	a	9,12
20	1100	a	34
20	~1100	a	7,8
0	~1100	a	12
		a	12

[a]Not reported, presumably autogenous.

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Table XXXVII. Trichloroethylene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
87.0	760	7,8
40	147	18
40	146.8	9
30	95.0	18
30	94	9
25	~71	12
20	60	18
20	57.9	7,8
20	57.8	9
10	36.9	18
10	35.2	9
0	21.3	18
0	20.1	9

Table XXXVIII. Tetrachloroethylene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
25	400	a	34
25	150	a	9,12
25	103	a	23
20	150	a	7,8
20	90	a	23
0	140	a	12
0	40[b]	a	23

[a]Not reported, presumably autogenous. [b]Extropolated from data >~10°.

Table XXXIX. Tetrachloroethylene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
121.2	760	7,8
100	400	19
40.0	41	9
40	40.0	18
30	24.5	18
26.3	20	9
25	~19	12
20	14.3	18
20	14.0	7,8
13.8	10	9
10	8.10	18
2.4	5	9
0	4.4	18

Table XL. 1,2,3-Trichloropropane Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
30	1900	a	19
20	1900	a	25,26

[a]Not reported, presumably autogenous.

Table XLI. 1,2,3-Trichloropropane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
156.3	760	25
95.5	100	34
87.3	60	34
76	50	25
73.9	40	34
59.35	20	34
46	10	34
44	10	25
33.7	5	34
9	1	34

Table XLII. 1,2,2,3-Tetrachloropropane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
141.70	408.26	30
136.20	346.94	30
130.40	290.57	30
122.45	226.36	30
117.10	189.77	30
112.00	159.62	30
104.90	128.58	30
99.30	102.03	30
94.25	84.08	30
85.30	59.41	30
79.60	47.51	30
73.00	35.97	30
67.25	27.32	30
61.90	21.02	30
25	3.0[a]	30

[a]Calculated.

Table XLII. 1,2,2,3-Tetrachloropropane Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
141.70	408.26	30
136.20	346.94	30
130.40	290.57	30
122.45	226.36	30
117.10	189.77	30
112.00	159.62	30
104.90	128.56	30
99.30	102.03	30
94.25	84.08	30
85.30	59.41	30
79.60	47.51	30
73.00	35.97	30
67.25	27.32	30
61.90	21.02	30
25	3.0[a]	30

[a]Calculated.

Table XLIII. Allyl Chloride Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
40.0	3900	a	29
35.0	3700	a	29
30.0	3500	a	29
25	4000	760	39
25	1900	361	39
20	3600	a	27
20.0	3300	a	28,29
20	3000	a	19

[a]Not reported, presumably autogenous.

Table XLIV. Allyl Chloride Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
54.4	817	19
44.96	760	28
37.8	595	19
25	361.3	27
25	361.17	28
21	300	19

Table XLV. 2-Chloropropene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
25	826	19
10	370	19
0	220	19

000361

Table XLVI. cis-1-Chloropropene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
79	3217	27
59	1942	27
25	575	19
20	500	19
8	322	27
0	225	27

Table XLVII. trans-1-Chloropropene

Temperature, °C	Vapor pressure, mm Hg	Reference
79	2725	27
59	1536	27
25	487	19
20	408	19
8	301	27
0	171	27

Table XLVIII. 2,3-Dichloropropene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
59.0	3610	a	29
46.0	3080	a	29
26.0	2200	a	29

[a]Not reported, presumably autogenous.

Table XLIX. 2,3-Dichloropropene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
80	500	19,26
74.80	423.30	30[a]
73.90	407.73	30
71.00	378.93	30
69.30	368.10	30
67.55	329.10	30
64.60	297.08	30
61.00	260.52	30
60.90	258.52	30
59.50	237.52	30
59.00	233.46	30
55.65	240.37	30
55	200	19,26
54.90	198.61	30
51.65	173.97	30
46.60	140.72	30
41.00	111.20	30
40.60	108.90	30
37.75	94.10	30
34.70	83.48	30
20	43	19,26

Footnote for Table XLIX

[a]The constants A and B in the equation, log p = -(A/T) + B, given by Otopkov, et. al.,[30] are incorrect. See data book ES-WLD-41, pp 5,8 (1976).

Table L. cis-1,3-Dichloropropene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
20	2700	a	31

[a]Not reported, presumably autogenous.

Table LI. cis-1,3-Dichloropropene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
106.0	760	31
92.2	500	19
64.5	200	19
25	43	19
20	28.0	31

Table LII. trans-1,3-Dichloropropene Solubility in Water

Temperature, °C	Solubility, ppm	RCl pressure, mm Hg	Reference
20	2800	a	31

[a]Not reported, presumably autogenous.

Table LIII. trans-1,3-Dichloropropene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
111.0	760	31
95	500	19
67	200	19
25	34	19
20	18.5	31

Table LIV. cis-1,2,3-Trichloropropene[a] Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
150.7	760	19
67	100	19,26

[a]Stenger[40] reported vapor pressure of unspecified isomer as 100 mm at 157° and 1 mm at 35°.

Table LV. trans-1,2,3-Trichloropropene Vapor Pressure

Temperature, °C	Vapor pressure, mm Hg	Reference
137.8	760	19,26
75	100	19,26

Table LVI. Partition Coefficients (Henry's Law Constants) for Chlorohydrocarbons

Compound	Temperature, °C	Partition coefficient (C_{air}/C_{water}) Calcd., H_a	Found, H_e	Reference
CH_3Cl	20	0.33	0.30	7,8
CH_2Cl_2	25	0.10	0.11	11
	20	0.085[a]	0.12	7,8
$CHCl_3$	25	0.16	0.13	11
	20	0.13[b]	0.12	7,8
CCl_4	25	1.2	0.87	11
	20	0.97[c]	0.91	7,8
	d		1.08	4
CH_2ClCH_2Cl	25	0.050	0.040	11
	20	0.042[e]	0.0379	7,8
CH_3CCl_3	20	1.0	1.4	7,8
$CH_2=CHCl$	10	43	50	7,8
$CH_2=CCl_2$	20	6.6	6.3	7,8
$CHCl=CCl_2$	20	0.40	0.369	7,8
$CCl_2=CCl_2$	25	1.2	0.50	11
	20	1.0	0.82	7,8

[a]Calculated using solubility of 19600 ppm and vapor pressure of 358 mm. [b]Calculated using solubility of 8160 ppm and vapor pressure of 155 mm. [c]Calculated using solubility of 785 ppm and vapor pressure of 90 mm. [d]Not reported. [e]Calculated using solubility of 8460 ppm and vapor pressure of 65 mm.

Allyl chloride probably is the most reactive in water of all of the chlorohydrocarbons reported here. Extrapolation of reported solvolysis rate data to a hydrolysis rate in water at 25° indicated a half-life of ~9 days

Thus there should be no problem of disappearance of the chlorohydrocarbons from water due to reaction rather than evaporation during the relatively short times involved for the evaporation experiments.

A comparison of evaporation half-lives, calculated by the two methods described in this report, with other experimental data has recently been reported (41).

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CLINICAL EVALUATION OF

1,1,1-TRICHLOROETHANE LEVELS IN THE

ADIPOSE TISSUE OF RABBITS

Chemical Name (300 per name) 25	CAS No. (10) 24
1,1,1-TRICHLOROETHANE	71-55-6

A.K 5/31/85

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Lab code letters and report number

R-NO. LAB CODE LETTERS AND REPORT NO.

Division: TEXAS | Department: BIO-MEDICAL | Date: July 3, 1972

Author: T. G. Pullin and S. S. Sutherland, III

Problem Number:

CORP. RES. AND DEV. LAB. NO.

Title

Clinical Evaluation of 1, 1, 1, -Trichloroethane Levels in the Adipose Tissue of Rabbits

Informative summary with conclusions — PATENT DISCLOSURE NO. _____ DISCLOSURE PLANNED? _____

Nine albino New Zealand rabbits were exposed simultaneously in a chamber to 232-362 ppm CHLOROTHENE® in air. A control group was exposed simultaneously in a duplicate chamber to air. Three control and three experimental rabbits were sacrificed at the end of the 72 hour exposure and at 24 and 48 hours post-exposure.

Blood, fat and bone marrow samples were taken from each animal for blood tests, bone marrow counts and CHLOROTHENE® analysis.

The 72 hour exposure fat samples had CHLOROTHENE® concentrations ranging from 316-374 ppm. The CHLOROTHENE® levels in the 24 hour post-exposure samples ranged from 0-75 ppm. No CHLOROTHENE® was found in the control and 48 hour post-exposure samples.

No treatment related effects were detected by evaluations of body weight, blood tests and bone marrow counts.

The results indicated that CHLOROTHENE® is readily absorbed and desorbed from rabbit adipose tissue. No adverse effect on the hematopoietic system of the rabbits was seen after exposure to CHLOROTHENE® in the range of 232-362 ppm up to 72 hours.

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CLINICAL EVALUATION OF 1, 1, 1, - TRICHLOROETHANE LEVELS IN THE ADIPOSE TISSUE OF RABBITS

T. G. Pullin and S. S. Sutherland, III

INTRODUCTION

Numerous investigations have been conducted on the distribution of chlorinated hydrocarbon pesticides in our environment. Many of the studies, such as those by Radomski and Rey (1) centered around the development of procedures for determination of these pesticides in animal and human tissues. Radomski and Fiserova - Bergerova (2) developed a procedure for the determination of pesticides in tissues using the electron capture detector without prior clean-up of the tissues. Investigators, such as Fiserova - Bergerova, et al (3) and Hoffman (4) determined levels of pesticides in human tissues and, more important, improved the understanding of the significance of the chlorinated hydrocarbon pesticide residues in the human body.

There has been little attempt to determine whether chlorinated hydrocarbon solvent residues are present in human adipose tissue; and, if present, the significance of these residues. Thus, this study was undertaken to investigate chlo-

rinated hydrocarbon solvent levels in adipose tissue of animals. The data from this study is preliminary to the ultimate goal of determination of chlorinated hydrocarbon solvent levels in the adipose tissue of man and the relationship of these findings to his state of health.

MATERIALS AND METHODS

A. General Outline of Study

The objective of this study was to determine whether a chlorinated hydrocarbon solvent was absorbed and desorbed in adipose tissue of animals and to relate these findings to clinical data of the animals. This was accomplished by exposing rabbits in chambers to known concentrations of a solvent. Some rabbits were sacrificed and tissue specimens collected at the termination of exposure and others at frequent time intervals following the termination of exposure. Absorption and desorption were then observed by comparing results from the different sampling periods.

B. Selection of Compound Studied

The chlorinated hydrocarbon solvent chosen for this study was CHLOROTHENE® (1,1,1 - trichloroethane). Many of

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the reasons for this choice have been pointed out by Torkelson (5). CHLOROTHENE® has a high lipid solubility, a widespread industrial usage and is readily volatilized. Because of these reasons inhalation of the vapors by production and consumer workmen is of major concern. It is also economically important because of its widespread industrial usage.

C. Analytical Techniques

It was desirable to use analytical methods that required a minimum of sample preparation, gave rapid results and were specific and precise for CHLOROTHENE®. Since the literature was practically non-existent on analytical techniques for CHLOROTHENE® in adipose tissue, the methodology necessary to sample, analyze and assess CHLOROTHENE® in adipose tissue of animals had to be developed.

