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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.

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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,

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Regulatory Specialist
Regulatory and Legislative Issues
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rt

D-340

R & D REPORT

DOW CHEMICAL U.S.A.

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DATE ISSUED: January 27, 1977

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) Robert E. Bailey

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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R & D REPORT

DOW CHEMICAL U.S.A.

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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 INHIBITION 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
Swanwick(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	—	—
Bouchop(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

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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 scary 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.

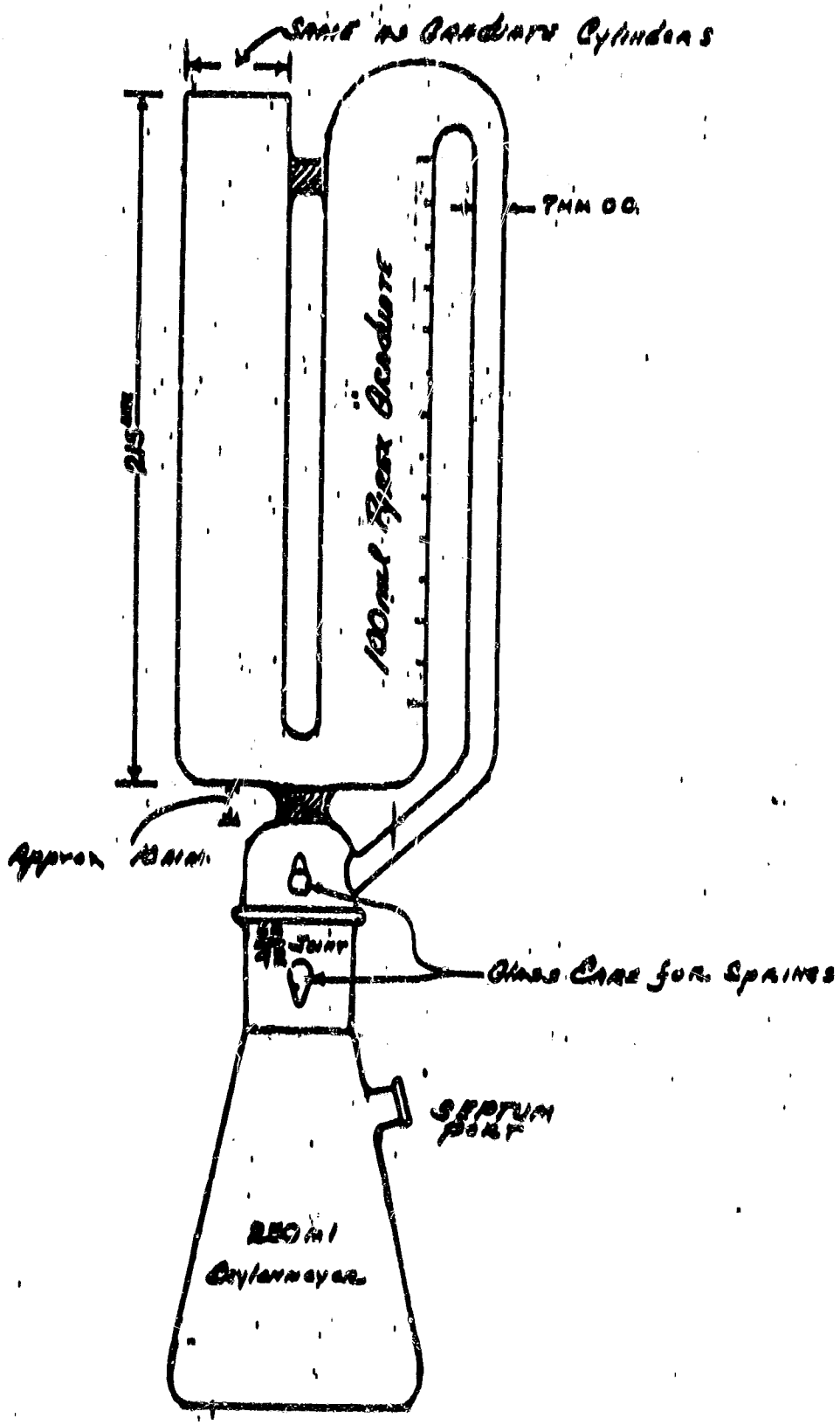

FIGURE 1. ANAEROBIC DIGESTER AND GAS MEASURING APPARATUS.

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.