1. CHLOROTHENE® Determination

The Varian Aerograph Gas Chromatograph, model 2100-20, was used to develop a gas chromatographic procedure to determine CHLOROTHENE® concentrations. The linear ranges of the flame ionization and Ni^{63} electron capture detectors for CHLOROTHENE® were determined as follows:

flame detector: 67 ppb - 670,000 ppm; Ni^{63} detector:

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0.8 ppb - 40 ppb. Due to the low linear range of the Ni^{63} detector, a calibration curve was constructed from 0.8 ppb to 800 ppb. The successful procedure for CHLOROTHENE® determination on this instrument was accomplished using isooctane as the solvent on a column packed with 10% squalane on chromosorb G, 80/100 mesh non-acid washed (NAW). The gas chromatographic parameters for this separation were:

Column size: 6' X 1/8" U shaped glass

Carrier gas: Pre-purified nitrogen, 25 ml/min, 65 psi

Injector port: 110° C

Column Temp: 80° C

Detector: 110° C

Electrometer: In general, electrometer settings were used which gave the maximum usable sensitivity.

Volumn of sample injected: 2 µl.

2. CHLOROTHENE Determination in Adipose Tissue

The method developed by Radomski and Fiserova - Bergerova (2) for the analysis of organic chloride pesticides in adipose tissue was modified and tested for the analysis of CHLOROTHENE® in pig, rabbit, and rat adipose tissue. The tissue surface was

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dried with filter paper and 200 ± 5 mg was weighed. The sample was then homogenized in 10 mls of isooctane containing 0.2 mg CHLOROTHENE® until the tissue was completely dispersed. A control sample was homogenized in 10 mls of isooctane only. The extract was poured into a capped centrifuge tube, and the volume adjusted with isooctane to 10 mls. After centrifuging, the supernatant was analyzed by the gas chromatographic procedure described above to determine if a successful separation could be obtained without prior clean-up. This procedure produced a successful separation of CHLOROTHENE® from pig, rabbit, and rat adipose tissue without prior clean-up. However, the results of the single extraction of the adipose tissue with isooctane (Table 1) showed that it was not sufficient to extract all of the CHLOROTHENE®.

a. Extraction Efficiency. Since the efficiency of the single extraction was insufficient to remove all CHLOROTHENE® from the tissue, repeated extractions were accomplished to determine if 100% recovery of CHLOROTHENE® could be obtained. Standards of CHLOROTHENE® in isooctane were made and added to individual 200 ± 5 mg fat samples. The modified Radomski

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Table 1
Single Extraction of Pig Adipose Tissue with Isooctane

CHLOROTHENE® Added ppm	Recovered %
10	96.8
10	86.3
10	82.1
5	72.8
5	68.7
2.5	86.7

Table 2
Repeated Extractions of Pig Adipose Tissue with Isooctane

	CHLOROTHENE® Added	
	10ppm	2.5ppm
% Recovery 1st Extraction	82.1	86.7
% Recovery 2nd Extraction	4.2	6.7
% Recovery 3rd Extraction	0	0
Total	86.3	93.4

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and Fiserova - Bergerova procedure described above was then used to determine the recovery percentage of the repeated extractions. The results (Table 2) showed that 100% recovery was not acquired.

b. Evaporative losses. It was thought that repeated extractions did not accomplish 100% recovery because of evaporative losses. So, with the objective of reducing these losses, two extractions using chilled materials were tried next. Standards and glassware were chilled, and the homogenization step was accomplished in an ice bath. The homogenate was then allowed to return to room temperature before the analysis was accomplished. Following this analysis, the sample was centrifuged and the supernatant discarded. The sediment was extracted again in 10 mls chilled isooctane, and an analysis was performed on the homogenate. Data from this procedure (Table 3) indicated that it was sufficient to extract all or virtually all of the CHLOROTHENE.

D. Exposure Equipment

The rectangular chambers used in this study were stainless steel and had a capacity of 196 cu. ft. A continuous air flow was maintained through the chamber by means of exhaust fans. Air entering the chambers was first drawn over

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Table 3
Repeated Extractions of Pig Adipose Tissue with Isooctane using Chilled Materials

CHLOROTHENE® Added	% Recovery 1st Extraction	% Recovery 2nd Extraction	Total
10ppm	96.8	2.1	98.9
5ppm	97.0	3.0	100
2.5ppm	93.3	0	93.3

evaporation pans to which a metered stream of CHLOROTHENE® was pumped by means of a Dual Syringe Feeder Pump (Modern Metalcraft, Midland, Michigan). The air - vapor mixture then passed into the chamber at one end, through two sets of baffle plates and out the opposite end. The air flow within the chamber was equilibrated from top to bottom by proper adjustment of the baffle plates.

The feeder pump was used to produce a flow rate of 200cc CHLOROTHENE®/ hour to the evaporation pans of the chamber. This flow rate was calculated to give an acceptable range around the desired 300 ppm CHLOROTHENE® in the chamber.

E. Analysis of the Chamber Atmosphere

1. Preparation of Standards.

A set of standards of CHLOROTHENE® in isooctane was made and analyzed by the gas chromatographic procedure described above. A calibration curve was constructed from the standards and was used to determine the concentration of CHLOROTHENE® in the chamber.

2. Sampling System.

Two liters of the chamber atmosphere were drawn through a scrubber containing 200 mls. of isooctane. A constant

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flow rate of 500 cc chamber atmosphere/min. was maintained through the scrubber by means of a vacuum pump. This flow rate was monitored by a calibrated rotameter. A sample from the scrubber was then analyzed by the gas chromatographic procedure previously described, and the CHLOROTHENE® concentration was determined from the standard calibration curve.

F. Experimental Procedure

Nine albino New Zealand rabbits were exposed simultaneously in the chamber to 232-362 ppm CHLOROTHENE® in air. A second control group was also exposed simultaneously in a duplicate chamber to room air. Three control and three experimental rabbits were sacrificed at the end of the 72 hour exposure and at 24 and 48 hours post-exposure.

Blood, fat and bone marrow samples were taken from each animal for blood tests, bone marrow counts and CHLOROTHENE® analysis. The blood was drawn by cardiac puncture just prior to sacrifice of the animal. The blood tests included complete blood count (CBC), blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalic transaminase (SGOT), alkaline phosphatase, total protein and chol-

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esterol. Pre- and post-exposure weights were recorded on each animal.

RESULTS AND DISCUSSION

The analysis of the 72 hour exposure fat samples showed CHLOROTHENE® concentrations ranging from 316-374 ppm. However, the CHLOROTHENE® concentrations in the 24 hour post-exposure fat samples ranged from 0-75 ppm. After 48 hours post-exposure no CHLOROTHENE® was found in the fat samples. Analysis of all control fat samples revealed no concentrations of CHLOROTHENE®.

No treatment-related effects of the experimental and control animals were detected by evaluations of blood tests (Table 4) and bone marrow counts (Table 5).

The body weights of the animals are shown in Table 6. The results indicated that CHLOROTHENE is readily absorbed into and desorbed from rabbit adipose tissue. However, detrimental effects from CHLOROTHENE® concentration in the adipose tissue were not implied by evaluation of body weight, blood tests and bone marrow counts.

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TABLE 4

BLOOD TESTS OF CONTROL AND EXPERIMENTAL RABBITS EXPOSED TO CHLOROTHENE®

DOSE:	316 - 374 ppm. CHLOROTHENE®								
Animal No.	1-1	2-1	3-1	1-2	2-2	3-2	1-3	2-3	3-3
Hours Following Test Termination	0	0	0	24	24	24	48	48	48
BLOOD COUNT									
Hemoglobin	14.1	12.0	12.9	13.1	13.3	12.4	12.4	13.7	12.4
Hematocrit	42	37	39	40.5	39	37	37	41.5	39
WBC	6,600	9,300	8,100	11,100	6,300	5,200	6,600	7,700	9,800
Neutrophils	24	15	13	32 31/1	36	28	36	36	20
Lymphocytes	75	79	87	67	62	71	53	61	77
Monocytes	0	0	0	1	2	0	4	1	2
Eosinophils	1	6	0	0	0	0	4	2	1
Basophils	0	0	0	0	0	1	3	0	0
RBC X 10^6	5.76	4.83	5.41	6.0	6.12	5.73	5.9	6.58	6.22
BUN	19.6	19.6	13.5	24.0	26.5	22.0	18.5	13.5	18.5
SGOT	18	18	22	40	26	37	22	37	18
SGPT	68	30	60	46	104	80	60	46	40
ALKALINE PHOSPHATASE	2.0	1.8	3.0	2.0	3.9	2.6	2.4	2.4	3.3
TOTAL PROTEIN	7.2	6.0	6.0	7.0	5.2	5.0	6.0	5.2	5.0
CHOLESTEROL	57.6	82	57.5	72.5	57.5	.65	82	41	72.5

TABLE 4 (Continued)

DOSE:	CONTROL							
Animal No.	4-2	5-1	6-1	4-3	5-2	6-2	5-3	6-3
Hours Following Test Termination	0	0	0	24	24	24	48	48
BLOOD COUNT								
Hemoglobin	12.9	13.3	12.4	12.9	9.2	12.4	12.9	13.0
Hematocrit	40	43	38	39.5	29	37.5	40	40.5
WBC	11,200	7,800	10,600	9,300	CL	9,200	7,500	9,300
Neutrophils	31	7	55	30	14	39	10	24
Lymphocytes	62	87	42	66	83	55	83	74
Monocytes	0	1	0	0	2	1	2	0
Eosinophils	6	5	2	2	1	5	2	1
Basophils	1	0	1	2	0	0	2	1
RBC X 10^6	6.23	6.0	5.19	5.89	CL	5.51	6.3	6.41
BUN	20.6	30	17.5	17.5	23.0	22.0	17.5	14.5
SGOT	40	40	25	14	26	37	14	55
SGPT	60	104	40	80	68	50	18	50
ALKALINE PHOSPHATASE	2.7	2.9	2.4	2.2	2.2	2.6	2.5	2.1
TOTAL PROTEIN	6.0	6.0	6.6	6.0	5.8	5.2	6.4	6.0
CHOLESTEROL	55	50	45	57.5	57.5	39	50	55

Animal 4-1 expired 25 hours after exposure due to dehydration.

CL - Clotted blood: unable to perform test.

TABLE 5

BONE MARROW COUNTS OF CONTROL AND EXPERIMENTAL RABBITS EXPOSED TO CHLOROTHENE

DOSE:	CONTROL							
Animal No.	4-2	5-1	6-1	4-3	5-2	6-2	5-3	6-3
Hours Following Test Termination	0	0	0	24	24	24	48	48
CELL TYPES								
Mitotic	0.4	0	0	0	0	0	0	0
Primitive	0	0	0	0.4	0.6	0	0	0
Blasts	1.8	0.8	2.0	3.2	4.4	1.2	4.0	1.2
Promyelocytes	0.4	0.4	2.0	0.8	0.8	0.8	0	1.6
Myelocytes	2.5	2.8	3.2	2.8	1.6	2.4	1.2	1.2
Metamelocytes	2.5	2.8	2.4	4.0	3.2	2.8	3.2	1.2
Band	5.8	7.2	11.6	10.0	8.8	10.0	7.6	5.6
Filamented	23.3	19.2	23.6	35.6	24.0	35.6	29.2	28.8
Lymphocytes	5.8	4.8	5.6	6.8	6.4	6.4	5.2	5.2
Plasma	0.4	0	1.6	0	0	0.4	0.4	0
Monocytes	0	0.4	0.4	0.8	0.4	0	1.2	0.4
Megakaryocytes	0	0	0	0	0	0	0	0.4
Double nucleated	0	0	0	0	0	0	0	0
Megaloblasts	0.4	0.4	0.8	0.8	0.4	0	2.4	0.4
Erythroblasts	2.1	8.4	6.0	5.6	4.0	4.4	9.2	6.4
Normoblasts	54.6	52.8	40.8	29.2	45.6	36.0	36.4	47.6

TABLE 5 (Continued)

DOSE:	316 - 374 ppm. CHLOROTHENE®								
Animal No.	1-1	2-1	3-1	1-2	2-2	3-2	1-3	2-3	3-3
Hours Following Test Termination	0	0	0	24	24	24	48	48	48
CELL TYPES									
Mitotic	0	0.4	0	0	0	0	1.2	0	0
Primitive	0	0	0	0	0.8	0	0	0.4	0.4
Blasts	2.0	0.8	0.8	1.2	0.8	1.6	2.8	1.6	2.0
Promyelocytes	1.6	0.8	1.6	1.6	0.4	2.4	1.2	0.8	1.2
Myelocytes	0.8	2.4	2.4	1.6	1.2	1.2	2.4	4.0	2.4
Metamyelocytes	2.0	2.4	2.0	2.8	2.0	3.2	2.4	3.2	2.8
Band	3.6	8.8	8.4	6.8	4.4	4.4	8.4	2.4	6.3
Filamented	19.6	20.4	8.0	24.0	25.6	33.6	28.0	28.4	24.8
Lymphocytes	4.4	7.2	6.4	2.8	4.0	6.8	9.2	4.4	4.8
Plasma Cells	0.8	1.6	0.4	0	0.8	0.4	0.8	0.4	0
Monocytes	0	0.4	0	0.4	0	0.4	0.4	0.4	1.2
Megakaryocytes	0.4	0	0	0	0	0.4	0.4	0.4	0
Double nucleated	0	0.4	0	0	0	0	0	0	0
Megaloblasts	0.4	0	0.4	1.2	0.4	0.4	0.4	1.2	1.6
Erythroblasts	5.6	5.6	4.0	6.4	11.2	6.4	6.8	9.6	8.4
Normoblasts	58.8	48.8	55.6	51.2	48.4	38.8	35.6	42.8	43.6

TABLE 6

BODY WEIGHTS OF CONTROL AND EXPERIMENTAL RABBITS EXPOSED TO CHLOROTHENE®

	Animal No.	Weight in Grams				Change	
		Pre-Exposure	1 Hour Post Exposure	24 Hours Post Exposure	48 Hours Post Exposure	GMS.	%
	1-1	2975	2873	-	-	-102	3.4
	2-1	3140	3136	-	-	-6	0.2
	3-1	2730	2738	-	-	+8	0.3
16 - 374	1-2	3120	3017	3008	-	-112	3.6
ppm	2-2	2490	2530	2598	-	+106	4.3
HLOROTHENE®	3-2	2464	2470	2557	-	+93	3.8
	1-3	2908	2840	2944	2986	+78	2.7
	2-3	2612	2624	2677	2694	+82	3.1
	3-3	2715	2757	2828	2924	+209	7.8
	4-2	2090	2180	-	-	+90	4.3
	5-1	2943	3091	-	-	+148	5.0
	6-1	2864	2801	-	-	-63	2.2
ONTROL	4-3	2364	2365	2473	-	+109	4.6
	5-2	3110	2960	3125	-	+15	0.5
	6-2	3400	3165	3128	-	-272	8.0
	5-3	2987	2994	3089	3063	+76	2.5
	6-3	3360	3209	3434	3485	+125	3.7

SUMMARY

Nine albino New Zealand rabbits were exposed simultaneously in a chamber to 232-362 ppm CHLOROTHENE® in air. A control group was exposed simultaneously in a duplicate chamber to air. Three control and three experimental rabbits were sacrificed at the end of the 72 hour exposure and at 24 and 48 hours post-exposure.

Blood, fat and bone marrow samples were taken from each animal for blood tests, bone marrow counts and CHLOROTHENE® analysis.

The 72 hour exposure fat samples had CHLOROTHENE® concentrations ranging from 316-374 ppm. The CHLOROTHENE® levels in the 24 hour post-exposure samples ranged from 0-75 ppm. No CHLOROTHENE® was found in the control and 48 hour post-exposure samples.

No treatment related effects were detected by evaluations of body weight, blood tests and bone marrow counts.

The results indicated that CHLOROTHENE® is readily absorbed and desorbed from rabbit adipose tissue. No adverse effect on the hematopoietic system of the rabbits was seen after exposure to CHLOROTHENE® in the range of 232-362 ppm up to 72 hours.

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MIDLAND, MICHIGAN

DEPARTMENT: Chemicals Laboratory

DATE: 3-10-7[illegible]

AUTHOR: Corwin J. Bredeweg

TITLE: PHOTOCHEMICAL REACTIVITY OF TRICHLOROETHYLENE AND OTHER CHLORINATED SOLVENTS IN THE PRESENCE OF OTHER ORGANIC MATERIALS IN A SIMULATED

INFORMATIVE SUMMARY WITH CONCLUSIONS

ATMOSPHERIC ENVIRONMENT AS RELATED TO AIR POLLUTION

The presence of other organic materials and nitric oxide reduces the photochemical reactivity of trichloroethylene. It is postulated from the data that in a real atmosphere, e.g., over Los Angeles, where the ratio of organic contaminants to trichloroethylene is quite large, the photochemical smog symptoms resulting from trichloroethylene should be quite negligible. Vinyl chloride, vinylidene chloride and cis-dichloroethylene appear to behave like trichloroethylene whereas the photochemical reactivity of methylene chloride and methylchloroform are not significantly affected by the presence of other organic materials. Only trichloroethylene increases the photochemical reactivity of perchloroethylene.

The change in photochemical reactivity for hydrocarbons in mixtures with only hydrocarbons is generally less than that experienced for trichloroethylene.

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I. INTRODUCTION

In an earlier report[1] an attempt was made to determine the contribution of chlorinated solvents to photochemical smog formation when these solvents were subjected to a simulated atmospheric environment which contained oxides of nitrogen. There were indications that stabilized formulations of trichloroethylene were less reactive than unstabilized trichloroethylene which suggests that the stabilizers became involved in the photochemical oxidation process. Altshuller[2] has also reported that the presence of trans-2-butene with 1-butene and NO_2 caused an increase in photochemical reactivity of 1-butene whereas the presence of mesitylene with 1-butene and NO_2 caused a decrease in reactivity of the 1-butene. Since the order of increasing photochemical reactivities of these materials by themselves with NO_2 is mesitylene < 1-butene < trans-2-butene, Altshuller concluded that leveling effects should exist when combinations of materials are present, e.g., organic contaminants in the atmosphere. That is, the presence of slow-reacting hydrocarbons will decrease the reactivity of the faster reacting hydrocarbons and conversely, the presence of fast-reacting hydrocarbons will increase the reactivity of slower-reacting hydrocarbons. However, the reactivity of the second component in these reactions with 1-butene is unknown.

It was, therefore, of interest to determine how the photochemical reactivity of the chlorinated solvents, particularly trichloroethylene, is affected by the presence of other organic materials. This type of data is important in evaluating how the chlorinated solvents, as well as other materials, behave in a real atmosphere which contains a mixture of slow- and fast-reacting components.

Notably, most solvent regulations, such as in Los Angeles, suffer a deficiency in that they have been based on experimental data which involved studies on reactivities of one component at a time rather than competitive types of reactions.

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II. EXPERIMENTAL

The design of the photochemical smog chamber and the reaction conditions as well as the analytical techniques have been previously described.[1] Briefly, a typical run in the chamber involving a single component is carried out as follows: The chamber is evacuated to ca. 0.2 mm and charged with 47.9 µl NO (5 ppm), 90 µl H_2O (35-40% relative humidity), pure air and 0.34 µl trichloroethylene (10 ppm). The chamber temperature was maintained at 26-28° and the sunlight was simulated with RS-275 watt sunlamps. The rate of reaction or solvent disappearance was determined by VPC. The data are given as solvent half-life or the percent that reacted in a given period of time. Lightly stabilized trichloroethylene (HI-TRI® - which contains 20 ppm diisopropylamine) was employed in this study. In most cases the ratio of organic materials to NO was kept near 2:1 because the highest reactivity generally occurs at this ratio.[3]

All data concerning solvent reactivity alone came from single component experiments which were previously reported.[1] These data are sometimes referred to as "normal" reactivities. The photochemical reactivity data determined from combination experiments are generally compared with the "normal" reactivity data of each component in order to determine if either component experienced any change in reactivity rate.

III. RESULTS AND DISCUSSION

1. Effect of Photochemical Reactivity of Trichloroethylene in Combination with Other Organic Materials

The photochemical reactivity of trichloroethylene has been investigated in combination with other organic materials with a wide range of reactivities, as shown in Table I. In all combina-

tions, except with methylchloroform and possibly perchloroethylene, the photochemical reactivity of trichloroethylene has decreased. In the extreme case with cyclohexane, the reactivity of trichloroethylene has been reduced by as much as 2 1/2 times. In fact, the reactivity of trichloroethylene with cyclohexane is about one-half that of cyclohexane in the combination experiments and is somewhat less than that for the "normal" reactivity of cyclohexane.

It should also be noted that in nearly all cases, the photochemical reactivity of the other organic materials are substantially increased over that of their "normal" reactivity. In no case is there a reduction in reactivity; however, with ethylene, cyclohexene, ethyl acetate and isobutyl alcohol, there is essentially no change. The most dramatic changes in reactivity of the other organic material appears to occur with perchloroethylene, butylene oxide, t-2-butene and n-butyl alcohol.

Thus in combination or in competitive experiments, it can be shown that materials such as trichloroethylene, which are supposedly reactive and controlled by Los Angeles air pollution standards, are less reactive than materials, such as cyclohexane or n-butyl alcohol, which are supposedly unreactive by Los Angeles standards. Thus, in an atmosphere which contains a wide variety of reactive materials, it cannot be stated or determined, particularly where trichloroethylene is involved, which organic material(s) are responsible for photochemical smog production by investigating only single component systems. On the other hand, it must be recognized that if a material such as trichloroethylene acts as a catalyst in causing other materials to produce photochemical smog, then the catalyst or trichloroethylene could still be considered a bad offender.

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TABLE I

PHOTOCHEMICAL REACTIVITY OF TRICHLOROETHYLENE IN COMBINATION WITH OTHER MATERIALS[a]

Solvent	Solvent Reactivity Alone[b]	Solvent Reactivity Combination[b]	$CHCl=CCl_2$ Reactivity[b]
None	——	——	3.5
CH_3CCl_3	<5% (23.5 hr.)	13% (16.9 hr.)	~3.5
$CCl_2=CCl_2$	44% (18.5 hr.) [14.2][c]	5	2-3.5
$CH_2=CH_2$	2.9	2.5	4.5-5
Dioxane	3.4	2.4	~6.0
MEK	33% (6 1/2 hr.) [9.9][c]	7.4	5.8
Cyclohexone	0.87	0.82	6.1
EtOAc	24% (7 hr.) [14.6][c]	12.5	6.1
CH_3OH	——	42% (7 hr.) [8.3][c]	7.0
Butylene oxide	47% (14.9 hr.) [15.9][c]	6.4	7.0
Toluene	6.75	5.2	7.6
Iso-BuOH	3.5	3.3	~7.0
t-2-Butene	0.3	0.16	30% (5 hr.) [8.3][c]
n-BuOH	6.5	3.0	~6.5
Cyclohexane	6.9	4.9	8.7

(a) For solvent reactivity alone, the chamber was charged with 10 ppm solvent, 5 ppm NO and 35% R.H. in air. For reactivity of solvents in combinations, the chamber was charged with 10 ppm HI-TRI®, 10 ppm solvent, 10 ppm NO and 35% R.H. in air.

(b) Rates are given as half-lives in hours or as the percent which reacted in a given period of time.