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% OV-200 on 100-120 mesh chromosorb WAW-DMCS
Carrier Gas	20 cc/min N_2
Reaction Gas	3 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 0.13 g powdered activated carbon. Similarly, 3 flasks were purged with 1.5 l (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 (6.4-7.8) (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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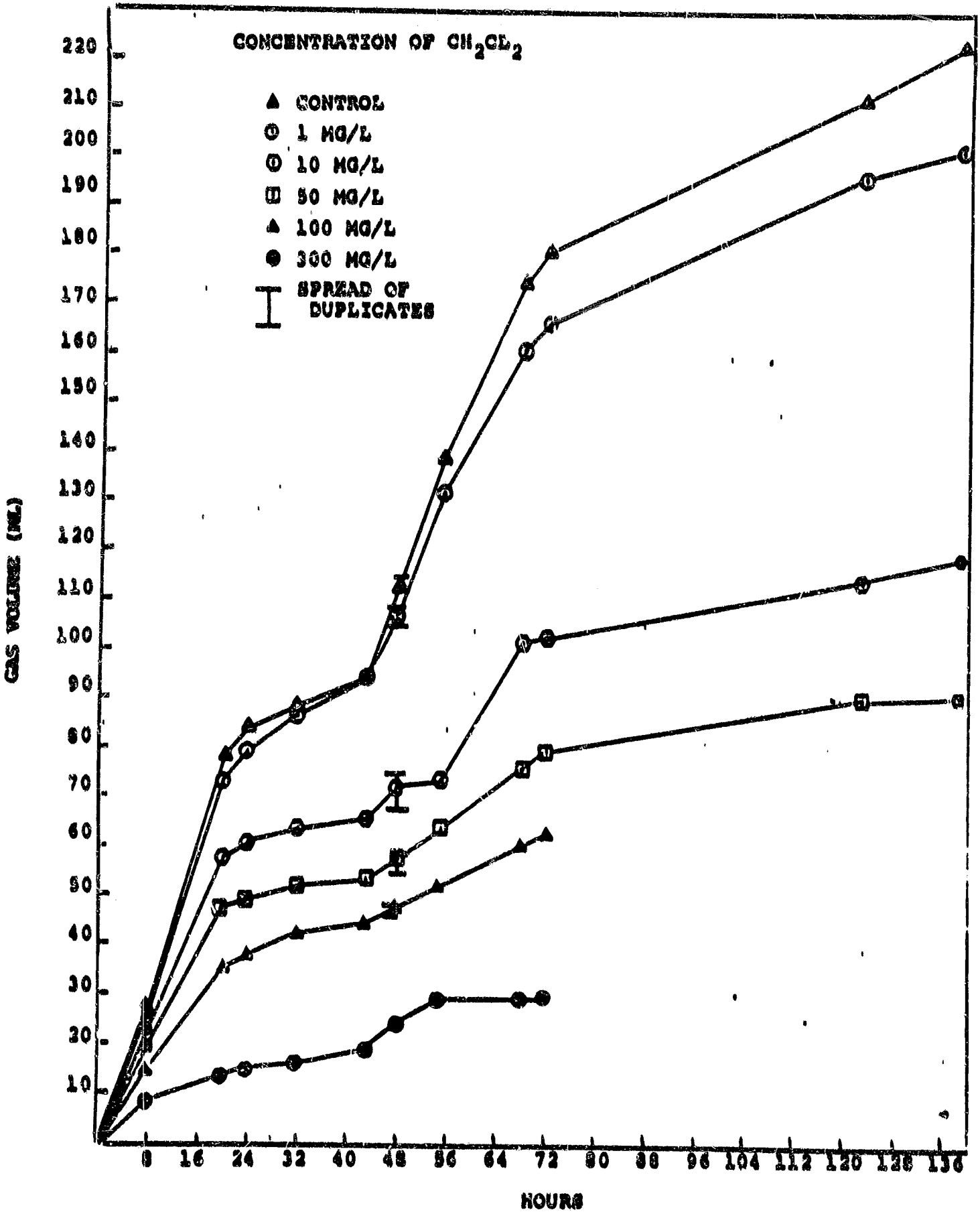

FIGURE 2. GAS PRODUCTION OF METHYLENE CHLORIDE (CH_2CL_2) INHIBITED SLUDGE.

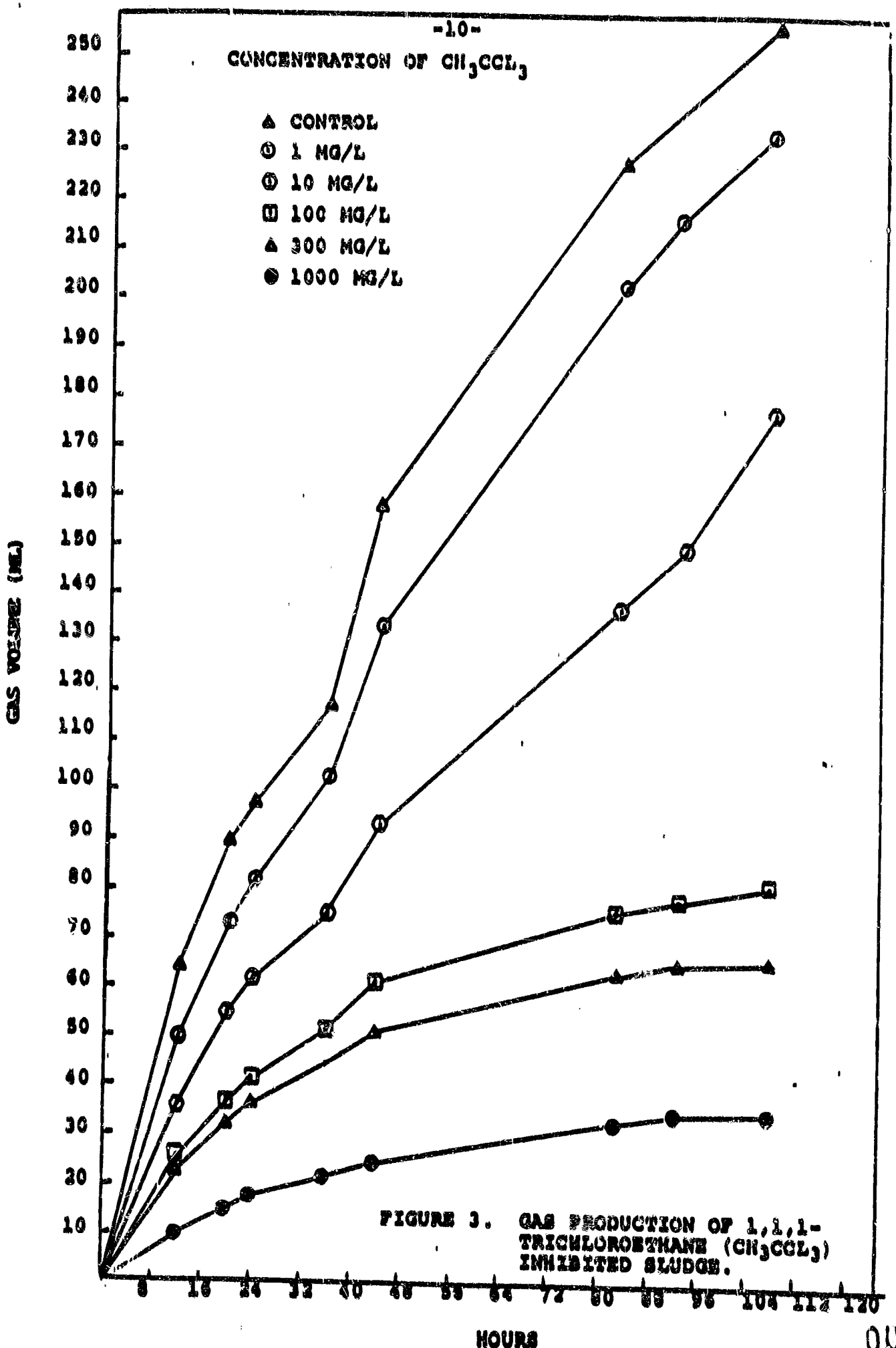

FIGURE 3. GAS PRODUCTION OF 1,1,1-TRICHLOROETHANE (CH_3CCL_3) INHIBITED SLUDGE.

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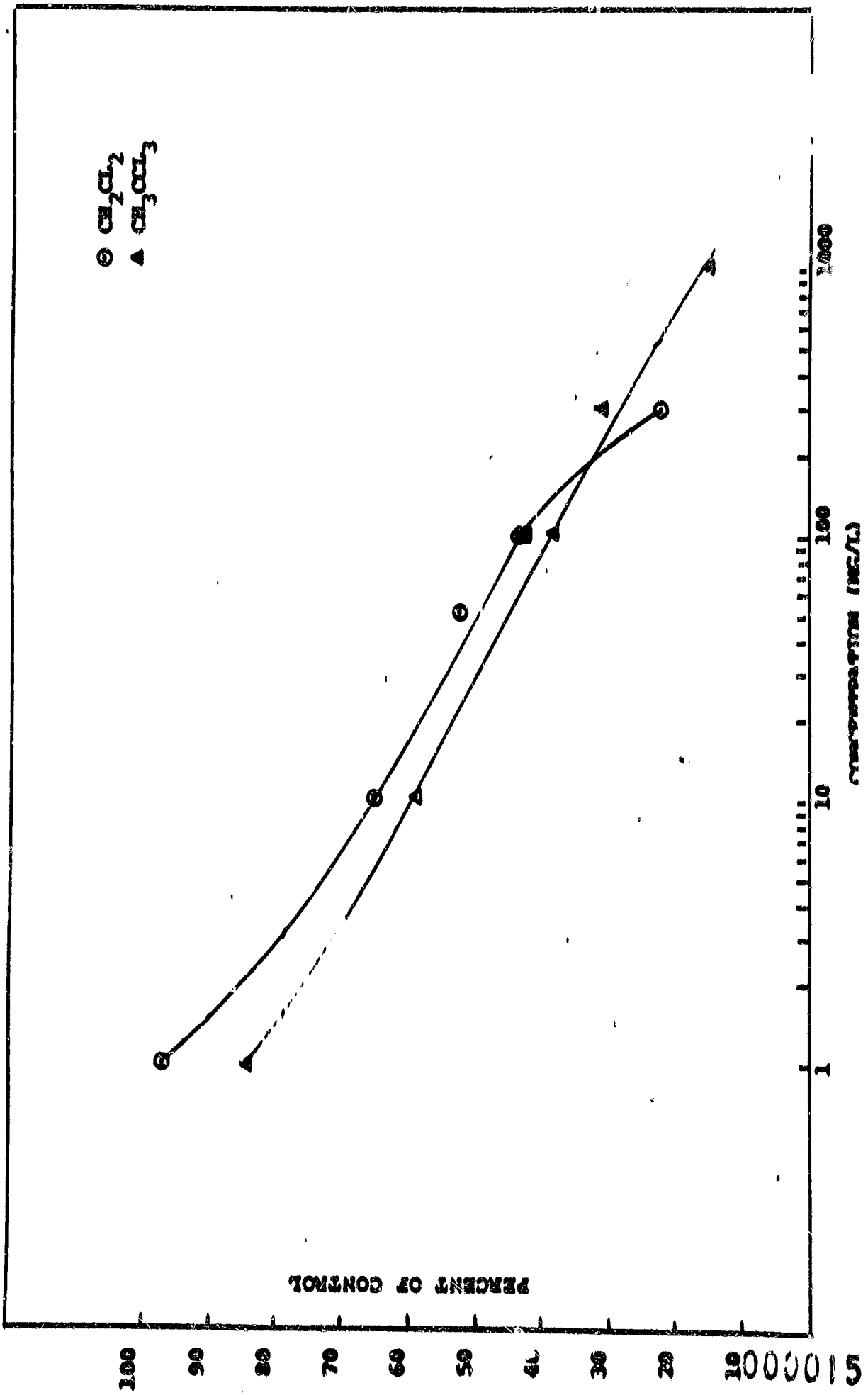

FIGURE 6. GAS PRODUCTION AS PERCENT OF CONTROL VERSUS CONCENTRATION IN MG/L MIXED LIQUOR.