(c) Estimated $t_{1/2}$

F. Effect of Photochemical Reactivity of Methylene Chloride, Methyl Chloroform and Perchloroethylene in Combination with Other Organic Materials

Since the reactivity of trichloroethylene is significantly affected by the presence of other organic materials, it was of interest to determine if the photochemical reactivity of other chlorinated solvents, such as methylene chloride, methylchloroform and perchloroethylene are similarly affected. As illustrated in Table II, combinations were chosen which involved both fast and slow reacting materials.

In general, the reactivity of these solvents as well as the other organic materials is not appreciably affected. In only two cases, the combinations of CH_2Cl_2 with cyclohexane and CH_3CCl_3 with ethylene, there is the possibility of some interaction. However, in both cases the reactivity of only one of the solvents is affected whereas the other remains "normal." This could be experimental error, but if it is real, the increased reactivities of the chlorinated solvents are still very small and quite insignificant, i.e. compare reactivities with a slow reactor such as cyclohexane alone.

Although perchloroethylene is not affected by the materials listed in Table II, it becomes considerably more reactive when trichloroethylene is present (see Table I).

Since these three chlorinated solvents are already quite unreactive by themselves, a reduced reactivity is not expected to be observed or detected in competitive experiments as was the case for a more reactive material such as trichloroethylene. Although an increase in reactivity is possible, it is generally not observed.

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TABLE II

REACTIVITY OF METHYLENE CHLORIDE, METHYL CHLOROFORM AND PERCHLOROETHYLENE IN COMBINATION WITH OTHER MATERIALS[a]

Solvent	CH_2Cl_2[b, c]	CH_3CCl_3[b, c]	$CCl_2=CCl_2$[b, c]
None	<5% (21 hr.)	<5% (23 1/2 hr.)	38% (6 1/3 hr.)
Cyclohexane	~13% (6 3/4 hr.)	~5% (7 hr.)	26% (6 1/2 hr.)
Ethylene	<5% (5 1/2 hr.)	11% (5 3/4 hr.)	32% (6 hr.)
t-2-Butene	<5% (2 1/2 hr.)	<5% (2 hr.)	29% (6 2/3 hr.)

Solvent	Cyclohexane[b, c]	$CH_2=CH_2$[b, c]	t-2-Butene[b, c]
None	50% (7 hr.)	50% (4 hr.)	50% (~15 min.)
CH_2Cl_2	50% (6 3/4 hr.)	50% (3 1/2 hr.)	50% (13 min.)
CH_3CCl_3	39% (7 hr.)	50% (3 1/2 hr.)	50% (12 min.)
$CCl_2=CCl_2$	43% (6 1/2 hr.)	50% (3 7/8 hr.)	50% (15 min.)

(a) See footnote a of Table I.

(b) See footnote b of Table I.

(c) Rates listed are for those materials at the head of the column. The competitive material is at the left under the solvent heading.

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3. Effect of Photochemical Reactivity of Ethylene, t-2-butene and Cyclohexene in Combination with Other Organic Materials

Since the photochemical reactivity of trichloroethylene was significantly reduced by the presence of a wide variety of organic materials, it was of interest to determine if other olefinic materials would experience similar results. Ethylene, which is normally moderately reactive, shows a slight reduced photochemical reactivity in combination with a wide variety of materials (see Table III). The reactivity of the other organic materials in conjunction with ethylene generally increased, particularly in the case of n-butyl alcohol. However, with t-2-butene, no change is observed.

The photochemical reactivity of t-2-butene, which is very reactive by itself, is only slightly changed, if any, by the presence of other organic materials (see Table IV). In contrast, the reactivity of the competitive materials have changed, e.g., n-butyl alcohol and cyclohexene increase in reactivity while ethylene is slightly reduced.

In a competitive experiment involving cyclohexene and toluene, only the reactivity of cyclohexene appears to be slightly changed (see Table IV).

4. Effect of the Photochemical Reactivity of Cyclohexane in Combination with Other Organic Materials Including All of the Chlorinated Ethylenes

In competitive experiments of cyclohexane with methyl-chloroform, methylisobutylketone (MIBK) or ethylene, some minor variations appear in the photochemical reactivities with the most significant change being with ethylene (see Table V).

When the chlorinated ethylenes are involved in competitive experiments with cyclohexane, some significant changes are observed (see Table V). In all cases excepting perchloroethylene,

TABLE III

PHOTOCHEMICAL REACTIVITY OF ETHYLENE IN COMBINATION WITH OTHER MATERIALS[a]

Solvent	Solvent Reactivity Alone[b]	Solvent Reactivity Combination[b]	$CH_2=CH_2$ Reactivity[c]
None	———	———	[illegible]
Butylene oxide	47% (14.9 hr.) [15.9][c]	12.4	[illegible]
n-BuOH	6.5	3.8	3.
Cyclohexane	6.9	6.1	4.1
Cyclohexene	0.87	0.73	4.1
t-2-Butene	0.3	0.37	31% (3.0 hr.) [3.3][c]

a. See footnote a of Table I.
b. See footnote b of Table I.
c. Estimated $t_{1/2}$.

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TABLE IV

PHOTOCHEMICAL REACTIVITY OF t-2-BUTENE AND CYCLOHEXENE IN COMBINATION WITH OTHER MATERIALS[a]

Solvent	Solvent Reactivity Alone[b]	Solvent Reactivity Combination[b]	t-2-Butene Reactivity[b]
None	---	---	0.30
$CH_2=CH_2$	2.9	315 (2.9 hr.) [3.2][c]	0.37
Cyclohexene	0.87	0.37	0.37
n-BuOH	6.5	344 (2.9 hr.) [4.1][c]	0.32

Solvent	Solvent Reactivity Alone[b]	Solvent Reactivity Combination[b]	Cyclohexene Reactivity
None	---	---	0.87
Toluene	6.8	6.8	1.0

a. See footnote a of Table I.
b. See footnote b of Table II.
c. Estimated $t_{1/2}$.

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TABLE V

PHOTOCHEMICAL REACTIVITY OF CYCLOHEXANE IN COMBINATION WITH OTHER MATERIALS INCLUDING CHLORINATED ETHYLENES[a]

Solvent	Solvent Reactivity Alone[b]	Solvent Reactivity Combination[b]	Cyclohexane Reactivity[b]
---	---	---	6.9
CH_3CCl_3	<5% (23.5 hr.)	<5% (7 hr.)	39% (7 hr.)
MIBK	3.5	3.9	8.3
$CH_2=CH_2$	2.9	4.1	6.1
---	---	---	---
$CH_2=CCl_2$	2.1	3.8	5.3
$CH_2=CHCl$	4.3	5.5	5.4
CHCl=CHCl (cis)	3.0	13.1	5.4
$CHCl=CCl_2$	3.5	8.7	4.9
$CCl_2=CCl_2$	38% (6 1/3 hr.)	26% (6 1/3 hr.)	43% (6 1/3 hr.)

a. See footnote a of Table I.

b. See footnote b of Table I.

the photochemical reactivity of cyclohexane is increased, while that of the chlorinated ethylenes is decreased. Tetrachloroethylene is more than four times less reactive than "normal."

5. Photochemical Reactivity of DOWANOL® PM in Combination With Chlorinated Solvents

The combination of DOWANOL PM and chlorinated solvents has been considered as a base formulation for the "Vapor Reflow Process." This process is used by General Motors for repairing scratched painted surfaces. The photochemical reactivity of DOWANOL PM may be slightly increased, if any, in the presence of methylene chloride or trichloroethylene (see Table VI). Methylene chloride seems unaffected by the DOWANOL PM, while trichloroethylene is greatly reduced in reactivity.

6. Effect of the Photochemical Reactivity of Trichloroethylene in Combination with a Real "Polluted" Atmosphere

A real atmospheric environment was simulated by adding trichloroethylene to a chamber containing a number of materials with varying reactivities (see Table VII). It was again observed that many of the reactivities had changed from their "normal" values. Trichloroethylene, in particular, was reduced in reactivity by more than a factor of three. It was also interesting to note that the reactivities of cyclohexane and t-2-butene were identical to that found in a simple competitive experiment (see Table IV).

7. Effect of the Trichloroethylene Cyclohexane Ratio on the Photochemical Reactivity of the Two Solvents

Trichloroethylene can reasonably be expected, under real atmospheric situations, to exhibit concentration variations with

TABLE VI

PHOTOCHEMICAL REACTIVITY OF DOWANOL PM IN COMBINATION WITH CHLORINATED SOLVENTS[a]

Solvent	Solvent Reactivity Alone[b]	Solvent Reactivity Combination[b]	DOWANOL PM Reactivity[b]
None	—	—	3.1
CH_2Cl_2	<5% (21 hr.)	0% (7 hr.)	2.6
$CHCl{=}CCl_2$ (HI-TRI®)	3.5	32% (6.7 hr.)	2.5

a. See footnote a of Table I.

b. See footnote b of Table I.

TABLE VII

PHOTOCHEMICAL REACTIVITY OF TRICHLOROETHYLENE IN COMBINATION WITH A WIDE VARIETY OF ORGANIC MATERIALS

t-2-Butene + + $CH_2{=}CH_2$ + Butylene oxide + $CHCl{=}CCl_2$ + NO $\frac{hv}{35\%}$ R.H.

ppm	10	10	10	10	10	25
Norm. React. $t_{1/2}$ (hr.)	0.30	0.87	2.9	~15	3.5	
Actual React. $t_{1/2}$ (hr.)	0.35	0.35	3.5	12.9	11.1	

other organic materials ranging from near 1 (at the ozonation point for trichloroethylene) to near zero (some distance downwind from the ozonation point).

In order to demonstrate what might happen to the photochemical reactivity of trichloroethylene in this environment, cyclohexane (an unreactive compound) was chosen as a model organic contaminant in a trichloroethylene atmosphere. By holding the NO concentration constant, it was found that the half-life of trichloroethylene was increased from 3.5 hr. (only trichloroethylene present) to more than 20 hr. [ratio of trichloroethylene to cyclohexane is 0.3 (see Table VIII)]. A half-life of 20 hr. or more indicates that the trichloroethylene would be very unreactive, much less than cyclohexane ($t_{1/2}$ = 6.9 hr.). In contrast, the photochemical reactivity of cyclohexane was found to increase markedly from $t_{1/2}$ of 6.9 hr. to < 1 hr. as the concentration of cyclohexane was decreased (relative to trichloroethylene). A half-life of less than 1 hr. indicates that the cyclohexane is very reactive, approaching that of olefinic materials.

The photochemical reactivity of each component in the trichloroethylene-cyclohexane system is also dependent on the NO concentration. In comparing the two sets of experiments involving 20 ppm total organic with 5 and 10 ppm NO and 12 ppm total organic with 5 and 10 ppm NO, the former set exhibits slower overall reactivity for both components (see Table VIII). In fact, at the 5 ppm NO level, trichloroethylene appeared to cease reacting suggesting that all the NO or NO_2 had been consumed.

8. Effect of the Ratio of Trichloroethylene to Butylene Oxide on the Photochemical Reactivity of the Two Solvents

In a series of experiments similar to those described in Section 7, butylene oxide (which has a very low "normal" reactivity) was used as a competitive material with trichloroethylene at various ratios. Similarly, a trend was observed where the photochemical

TABLE VIII

PHOTOCHEMICAL REACTIVITY OF DIFFERENT RATIOS OF TRICHLOROETHYLENE TO CYCLOHEXANE

$CHCl=CCl_2$/ (ppm)	NO (ppm)	$t_{1/2}$ $CHCl=CCl_2$ (hours)	$t_{1/2}$ (hours)
0/10 = 0	5	----	6.9
2/10 = .2	5	19-39(a)	6.7
10/10 = 1	5	>>16(a)	5.5
[10/10 = 1	10	8.7	4.9]
10/2 = 5	5	5.6	2.2
10/.2 = 50	5	3.5	1.3
10/.1 = 100	5	2.7	<0.9
10/.01 = 1,000	5	3.3	----
10/0 = ∞	5	3.5	----
10/10	10	8.7	4.9
10/10	5	>>16(a)	5.5
10/2	10	6.6	2.8
10/2	5	5.6	2.2

(a) Estimated

reactivity of trichloroethylene decreased as its concentration decreased and the photochemical reactivity of the butylene oxide increased as its concentration decreased (see Table IX).