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

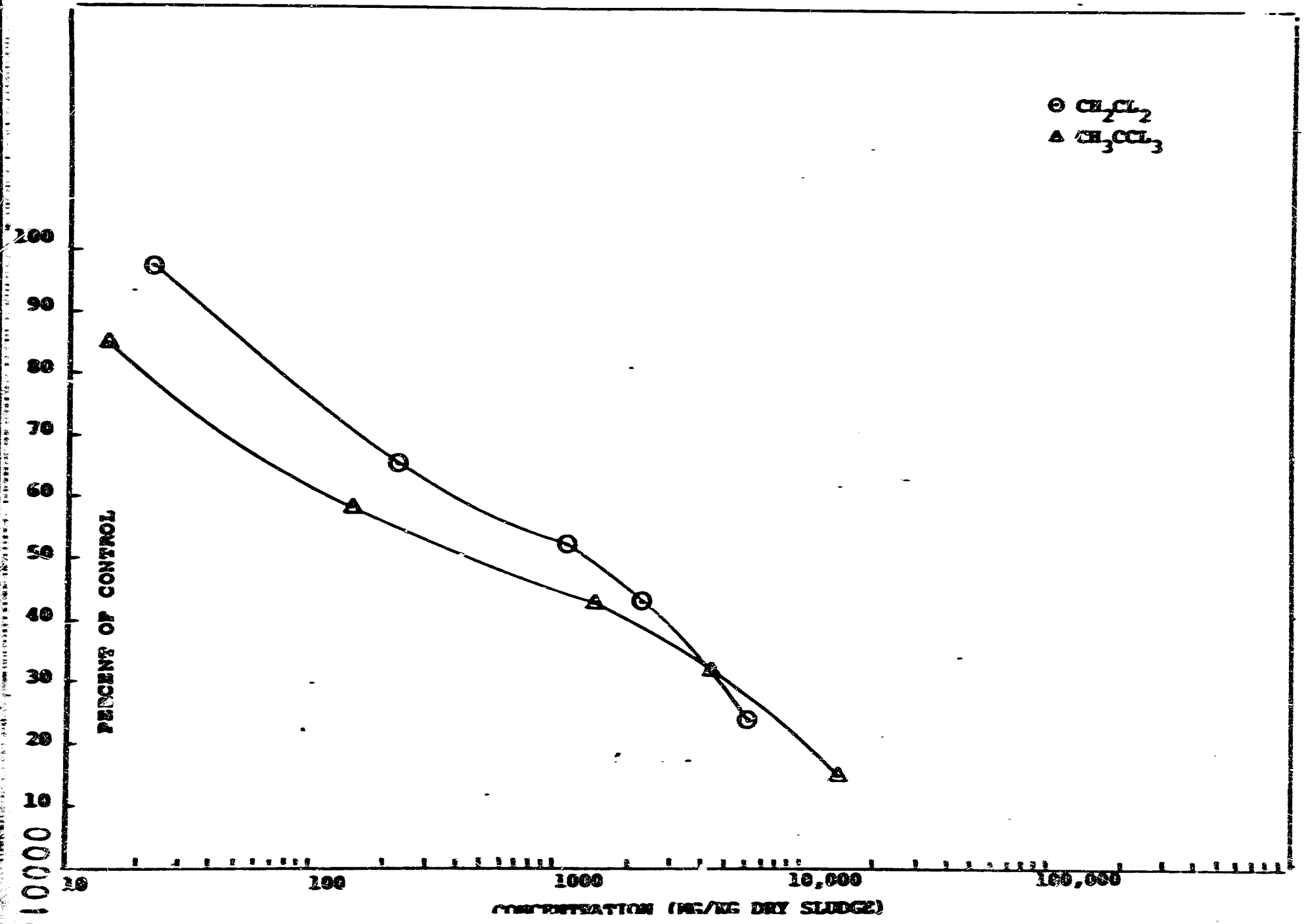

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TABLE II

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	158 ml	133 ml. 84%	93 ml. 59%	—	61 ml. 39%	50.5 ml. 32%	26.5 ml. 16%

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

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TABLE XXI

SOLIDS CONTENT OF SLUDGES USED WITH VARIOUS COMPOUNDS

Compound	Mixed Liquor Solids, g/l	Volatile Solids, g/l
CN_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.

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TABLE V

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 48 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	126	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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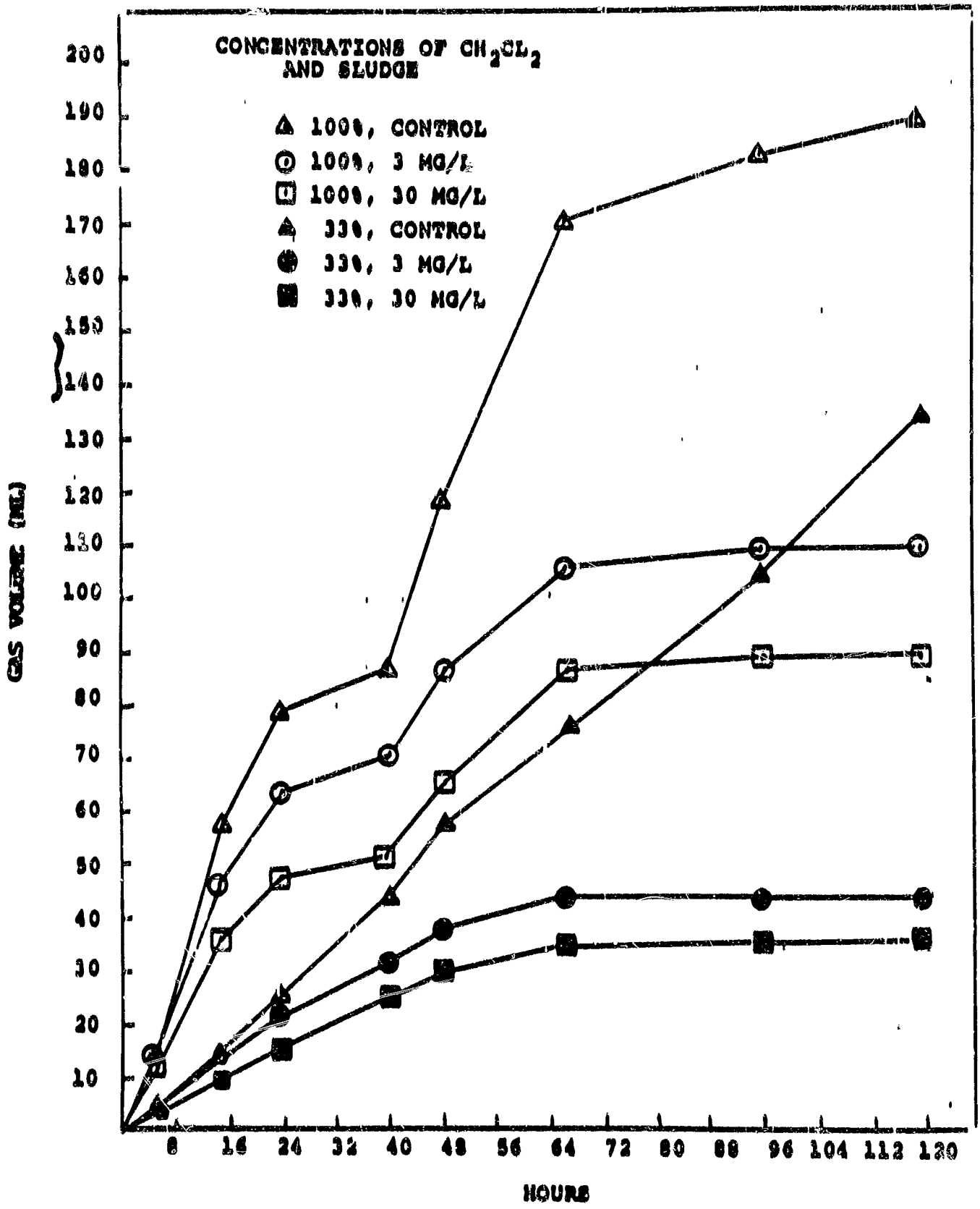

FIGURE 8. GAS PRODUCTION OF METHYLENE CHLORIDE (CH_2CL_2) INHIBITED SLUDGE AT TWO SLUDGE CONCENTRATIONS.

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Analyses

Methylene chloride were analysed 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	1.1 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, mg	Concentration in Sludge, mg/l	Recovery from Sludge, mg	Total Recovery, mg	Accountability, %
Control, 100%	0	189	0	0	0	0	—
3 mg/l, 100%	0.11	66.5	12.55	1.36	204	216.5	68
30 mg/l	1.75	88.5	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, 26 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	0.0092
Al	0.060
Ca	0.22
Mg	0.054
Mn	0.0018
Cr	0.0006
Sn	0.015
Ti	0.010
Sr	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 3000 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	0.12	--
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).

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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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Chemical Name (300 per name)	CAS No. (10)
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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. GIELANSKI, M.D., C.M.
Director

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NINETY DAY PERCUTANEOUS ABSORPTION 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

Continued

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.

Continued

Ninety Day Percutaneous Absorption Study In Rabbits With
Dow Chemical Co. - Chlorothene & 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.

Continued

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.

[illegible]

[illegible]	No.	Daily Dose (mg./kg.)	RBC $X10^6$	WBC $X10^3$	Hb. g./100 cc.	Differential (%)				
						N	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	-	[illegible]	[illegible]	10.5	[illegible]	[illegible]	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

000007

[illegible]

Group	Group No.	Daily Dose (mg./kg.)	Average Body Weight (g.) Initial	30-day	60-day	Weight Gain (g.)
Control	1	-	2570	2170	2510	140
Chloroform	2	15	1970	[illegible]	[illegible]	[illegible]
"	3	50	2190	2500	[illegible]	700
"	4	100	[illegible]	2560	2910	[illegible]
"	5	200	[illegible]	[illegible]	[illegible]	[illegible]
"	6	500	[illegible]	2100	[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	[illegible]	2970	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]

000008

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 (%) E	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	4.88	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

000009

[illegible]

[illegible]	[illegible]	[illegible] (ppm)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]										
	1		1.99	0.31	0.25	4.52	0.75	0.09	0.45	0.37
	7		3.52	0.37	0.21	3.59	0.26	0.11	0.09	0.25
	Average		2.61	0.35	0.23	4.10	0.51	0.10	0.27	0.31
[illegible]										
	2	15	2.30	0.35	0.25	3.85	0.83	0.08	0.17	0.29
	3	50	2.34	0.35	0.26	4.67	0.59	0.08	0.08	0.27
	4	100	2.41	0.31	0.27	5.51	0.81	0.07	0.12	0.31
	5	200	2.55	0.35	0.29	5.37	0.81	0.06	0.09	0.27
	6	500	1.91	0.35	0.25	4.22	0.18	0.05	0.06	0.19
Methylene Chloride										
	8	15	2.33	0.39	0.28	3.31	0.71	0.06	0.26	0.35
	9	50	3.06	0.32	0.21	3.85	0.71	0.05	0.07	0.21
	10	100	2.50	0.32	0.26	4.86	0.81	0.04	0.22	0.30
	11	200	2.03	0.35	0.19	5.05	0.71	0.05	0.09	0.31
	12	500	2.05	0.35	0.25	3.05	0.71	0.05	0.36	0.27
[illegible]										
	13	15	2.73	0.35	0.30	5.20	0.80	0.05	0.31	0.31
	14	100	2.43	0.35	0.26	5.50	0.75	0.04	0.09	0.30
	15	500	2.11	0.30	0.25	3.91	0.70	0.05	0.10	0.30