TABLE IX

PHOTOCHEMICAL REACTIVITY OF DIFFERENT RATIOS OF TRICHLOROETHYLENE TO BUTYLENE OXIDE

$CHCl=CCl_2$/ Butylene Oxide (ppm)	NO (ppm)	$t_{1/2}$ $CHCl=CCl_2$ (hours)	$t_{1/2}$ Butylene Oxide (hours)
0/10 = 0	5	---	473 (14.9 hr.) [15.9](a)
10/10 = 1	10	7.0	6.42
10/2 = 5	5	4.2	4.08
10/0 = ∞	5	3.5	---

(a) Estimated $t_{1/2}$

IV. CONCLUSIONS

(1) In the presence of other organic materials, the photochemical reactivity of trichloroethylene itself as well as the organic materials is affected in comparison to "normal" reactivities of these materials.

(2) In competitive experiments, the photochemical reactivity of trichloroethylene is reduced by as much as 2 1/2 times (e.g., the case with cyclohexane) while the photochemical

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reactivity of the competitive material is generally increased by as much as a factor of two (e.g., the case with butylene oxide, t-2-butene and n-butyl alcohol).

(3) If the ratio of trichloroethylene to the organic materials (cyclohexane) is <1, trichloroethylene becomes quite unreactive and the reactivity of the organic material is not significantly affected. It follows that in a real atmosphere (e.g., over Los Angeles) where the ratio of organic contaminants to trichloroethylene is quite large, the photochemical smog symptoms resulting from trichloroethylene should be quite negligible.

(4) If the ratio of trichloroethylene to the other organic materials (cyclohexane) is >1, trichloroethylene appears to behave as a catalyst in causing an increased rate of decomposition of the organic materials. In this case, trichloroethylene should be considered as a bad offender; however, for the most part, this situation is not typical.

(5) Vinyl chloride, vinylidene chloride and cis-dichloroethylene behave like trichloroethylene.

(6) Only trichloroethylene increases the photochemical reactivity of perchloroethylene. No other organic material was found to alter its reactivity.

(7) The photochemical reactivities of methylene chloride and methylchloroform are not significantly affected by the presence of any other organic materials.

(8) The photochemical reactivities of ethylene, t-2-butene, cyclohexene and cyclohexane are generally less affected by other organic materials, excluding the chlorinated ethylenes, than that experienced with trichloroethylene.

(9) There is some indication of leveling effects[3] when only hydrocarbons are involved. When trichloroethylene is one

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of the components in the combination experiments, the leveling effect is not an important factor, whereas other catalytic effects may be present.

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VI. INDEX HEADINGS

(1) Photochemical reactivity
(2) Photochemical smog
(3) Air pollution
(4) Trichloroethylene with other org. materials, photochem. react. of
(5) Methylene chloride " " " " " " "
(6) Methylchloroform " " " " " " "
(7) Perchloroethylene " " " " " " "
(8) Ethylene " " " " " " "
(9) t-2-Butene " " " " " " "
(10) Cyclohexene " " " " " " "
(11) Cyclohexane " " " " " " "
(12) Vinyl chloride " " " " " " "
(13) Vinylidene chloride" " " " " " "
(14) cis-Dichloroethylene" " " " " " "
(15) DOWANOL PM " " " " " " "

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EVALUATION OF TERATOGENICITY AND NEUROTOXICITY WITH MATERNAL INHALATION EXPOSURE TO METHYL CHLOROFORM PREPARED BY DEPT OF ENVIR HEALTH UNIV OF CINCINNATI

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METHYL CHLOROFORM	71-55-6

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EVALUATION OF TERATOGENICITY AND NEUROTOXICITY WITH MATERNAL INHALATION EXPOSURE TO METHYL CHLOROFORM

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ABSTRACT

Female Long-Evans rats were exposed by inhalation to 2100 ± 200 ppm of methyl chloroform (MC) to determine whether exposure before mating and during pregnancy was more detrimental to the offspring than exposure either before mating or during pregnancy alone. Four groups were exposed for two weeks before mating and through day 20 of gestation in a two by two factorial design: (1) MC exposure both before and during pregnancy; (2) MC before mating alone; (3) MC during pregnancy alone, and; (4) filtered air both before and during pregnancy. At term, half of each group was sacrificed and assessed for maternal toxicity, embryotoxicity and teratogenicity while the other half delivered their young for later behavioral and carcinogenicity evaluation.

No significant differences were found in measurements of maternal toxicity or embryotoxicity except for a decrease in fetal body weight in dams exposed during pregnancy alone. A significantly increased incidence of skeletal and soft tissue variations were seen in fetuses from the group exposed both before mating and during pregnancy. These variations in fetal morphology were indicative of developmental delay rather than true malformations.

Postnatal evaluation included body weight and survival measurements, and neurobehavioral tests of open field activity (21 days of age), running wheel activity (40-110 days of age), and an amphetamine challenge test (110-120 days of age). All surviving offspring were autopsied at 12 months of age and examined for gross lesions. No significant treatment effects were ascertained with any of these measurements. We conclude that inhalation exposure of female rats before mating and/or during pregnancy to 2100 ± 200 ppm of MC did not exert a persistent detrimental effect on any of the parameters measured. Reversible

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developmental delays were observed in fetal body weight with pregnancy exposure only, and in fetal morphology with exposure before mating and during pregnancy, which had no influence on postnatal outcome.

INTRODUCTION

Methyl chloroform (1,1,1-trichloroethane, MC) is a saturated, lipophilic, trichlorinated hydrocarbon with a molecular formula of CH_3CCl_3. It has enjoyed increasing popularity as a solvent in recent years because of its reported low toxicity (Stewart, 1968). In 1976, the United States produced 630 million pounds of MC primarily for cold-cleaning, dip-cleaning, and bucket-cleaning of metals to remove grease, oil and wax (Parker et al., 1979). A secondary, but rapidly increasing use, is as the propellant in aerosol products for medicinal and household purposes (Kyle et al., 1976). The current Federal occupational exposure limit for MC is 350 ppm (1.9 mg/l) determined as a time-weighted average for eight hour exposure period (NIOSH, 1976).

Methyl chloroform has adverse effects on several organ systems. A functional depression of the central nervous system occurs, typical of anesthetic gases, that is proportional to the level of exposure (Stewart, 1968). Mild liver dysfunction has been observed in laboratory animals when MC was administered at near-lethal doses (Klaassen and Plaa, 1969). In a study conducted by Prendergast et al. (1967), 30 day repeated 8 hr. exposures (2300 ppm) and 90 day continuous exposures (370 ppm) were performed with rats, guinea pigs, rabbits, and dogs. Gross and histopathologic examinations did not reveal any abnormalities attributable to these treatments.

Humans exposed to 900-1000 ppm experienced transient, mild eye irritation and prompt, though minimal, impairment of coordination (Stewart et al., 1961; Torkelson, et al., 1958). Above 1700 ppm, obvious disturbances of equilibrium in humans have been observed (Stewart et al., 1961). Exposures of this magnitude also can induce headache and exhaustion. There have been eleven reported

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fatalities among workers exposed in solvent tanks (Avlado et al., 1976), where the concentration of MC was estimated to be as high as 5000 ppm. Concern has also been expressed over misuse of the aerosols in the home, where concentrations may reach 10,000 ppm in small, poorly ventilated areas, such as closets.

Some of these uses may result in the exposure of women of child bearing age, both at home and in the workplace. In an epidemiologic study by Kramer et al. (1978) peak concentrations were found to occasionally exceed 1000 ppm in a textile plant, where the solvent was used mainly by women for general cleaning. MC was one of several solvents tested for teratogenic potential by Schwetz et al. (1975). Pregnant mice and rats were exposed by inhalation for 7 hrs/day on days 6-15 of gestation to 675 ppm of MC. At term, the dams were sacrificed and the fetuses collected. Evaluation of maternal toxicity, embryotoxicity and teratogenicity all produced negative results. The only significant finding was an increase in the absolute liver weight of maternal rats but not mice, exposed to MC.

The short-term exposure period utilized in the Schwetz et al. (1975) study is typical of the conventional approach used in teratology studies. However, this exposure period may not be adequate to predict the teratologic potential of compounds which are metabolized by mixed function oxidase enzymes to reactive metabolites which could accumulate. Most of the parent compound is eliminated unchanged via the lung but a small percentage is metabolized by these enzymes to trichloroethanol and trichloroacetic acid (Stewart, 1960). These reactive metabolites could accumulate with repeated exposure producing consequences previously undetected.

One aim of the present study was to determine whether subchronic exposure of maternal animals to 2100 ppm of MC before mating as well as throughout

pregnancy was more detrimental to the embryo than exposure either before or during pregnancy. This sub-chronic paradigm more closely mimics the occupational exposure pattern of women and may also be necessary for detection of compounds that cause toxic reactions via the accumulation of active metabolites with repeated exposure.

The currently accepted protocol for teratology testing (i.e., sacrifice of pregnant dams at term following exposure during the organogenesis period, and examination of maternal and fetal tissue) is appropriate for investigation of the potential of highly toxic compounds administered acutely to induce birth defects, but may be of questionable value in identifying environmental agents which exert toxic effects only after low level, chronic exposure. In the latter case, postnatal behavioral evaluation may be a more sensitive index of reproductive toxicity. Several studies have found behavioral alterations as a result of chronic or acute exposure to MC in adult humans (Aviado, et al., 1976; Parker, et al., 1979; Torkelson, et al., 1970). However, systematic investigation of the behavioral effects in postnatal animals following prenatal exposure to MC have yet to be reported. Thus, a second aim of the present study was to ascertain whether offspring of dams exposed to MC prior to and/or during pregnancy possessed neurobehavioral deficits.

The final aim of this study was to ascertain if induction of transplacental carcinogenesis occurred with prenatal exposure to MC. A National Cancer Institute bioassay (NCI, 1977) has been conducted wherein no relationship was found between dosage groups, species, sex, type of neoplasm or site of occurrence in rats and mice exposed to MC by gavage. This study does not rule out the possibility of transplacental carcinogenic activity of MC, which has not been previously examined.

A vapor concentration of 2100 ppm was selected because it was a multiple (6X) of the threshold limit of 350 ppm but well below the 3500 ppm level reported as the maximum for repeated 6 hrs/day exposures causing no discernable organic injury to rats (Torkelson et al., 1958).

METHODS

Animals and Materials

Virgin female Long-Evans hooded rats were purchased from Charles River Breeding Laboratories. Animals were ear-notched for individual identification and toe clipped for treatment group identification and quarantined for two weeks after arrival. They were singly housed in stainless steel, wire-bottom cages in a temperature (74°F) and light-controlled room (12:12 light-dark cycle, light commencing at 7 a.m.). Standard laboratory rat chow (Purina 5001) and tap water were available ad lib. except during residence in exposure chambers.

At the beginning of the experiment, female rats weighing approximately 250 g were randomly assigned to 1 of 4 treatment groups, with 30 rats per group as follows:

A(++) - MC exposure before mating and during pregnancy;

B(+-) - MC exposure before mating, filtered air during pregnancy;

C(-+) - Filtered air before mating, MC during pregnancy; and

D(--) - Filtered air before mating and during pregnancy

The pre-mating exposure was conducted for 6 hrs. a day, 5 days a week for 2 weeks. Following the last day of exposure, the mating regimen began. Two estrous females were placed with 1 breeder male overnight. Vaginal smears were conducted the following morning prior to exposure. Day 1 of pregnancy was considered to be the day on which sperm were observed in the smears. The

pre-mating exposure condition was continued until pregnancy was confirmed. At this time, the pregnancy exposure regimen began and continued for 6 hrs. a day, 7 days a week through day 20 of pregnancy.

For daily inhalation exposure, rats were transferred to stainless steel wire mesh racks holding 15 rats in individual compartments. Details of the exposure chambers, method of vapor generation and monitoring system can be found in Dorfmueller et al., 1980. The technical grade solvent, Chlorothene VG[2], was manufactured and generously supplied by the Dow Chemical Company. Exposure concentrations were maintained at a time-weighted average of 2100 ± 200 ppm. Chamber concentrations were monitored every 10 minutes by a Naseline® gas chromatograph (model 1030A) with an automatic sampling pump.

Maternal and Fetal Observations

All female rats were observed daily throughout the exposure period and body weights recorded every four days, including day 1 and 21 of pregnancy. Exposure ended on day 20 of gestation and half of the dams in each treatment group were sacrificed on day 21 by ether anesthesia. Blood samples were withdrawn by cardiac puncture for SMA-6[3] determinations. Maternal livers were removed, weighed, and frozen. Uterine horns were exteriorized through an incision of the abdominal wall and the numbers of live, dead, and resorbed fetuses counted. Fetuses were removed, examined for external anomalies and their sex and weight recorded. Four fetuses from each litter were preserved in Bouin's solution for soft tissue analysis by the method of Wilson (1965), and four fetuses were placed in 95% alcohol for skeletal evaluation by the method described by Dawson (1926). Fetuses were selected for each analysis so as to represent both sexes and both uterine horns.

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Postnatal Measurements

The remaining half of the dams (6 in A, 10 in B, 8 in C, and 8 in D) were transferred to litter boxes consisting of polypropylene cages with stainless steel tops. Parturition occurred on the 22nd-23rd day of pregnancy. At 4 days after birth, all litters were randomly culled to eight with equal representation of male and female pups when possible. At weaning (20 days of age), each litter was reduced again by random selection to two males and two female pups which were housed in stainless steel wire bottom cages. Litter weights were taken at birth and 10 days of age, and individual weights at 20 day intervals from weaning to 320 days of age. Any offspring dying spontaneously during the 12 month maintenance period was immediately necropsied. Dams were sacrificed and autopsied after weaning. All surviving offspring were autopsied at 12 months and examined for gross lesions.

Neurobehavioral Tests

Open Field Activity Measurements (21 days of age)

One male and female per litter (32 litters total) were randomly selected and individually tested for a period of 1.5 hours in a photocell activity cage measuring 29 x 19 x 13 cm at 21 days of age. The activity period was divided into 18 consecutive 5 minute testing intervals. Ten cages of animals were run simultaneously and monitored by a Kim-1 microprocessor. The pups were assigned to test cages by a balanced design to control for potential differences in testing conditions across cages. The measure of general activity was reflected in the number of times photocell circuits were broken by the exploratory behavior exhibited by the rats. Data were recorded automatically by a Kim-1 microprocessor at the end of each 5 minute period. Following testing animals were ear-notched and toe-clipped for future identification, and the same animals were tested in the running wheels.

Running Wheel Activity Measurements (40 to 110 days of age)

Beginning at approximately 40 days of age, one male rat per litter (32 litters total) was placed in a Wahmann (LC34) running wheel. The rats were allowed to live continuously in the wheels for 10 weeks. Food and water were available ad libitum in the attached home cages. The number of wheel revolutions was recorded every 2 hours during the last 12 days in the running wheel, while cumulative food and water consumption were collected weekly during the entire study. Body weights were also recorded once a week. On day 110, group A and B animals were removed from the running wheels and placed in standard stainless steel wire-bottom cages with food and water available ad libitum.

Amphetamine Challenge (110-130 days of age)

At the end of the chronic running wheel testing, an amphetamine challenge test was conducted on male offspring in the C (pregnancy exposure, 10 animals) and D (filtered air controls, 10 animals) groups. All testing occurred during a 2 hr period between 10 a.m. and 12 p.m. each day. Rats were first locked into their home cages with food and water ad libitum for 24 hrs. The next day, rats were injected S.C. with saline and locked into the running wheels for 2 hrs. The number of wheel revolutions was recorded by a Kim-1 microprocessor for every 5 minute period. At the end of the 2 hr. period, the rats were again locked into their home cages until the following day, when a low dose of D-amphetamine sulfate (1 mg/kg) was administered, and wheel revolutions recorded. The rats were allowed a 4 day rest period and were then tested with a high dose of amphetamine (3 mg/kg) using the same protocol. At the end of the amphetamine challenge test, rats were returned to stainless steel wire bottom cages where they remained until the 12 month autopsy.

Control

Data Analysis

The litter was considered to be the experimental unit of analysis in the teratology study. Maternal toxicity (Table 1) and embryotoxicity (Table 2) data were analyzed by two way unweighted means analysis of variance to assess the influence of premating vs. pregnancy exposure (AC vs. BD). Significant ANOVA effects ($p < .05$) were explored via Newman-Kuels post hoc analysis (Weiner, 1962). In order to accomodate the presence of zero values in many of the skeletal (Table 3) and soft tissue (Table 4) anomaly categories, Kruskal-Wallis one-way analysis of variance by ranks was performed on these data sets (Ferguson, 1971). Ranks were based on the percentage of the examined litter exhibiting the anomaly.

One- and two-way (AC vs. BD) analysis of variance with repeated measures were used to assess weight treatment, sex and trial effects in the behavioral data. Significant effects were explored via Newman-Kuels post hoc analysis. When appropriate, data sets were first transformed via a square root transformation prior to performing analysis of variance.

RESULTS

Table 1 contains the maternal toxicity data. There was an unequal number of dams in each treatment group, due to either failure of a female to mate or inaccurate assessment of day 1 of pregnancy. In the latter case, the dam was not included in the teratologic analysis. The mean length of pregestation exposure was approximately 21 days, and did not vary significantly between treatment groups.

Female rats exposed to 2100 $\pm$ 200 ppm of MC before mating and/or during pregnancy failed to exhibit signs of toxicity. Animals exposed before mating

(Groups A and B) had significantly greater weight gains during pregnancy than Groups C, with exposure during pregnancy alone, and Group D, the filtered air control. Insofar as maternal liver weights and clinical blood chemistry tests (data not shown) were not influenced by treatment, it is unlikely that this elevation in weight gain during pregnancy with pregestational exposure is indicative of maternal toxicity.

Table 2 contains the results from the embryotoxicity measurements. There were no significant treatment effects in the number of corpora lutea, implantation sites, live fetuses, or resorptions in litters. Thus, MC was not embryolethal under these conditions. There was a significant depression in total fetal body weight and male fetal body weight in the pregnancy exposure groups, A and C, indicative of embryotoxicity.

Results from skeletal analysis of term fetuses are contained in Table 3. No major malformations were observed in any of the fetuses examined. The anomalies listed in Table 3 are either variations of normal (delayed ossification of sternum, rudimentary ribs) or minor skeletal anomalies (reduced clavicle, missing 13th rib, wavy ribs, missing 26th and 27th vertebrae, and incomplete braincase closure) (Palmer, 1977). There was a significant elevation in the total number of these anomalies in the A group. The most frequently observed anomalies were delayed ossification of the sternum and reduced clavicle. The latter was seen in six fetuses from six litters, and has not been previously observed in this laboratory.

Table 4 contains results from the soft tissue examination. Blood in the pericardium and small kidneys were the most frequently observed soft tissue anomalies. These are considered to be minor visceral anomalies (Palmer, 1977). When total anomalies were summed, Group A was found to be significantly elevated over the control, group D. The kidney abnormalities may be treatment-related insofar as they were found only in litters from dams exposed to methyl chloroform.

Body weight measurements of offspring from birth to 120 days of age are shown in Figure 1. Two separate analyses were performed on the data because litter weights were taken at birth and at 10 days of age, whereas from days 20 to 120 individual weights were recorded. The mean weights at birth were 6.0, 5.8, 5.9, and 5.8 gm for group A, B, C, and D, respectively. No significant treatment effects were observed in postnatal body weight measurements from birth to 10 days of age nor from 20 to 120 days of age.

Separate weights were maintained on male offspring subjected to running wheel measurements and on all offspring from 120 days to 320 days of age. No treatment-related differences in weight gain were observed in any of these animals.

Results from the open field measurements of offspring at 21 days of age are presented in Figure 2. Data from male and female offspring were pooled for presentation purposes due to a lack of sex-related differences in activity levels at this age. No significant differences were found in the ambulatory response of these offspring when transfered from their home cage to a novel environment.

Results from the running wheel test are presented in Figure 3. Daily activity for the last 12 days of a 10 week period is displayed. No significant differences were found in running wheel activity or in food and water consumption during this period.

Figure 4 contains results from the amphetamine challenge test conducted on group 3 (pregnancy exposure) and group D (filtered air controls) that had been previously tested in the running wheels. No significant differences were observed in baseline measurements of offspring exposed to saline, or to the high dose (2 mg/kg) of D-amphetamine sulfate. The same trend was observed in the low dose (1 mg/kg) amphetamine test.

The survival rates for offspring from 1 to 12 months of age are displayed in Table 5. Animals dying spontaneously during the 12 month period had inflammation and consolidations of the lung, or died from causes not discernable at gross autopsy. No significant treatment-related trends are evident in the survival data.

Results from the 12 month autopsy are presented in Table 6. All gross lesions indicative of a disease state were recorded. A variety of gross lesions were observed but none of them could definitively be attributed to treatment. The lesions observed in treatment groups occurred at a level comparable to controls.

The kidneys were the most frequently affected organ system. The majority of lesions observed consisted of pale, hollow and fluid-filled kidneys indicative of hydronephrosis. None of the offspring with hydronephrosis were littermates. The kidney lesions were equally distributed across groups, and were not judged to be due to treatment.

The predominant lesion in the female reproductive tract was cystic ovaries, with thickened uterine horns occurring to a lesser extent. In the male reproductive tract, yellow nodular masses in the fat pad around the testes were the most frequent observation, with enlarged prostate observed three times.

Hemorrhagic sites were noted four times in the liver, and pale, fatty and grainy-appearing livers were observed three times each. All of these lesions occurred across treatment groups.

Enlarged pituitaries were encountered, as well as hemorrhagic sites in the pituitary, G.I. tract and adrenals. Consolidations and hemorrhagic sites were noted in the lungs. None of these lesions occurred at a sufficient magnitude to indicate a clear treatment-related effect. Growths were observed in subcutaneous, inguinal and mesentary tissue. The majority of lesions observed were not

sufficiently indicative of neoplastic growth to warrant suspicion of transplacental carcinogenic activity.

DISCUSSION

The present study was designed to determine whether exposure to methyl chloroform before mating and during pregnancy was more detrimental to the offspring than exposure before mating or during pregnancy alone. The most notable effects of exposure to 2100 ± 200 ppm of methyl chloroform were depressions in fetal body weight in the A and C groups, and elevations of total skeletal and soft tissue anomalies in the A group. Maternal toxicity and embryolethality were not observed.