000010

[illegible] - [illegible]
Organ Weight Summary (g./100 g. Body Wt.)
[illegible]

Rabbit No.	Group No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Control										
1	1		2.[illegible]4	0.40	0.2[illegible]	7.0[illegible]	1.0[illegible]	0.05	0.10	0.[illegible]
2	7		2.[illegible]	0.52	0.25	[illegible].[illegible]	1.0[illegible]	0.07	0.0[illegible]	0.[illegible]
	Average		2.1[illegible]	0.46	0.27	7.73	1.0[illegible]	0.0[illegible]	0.0[illegible]	0.[illegible]
Chloroform										
2	2	15	2.4[illegible]	0.35	0.3[illegible]	4.61	0.90	0.0[illegible]	0.20	0.[illegible]
1	3	50	2.68	0.42	0.3[illegible]	5.48	0.75	0.07	0.19	0.33
4	4	100	2.64	0.38	0.2[illegible]	4.2[illegible]	0.7[illegible]	0.07	0.2[illegible]	0.36
	5	200	2.62	0.39	0.3[illegible]	4.9[illegible]	0.[illegible]	0.0[illegible]	0.25	0.3[illegible]
	6	500	2.5[illegible]	0.45	0.3[illegible]	5.3[illegible]	0.[illegible]	0.[illegible]	0.22	0.3[illegible]
Methylene Chloride										
1	3	15	2.[illegible]	0.48	0.27	4.10	0.75	0.0[illegible]	0.1[illegible]	0.3[illegible]
2	9	50	2.93	0.30	0.27	3.6[illegible]	0.6[illegible]	0.0[illegible]	0.2[illegible]	0.3[illegible]
1	10	100	2.47	0.46	0.30	5.26	0.[illegible]	0.11	0.12	0.[illegible]
4	11	200	2.25	0.39	0.26	4.65	0.[illegible]	0.11	0.11	0.[illegible]
3	12	500	2.33	0.40	0.27	5.13	0.[illegible]	0.0[illegible]	0.2[illegible]	0.[illegible]
Isopropyl Alcohol										
3	13	15	2.50	0.39	0.30	3.56	0.9[illegible]	0.0[illegible]	0.2[illegible]	0.3[illegible]
4	14	100	2.53	0.35	0.24	3.8[illegible]	0.7[illegible]	0.0[illegible]	0.2[illegible]	0.[illegible]
1	15	500	3.13	0.35	0.29	5.66	0.7[illegible]	0.0[illegible]	0.17	0.[illegible]

000011

[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	0.3[illegible]	0.27	4.35	0.63	0.0[illegible]	0.17	0.30
[illegible]	7		3.74	0.33	0.24	4.45	0.69	0.04	0.19	0.[illegible]
3	7		3.28	0.43	0.28	4.26	0.65	0.05	0.19	0.33
	Average		3.28	3.67	0.26	4.30	0.55	0.04	0.18	0.2[illegible]
Chlorothiazide										
[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	0.2[illegible]
	Average		3.68	0.30	0.25	4.65	0.72	0.04	0.25	0.2[illegible]
[illegible]	3	50	3.20	0.34	0.24	5.49	1.09	0.47	0.25	0.3[illegible]
[illegible]	3	50	3.22	0.40	0.29	5.48	0.85	0.62	0.20	0.32
	Average		3.21	0.37	0.27	5.49	0.95	0.55	0.25	0.3[illegible]
[illegible]	4	100	3.48	0.32	0.26	5.20	0.65	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	0.2[illegible]
[illegible]	5	200	3.02	0.32	0.23	4.81	0.85	0.03	0.18	0.30
[illegible]	5	500	3.16	0.28	0.22	4.23	0.82	0.06	0.19	0.32
3	6	500	3.06	0.32	0.24	4.31	0.74	0.0[illegible]	0.21	0.[illegible]
	Average		3.11	0.29	0.23	4.27	0.7[illegible]	0.0[illegible]	0.2[illegible]	0.[illegible]

[illegible] SKIN CONTACT - [illegible]
Organ Weight Summary (Gm./100 Gm. 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	3.95	0.60	0.03	0.17	0.22
3	6	15	4.22	0.26	0.19	3.81	0.56	0.03	0.18	0.23
	Average		4.41	0.25	0.26	3.88	0.58	0.03	0.15	0.25
1	4	50	3.08	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	0.27
3	10	100	3.36	0.32	0.22	4.60	0.76	0.04	0.13	0.23
4	10	100	4.24	0.25	0.21	3.99	0.60	0.03	0.18	0.24
	Average		3.86	0.29	0.22	4.30	0.68	0.04	0.18	0.23
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	3.98	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	[illegible]	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	0.60	0.04	0.25	0.32
	Average		3.12	0.31	0.25	4.52	0.73	0.04	0.22	0.31