Insofar as the anomalies observed in term fetuses were not major malformations, but rather minor malformations and variations of normal, teratogenic activity is not strongly implied. The variation of normal anomalies involve reduced ossification of the skeleton and are most likely indicative of a transient stage of development. These are structural changes occurring in more than 5% of the population, including treatment as well as control groups. The anomalies classified as minor skeletal malformations are small, relatively rare structural changes not obviously detrimental to the offspring. Insofar as variations of normal and minor malformations can involve different degrees of incomplete ossification which is reversible postnatally, the distinction between the two categories is not always clear, and the impact on the offspring uncertain (Palmer, 1977). Given the depressed body weight of fetuses in the A group, it is likely that both categories of anomalies are indicative of a reversible developmental delay. Thus, the presence of these anomalies is more indicative of embryotoxicity than teratogenicity.

In a study conducted by Schwetz et al. (1975), in which maternal rats and mice were exposed to 875 ppm of methyl chloroform by inhalation on days 6-15 pregnancy, an increase in maternal liver weight was the only significant effect observed in rats but not mice. No indications of maternal toxicity were obtained in the present study, while embryotoxicity in the form of depressed fetal body weight and elevation of variations and minor malformations in term fetuses were observed. The longer exposure period and higher exposure level utilized in this study may be responsible for these effects.

Postnatal evaluation included body weight and survival measurements, and neurobehavioral tests of open field activity (21 days of age), running wheel activity (40-110 days of age), and an amphetamine challenge test (110-120 days of age). All surviving offspring were autopsied at 12 months of age and examined for gross lesions. None of these parameters have previously been measured in offspring exposed prenatally to MC. The behavioral measurements assume particular importance insofar as a functional depression in central nervous system function, manifested as impairment of coordination, is the most prevalent effect seen in humans occupationally exposed to MC (Stewart et al., 1961; Stewart, 1968). These same symptomologies may not result from prenatal exposure. This is especially true since previous reports have dealt with concurrent effects of MC exposure, while we were interested in residual effects resulting from prenatal exposure. Having little or no information on how MC may affect the developing nervous system, we concentrated our efforts on neurobehavioral assessment of motor performance and response to a drug challenge. These measures are amongst the most widely used, and appear to be the most useful in assessing general neurobehavioral maturation (Geller et al., 1979).

Significant, treatment-related effects were not observed in any of the postnatal parameters measured. Offspring from treatment groups responded

similarly to controls in measurements of body weight and survival, as well as in the battery of neurobehavioral tests. A variety of lesions were observed in the 12-month autopsy, none of which could definitively be attributed to treatment. A fairly high background rate of hydronephrosis (approximately 22%) was found across groups. This finding suggests that the kidney dysplasias observed in soft tissue examination of term fetuses (Table 4) in treatment groups alone was not a persistent lesion. Likewise, the depression in fetal body weights of fetuses in the pregnancy exposure groups (A and C, Table 2) on day 21 of gestation did not persist to birth (days 22-23), at which time body weights across groups were not significantly different. The kidney dysplasias may have been a transient result of an overall slight retardation in fetal growth rate. Historical control data from this lab (Manson et al., 1981) has indicated that Long-Evans hooded rats have a high background rate of hydronephrosis. This rate can reach as high as 40% in animals at 18 months of age.

The postnatal examination allows us to conclude that prenatal exposure to 2100 ± 200 ppm of MC did not exert a persistent detrimental effect on any of the parameters measured. The depression in fetal body weight and elevation in skeletal and soft tissue variants did not influence postnatal weight gain, survival, neurobehavioral function, or the incidence and type of gross lesions in offspring at 12 months of age. No differences in outcome were observed in offspring from mothers exposed before mating and during pregnancy compared with those whose mothers were exposed during pregnancy alone.

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FOOTNOTES

[1]This research was supported by grant #ES01601 from NIEHS and Center for the Study of the Human Environment #ES00159.

[2]The technical grade solvent contained approximately 95% 1,1,1-trichloroethane and 5% inhibitors and impurities. A detailed description of the minor components of these inhibited commercial solvents is available from Inorganic Chemicals R&D, The Dow Chemical Company, Midland, Michigan 48640.

[3]Standard Medical Analysis - 8: Clinical blood test measuring levels of serum enzymes and components indicative of liver and kidney dysfunction.

Table 1

Effects of Exposure to 2100 ppm Methyl Chloroform on Maternal Rats

	Groups			
	A	B	C	D
Exposure Before Mating	+	+	-	-
Exposure During Pregnancy	+	-	+	-
Number of Dams	20	11	13	15
Days Pre-exposed	20 ± 1[a]	21 ± 2	23 ± 2	21 ± 1
Body Weight (g)				
Initial	252.7 ± 4	266.7 ± 7	253.5 ± 6	252.5 ± 3
Day 1	265.0 ± 4	284.6 ± 9	274.5 ± 7	268.5 ± 5
Day 21	393.2 ± 6	424.1 ± 13	387.3 ± 12	390.8 ± 7
Weight Gain During Pregnancy (%)	48.7 ± 2[b]	49.3 ± 3[b]	41.3 ± 3	45.7 ± 2
Liver Weight				
Absolute (g)	13.6 ± .3	15.3 ± .9	13.7 ± .6	13.9 ± .4
Relative (% of body wt.)	3.5 ± .0	3.6 ± .1	3.5 ± .1	3.6 ± .1

[a] Mean ± S.E.M.

[b] A and B significantly elevated over C and D, $p < .05$. Two-way unweighted means analysis of variance.

Table 2

Evaluation of Methyl Chloroform Embryotoxicity

	Groups			
	A	B	C	D
Exposure Before Mating	+	+	-	-
Exposure During Pregnancy	+	-	+	-
Number of Litters	20	11	13	15
Number of Corpora Lutea/ Litter	14.2 ± .5[a]	14.6 ± .8	14.9 ± .5	15.7 ± 1.2
Number of Implantation Sites/Litter	13.6 ± .6	14.0 ± .9	14.1 ± .5	13.3 ± .6
Ratio Implantation Sites/ Corpora Lutea	.85 ± .0	.85 ± .0	.86 ± .0	.89 ± .0
Number of Live Fetuses/ Litter	12.8 ± .6	12.9 ± .8	12.9 ± .7	11.9 ± .8
Ratio Live Fetuses/ Implantation Sites	.86 ± .0	.86 ± .0	.87 ± .0	.87 ± .0
Number of Resorptions/ Affected Litters	1.45	2.00	1.67	2.50
Number of Litters With 2 or More Resorptions	3	2	4	4
Mean Fetal Body Weight(g)	5.1 ± .1[b]	5.3 ± .1	4.9 ± .1[b]	5.1 ± .1
Mean Male Fetal Body Wt. (g)	5.2 ± .1[b]	5.4 ± .1	5.0 ± .2[b]	5.3 ± .1
Mean Female Fetal Body Wt. (g)	5.0 ± .1	5.1 ± .1	4.8 ± .1	4.9 ± .1
Ratio Male:Female	1:1.2	1:0.9	1:1.3	1:1.1

[a]Mean ± S.E.M.

[b]A and C significantly depressed in relation to B and D, $p < .05$. Two-way unweighted means analysis of variance.

Table 3

Skeletal Anomalies In Fetal Rats After Maternal Methyl Chloroform Exposure

	Groups			
	A	B	C	D
Exposure before mating	+	+	-	-
Exposure during pregnancy	+	-	+	-
Number examined	70(20)[a]	45(11)	52(13)	62(15)
Delayed ossification of sternum	5(3)	1(1)	-	5(4)
Reduced clavicle	5(5)	-	1(1)	-
Missing 13th rib	1(1)	1(1)	-	-
Rudimentary 13th rib	2(2)	2(1)	-	-
Rudimentary 14th rib	3(1)	-	-	-
Wavy ribs	1(1)	1(1)	-	-
Missing 26th or 27th vertebrae	1(1)	-	-	-
Incomplete brain case closure	1(1)	3(2)	-	-
Total Anomalies[b]	19(8)	8(4)	1(1)	5(4)
Percent Normal litters	60	64	92	93

[a]Number of fetuses affected (Number of litters affected)

[b]Significant effect A > B, C, D $p < .001$ (Kruskal Wallis)

Table 4

Soft Tissue Anomalies In Fetal Rats After Maternal Methyl Chloroform Exposure

	Groups			
	A	B	C	D
Exposure before mating	+	+	-	-
Exposure during pregnancy	+	-	+	-
Number examined	71(18)[a]	40(10)	52(13)	63(16)
Hemorrhagic pericardium	3(3)	-	-	-
Small kidney	3(3)	-	-	-
Hydronephrotic kidney	-	2(2)	-	-
Enlarged renal medulla[b]	-	-	1(1)	-
Total Anomalies[c]	6(6)	2(2)	1(1)	-
Percent Normal litters	67	80	92	100

[a]Number of fetuses affected (Number of litters affected)

[b]Kidney a solid mass, no pelvis evident

[c]Significant effect A > D, $p < .05$ (Kruskal-Wallis)

Table 5

Survival of Offspring Exposed Prenatally To [illegible]

% Survival

Age (Months)	A	B	C	D
N	60	68	51	56
1	100	100	100	100
2				98
3				
4				
5				
6	98	97		96
7				
8				
9		96		93
10			96	
11				
12	98	95	96	93
N	59	65	49	52

only one of these corresponds to the ## autopsied as shown on Table 6. Why?

Table 6

Gross Lesions Observed at 12 Month Autopsy

Number Autopsied	60 (100)[a]	62 (100)	49 (100)	53 (100)
Organ:				
Kidneys	11 (18)	14 (23)	10 (20)	15 (28)
Male reproductive tract	4 (14)	8 (24)	3 (12)	6 (23)
Female reproductive tract	5 (16)	3 (11)	2 (9)	9 (33)
Liver	3 (5)	4 (6)	3 (6)	3 (6)
Pituitary	2 (3)	2 (3)	1 (2)	4 (8)
Lung	0 (0)	2 (3)	4 (8)	1 (2)
Brain	0 (0)	1 (2)	0 (0)	2 (4)
G.I. Tract	1 (2)	1 (2)	1 (2)	3 (6)
Adrenals	1 (2)	3 (5)	1 (2)	0 (0)
Spleen	1 (2)	1 (2)	1 (2)	0 (0)
Urinary bladder	1 (2)	1 (2)	0 (0)	1 (2)
Tissues:				
Subcutaneous	4 (7)	0 (0)	2 (4)	5 (9)
Inguinal	0 (0)	1 (2)	0 (0)	0 (0)
Mesentary	0 (0)	1 (2)	0 (0)	0 (0)
Number Total Lesions	33 (22)	42 (27)	28 (18)	49 (33)

[a]Number (%)

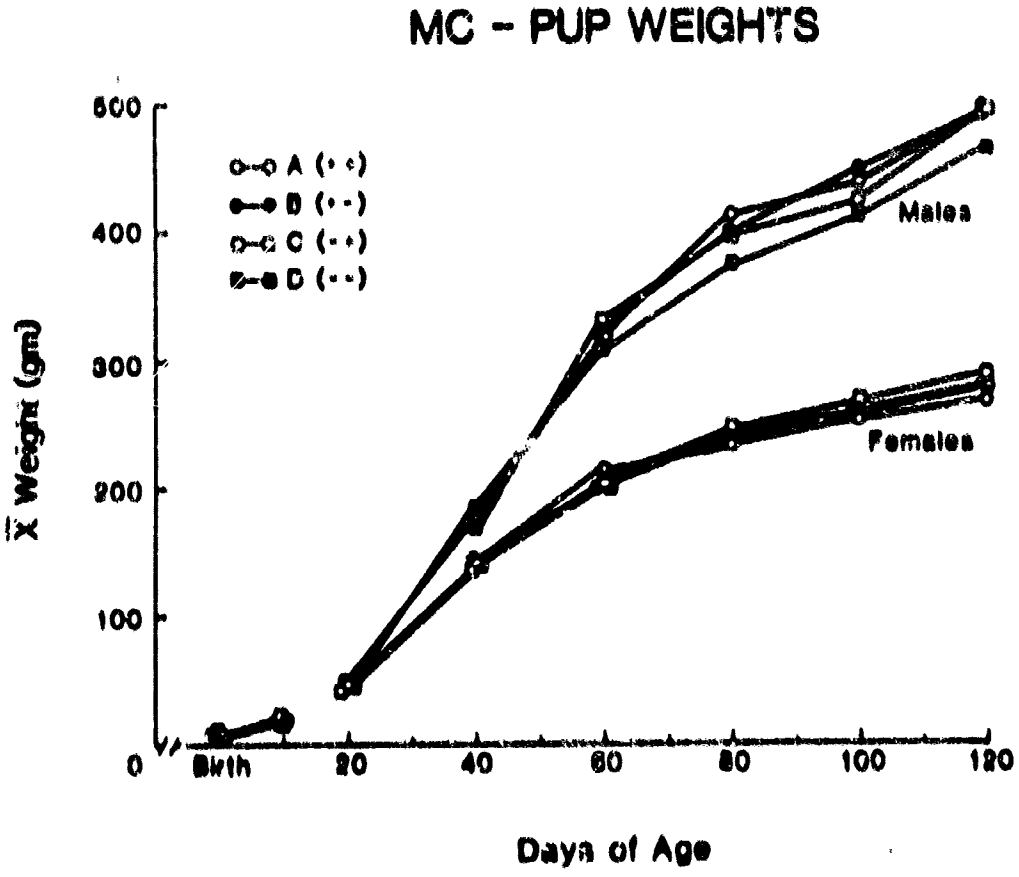

Figure 1. Mean Body Weights of Control and MC-Exposed Offspring from Birth to 120 Days of Age.

Weights of male pups assigned to running wheels are not included. See text for explanation. No significant differences were found with 2-way analysis of variance.

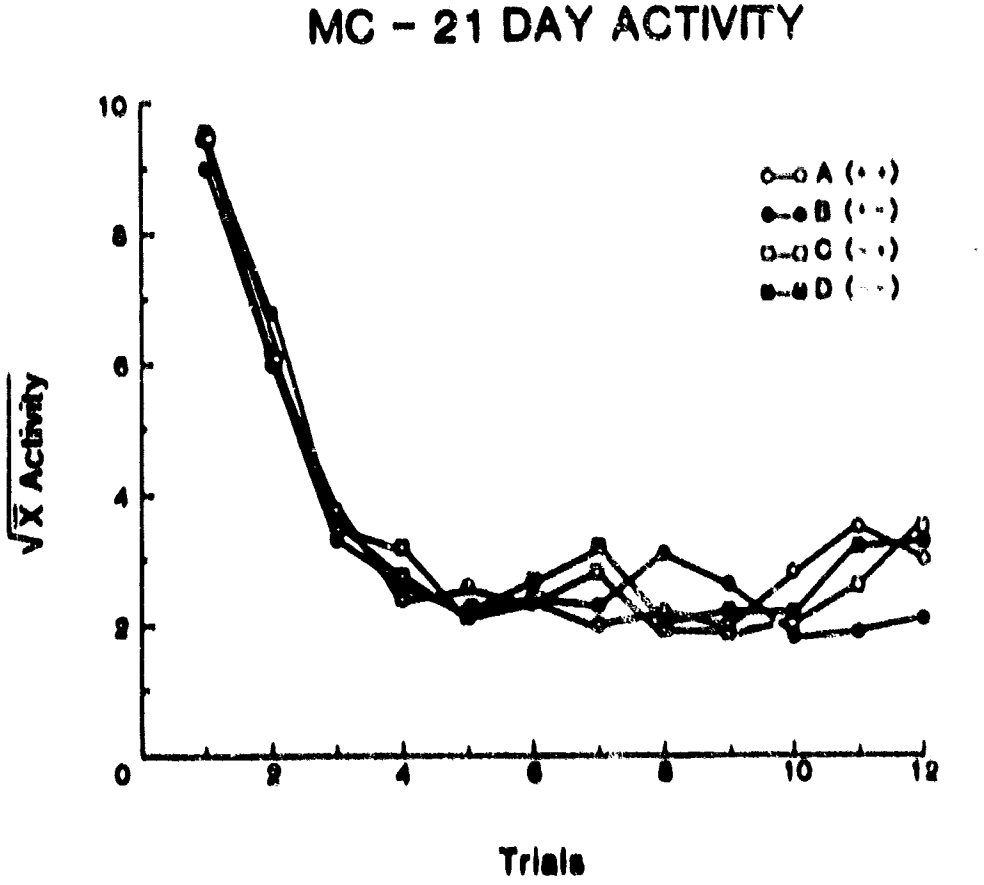

Figure 2. Open Field Activity Measurements of Control and MC-Exposed Offspring at 21 Days of Age.

Eight offspring per litter (6 littrave in A, 10 litters in B, 8 litters in C, and 8 litters in D) were tested. No significant differences were found with 2-way analysis of variance.

DAILY RUNNING WHEEL - METHYL CHLOROFORM

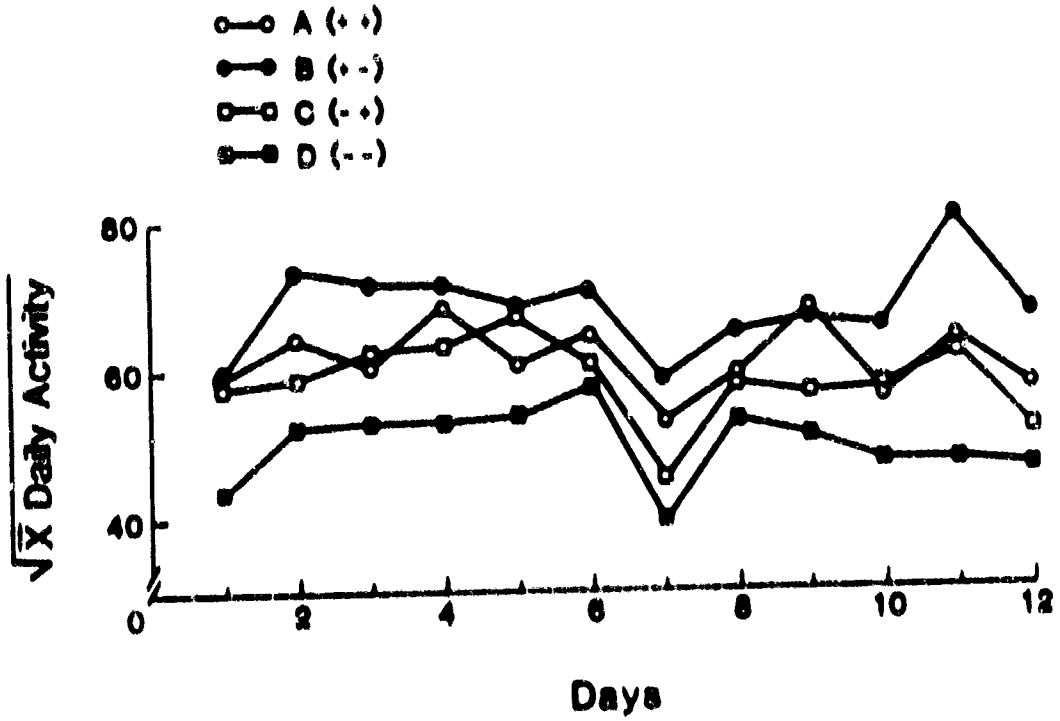

Figure 3. Running Wheel Activity Measurements of Control and MC-Exposed Offspring.

Activity levels for the last 12 days of a 10-week period (40-110 days of age) are presented. One male offspring per litter (6 litters in A, 10 in B, 8 in C and 8 in D) was tested. No significant differences were found with 2-way analysis of variance.

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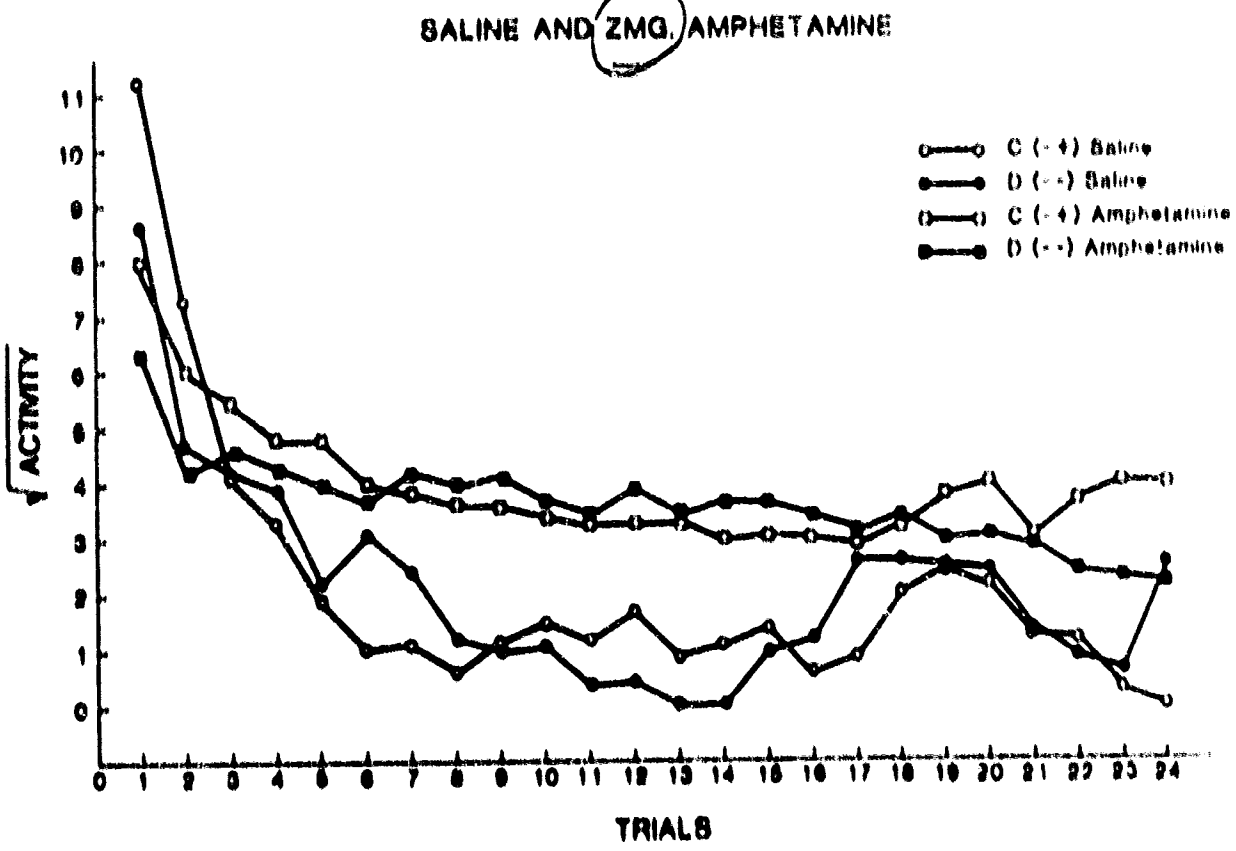

Figure 4. Amphetamine Challenge Test of Control and MC-Exposed Offspring from 110-120 Days of Age.

The response of 10 males in the C group and 10 in the D group to saline and 2 mg/kg of amphetamine sulfate is given. No significant differences were found by 1-way analysis of variance.