Group	No.	Dose (mg/kg)	Body wt. (kg)	Urea (g/100 g dry wt.)	Uric acid (g/100 g dry wt.)	Creatinine (g/100 g dry wt.)	Ammonia (g/100 g dry wt.)
1	1	15	2.82	0.34	0.27	1.5	0.63	0.06	0.17	0.35
	2		3.87	0.29	0.25	1.52	0.76	0.04	0.25	0.24
	3		3.75	0.33	0.24	1.46	0.69	0.05	0.19	0.28
	4		3.48	0.31	0.25	1.50	0.68	0.05	0.20	0.28
	5		3.26	0.43	0.28	1.26	0.65	0.03	0.19	0.33
	6		3.38	0.37	0.26	1.38	0.70	0.03	0.15	0.33
	7		3.31	0.37	0.26	1.36	0.70	0.03	0.16	0.33
Average			3.27	0.35	0.26	1.40	0.69	0.04	0.19	0.30
2	1	15	3.87	0.29	0.25	1.52	0.76	0.04	0.25	0.24
	2		3.48	0.31	0.25	1.50	0.68	0.05	0.26	0.23
	3		3.31	0.30	0.26	1.48	0.72	0.04	0.20	0.26
	4		3.20	0.34	0.24	1.49	1.03	0.07	0.25	0.35
	5		3.22	0.40	0.29	1.38	0.85	0.02	0.25	0.32
	6		3.21	0.37	0.27	1.44	0.83	0.03	0.28	0.33
	7		3.35	0.32	0.25	1.43	0.78	0.03	0.23	0.32
Average			3.31	0.34	0.26	1.46	0.81	0.04	0.25	0.29
3	1	15	3.50	0.32	0.26	1.32	0.66	0.07	0.13	0.26
	2		3.28	0.36	0.27	1.27	0.74	0.05	0.15	0.35
	3		3.35	0.35	0.25	1.32	0.70	0.03	0.16	0.33
	4		3.02	0.32	0.23	1.48	0.86	0.03	0.18	0.36
	5		3.16	0.26	0.22	1.23	0.82	0.09	0.15	0.32
	6		3.06	0.34	0.24	1.21	0.71	0.05	0.21	0.31
Average			3.11	0.39	0.23	1.25	0.78	0.05	0.17	0.32

[redacted] ON OTHER - [redacted]
Organ Weight: Grams (g./100 g. Body Wt.)
30 Day Exposure : 30 day post Period

Rabbit No.	Group No.	Dose (mg./kg.)	Body Wt. kg.	Lung	Heart	Liver	Kidney	Spleen	Testes	Brain
Tetraethyl 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
1	9	50	3.51	0.33	0.25	5.56	0.75	0.07	0.25	0.23
	Average		3.27	0.31	0.22	4.76	0.73	0.05	0.20	0.26
2	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	3.93	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
3	12	500	3.40	0.31	0.26	3.74	0.64	0.04	0.23	0.33
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	0.69	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	4.37	0.75	0.05	0.20	0.24
3	14	100	3.44	0.36	0.27	3.99	0.70	0.04	0.23	0.24
	Average		3.35	0.33	0.24	4.02	0.71	0.04	0.21	0.25
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	0.80	0.04	0.25	0.32
	Average		3.12	0.31	0.25	4.52	0.73	0.04	0.22	0.31

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INGESTION OF HALOGENATED HYDROCARBONS - METHODS FOR INTRATRACHEAL ADMINISTRATION

Rebecca R. Malek

November, 1977

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Bio-Medical Research
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Midland, Michigan 48640

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Preliminary work was done in our laboratory with halogenated hydrocarbons (methyl chloroform, methylene chloride, [illegible] to develop a method for intratracheal administration of halocarbons. Even these limited studies have shown that aspiration 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 - METHOD
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 Haudek (1975) 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 directs 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 #30 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 (~2 hr. later) for one group and before regaining consciousness in another group.

The sedative-muscle relaxant, xylazine (Rompun® purchased from Haver-Lockhart), was administered 1.67 mg/kg to one group of rats .

After administration, rats were observed for 15 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	Test Conditions		
	Anesthesia With Methoxyflurane	Anesthesia w/Methoxyflurane and Cannulation	Anesthesia With Cannulation (Control)
Methylene Chloride	0.1 ml/kg Died 0.2 ml/kg Lived	0.1 ml/kg Died 0.04 ml/kg Lived	0.2 ml/kg Died 0.1 ml/kg Lived
Methyl Chloroform	0.4 ml/kg Lived 0.2 ml/kg Lived	0.2 ml/kg Died 0.03 ml/kg Lived	0.4 ml/kg Died 0.1 ml/kg Lived

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.

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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PHOTOCHEMICAL REACTIVITY OF TRICHLOROETHYLENE AND OTHER CHLORINATED SOLVENTS IN THE PRESENCE OF OTHER ORGANIC MATERIALS IN A SIMULATED ATMOSPHERIC ENVIRONMENT AS RELATED

Chemical Name (100 per name)	25	CAS No. (10)	24
METHYLENE CHLORIDE		75-09-2	

Figure 3-7. Global Indexing Form

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MIDLAND, MICHIGAN | DEPARTMENT: Chemicals Laboratory | DATE: 3-16-72

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 a 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), 94 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 (ULI-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-

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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 experiment 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% (33.5 hr.)	13% (16.9 hr.)	~3.5
$CCl_2=CCl_2$	44% (12.5 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
Cyclohexene	0.87	0.82	6.1
MtOAc	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.) [13.9][c]	6.4	7.0
Toluene	6.7%	5.2	7.6
iso-BuOH	3.5	3.3	~7.8
t-2-Butene	0.3	0.16	30% (5 hr.) [8.3][c]
n-BuOH	6.5	3.0	~8.5
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-TRIO, 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 $t_{1/2}$

E. 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.

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 methylchloroform, 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,

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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[b]
None	———	———	3.9
Butylene oxide	47% (14.9 hr.) [15.9][c]	12.4	4.0
n-BuOH	6.5	3.8	3.7
Cyclohexane	6.9	6.1	4.1
Cyclohexene	0.87	0.73	4.1
t-2-Butene	0.3	0.37	31% (2.0 hr.) [3.2][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	31% (2.0 hr.) [3.2][c]	0.37
Cyclohexene	0.87	0.37	0.37
n-BuOH	6.9	34% (2.9 hr.) [4.3][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 $t_{1/2}$.

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]
—	—	—	0.9
CH_3CCl_3	<5% (7.5 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	3.6	5.0
$CH_2=CHCl$	4.3	5.5	5.4
CHCl=CHCl (cis)	3.0	13.1	8.1
$CHCl=CCl_2$	3.5	8.7	4.9
$CCl_2=CCl_2$	38% (6 1/3 hr.)	26% (6 1/2 hr.)	43% (6 1/2 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 cyclohexene 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
$CHCl=CCl_2$ (TRI-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 $\xrightarrow[35\%\ R.H.]{h\nu}$

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	0.34	3.5	12.9	11.1	

other organic materials ranging from near = (at the emission point for trichloroethylene) to hour zero (some distance downwind from the emission 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	2.3

(a) Estimated

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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	—	47% (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[2] when only hydrocarbons are involved. When trichloroethylene is one

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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Doc Title 23

SOLVENT OPTION TO MEET EPA AIR QUALITY REGULATIONS

Chemical Name (300 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75-09-2

878211066

SOLVENT OPTION TO MEET EPA AIR QUALITY REGULATIONS

Solvent-borne coatings for the rail car industry are currently being confronted with air quality regulations mandating severe reductions in volatile organic compound (VOC) emissions. The EPA guidelines for the emissions of VOC relative to this industry are a maximum of 3.5 pounds of solvent per gallon of paint at the spray gun.* In the past several years, new coating technologies, such as high solids and waterborne, have been developed to meet these regulations. An additional option is the use of compliance chlorinated solvents, 1,1,1-trichloroethane and methylene chloride. The physical properties of these solvents often allows them to be formulated in many coatings, using the same resins and pigments. These solvents are fast drying solvents and, with no flash point, may reduce fire hazards. Coatings formulated with chlorinated solvents will be similar in overall performance to conventional solvent-borne coatings, using the same proven resins, using the same application methods, and providing additional flexibility in meeting air compliance.

Air Quality Regulations

As a part of its guidelines of VOC emissions, the EPA has published a list of compounds which have been shown to be of negligible photochemical reactivity (or nonsmog producing), and thus do not need to be regulated or inventoried as VOC. The exempt compounds are methane, ethane, trichlorotrifluoroethane, 1,1,1-trichloroethane, and methylene chloride. Due to this definition, the solvents--1,1,1-trichloroethane and methylene chloride--may be placed in coating formulations and not increase the VOC emission rate.[1-7]

The exemption of these two solvents from air regulations is not present in some states. Following the EPA guidelines, however, over 80% of these states having VOC emissions plans, have exempted 1,1,1-trichloroethane, and over 50% have exempted methylene chloride (See Table 1).

Toxicological Data

1,1,1-Trichloroethane and methylene chloride are among the few solvents that have been widely investigated from a toxicity standpoint due to their history of use in areas such as metal degreasing, adhesives, and aerosol formulations. Studies in the area of mutagenicity, teratogenicity, carcinogenicity, chronic toxicity, epidemiology, and environmental effects lead to a favorable position for these products. Specifically, evaluation of the data to date does not indicate carcinogenic, mutagenic, or teratogenic risk to humans.

As a means of providing a guideline of health hazard, the Occupational Safety and Health Administration (OSHA) has set the current maximum exposure levels for various compounds. For the solvents of interest, these are shown in Table 2. These values are the maximum concentrations of the compound allowable for an eight-hour per day, five-day per week average known as time-weighted average (TWA). It can be seen that the TWA for chlorinated solvents is similar to conventional solvents.

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*This VOC level is a federal CTG guideline; state VOC emission levels may be lower.

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Flammability

Due to concerns of flammability, chlorinated solvents have replaced flammable solvents to reduce fire hazards of coatings. Both 1,1,1-trichloroethane and methylene chloride show no flash point by either open or closed cup test methods, in contrast to conventional solvents, as can be seen in Table 3.

In many cases, chlorinated solvents, particularly methylene chloride, have been responsible for a substantial increase in flash point, thus reducing the risk of fire. Use of such coatings may lead to increased insurability.

Physical Properties

Aside from the flammability characteristics of these two solvents, a knowledge of the physical properties is important in formulating. The physical properties of various solvents are given in Table 4.

1,1,1-Trichloroethane and methylene chloride both have specific gravities of 1.3, which enables pigments to be suspended longer and makes resuspension easier after settling. These solvents have high evaporation rates. These rates may be controlled by the addition of slower evaporating cosolvents, such as a glycol ether.

The solubility parameters are similar to conventional solvents, such that with cosolvents, they may be used to formulate with many common resin families, such as alkyds, acrylics, epoxies, urethanes, chlorinated rubber, etc.

The chlorinated solvents should not be grossly contaminated with water. Chlorinated solvents thermally degrade above 400°F to hydrochloric acid. These do not pose a problem in many rail car paint shops. The compatibility of chlorinated solvent should be evaluated in each paint operation.

In general, with low solids formulation, these solvents can replace the controlled solvent. The remaining VOC can be modified to optimize evaporation and rheology. With high solids formulations, the chlorinated solvents may be used as non-VOC thinners for viscosity adjustment.

Aluminum Spray Equipment is Not Recommended

Aluminum fluid handling systems should not be used with chlorinated solvent-based coatings. Aluminum is a reactive metal and has been shown to be an unsuitable material of construction for equipment handling a variety of materials--glycol ethers, alcohols, salt water, acids, bases, halogenated solvents, and coatings formulated with these products. Aluminum should not be used as a material of construction for pumps, valves, nozzles, and other handling equipment, nor should it be used for storage containers for chlorinated solvents or chlorinated solvent-based formulations.

Recommended materials of construction are regular steel, stainless steel, or black iron. Some spray equipment manufacturers have pumps and many other

components currently available which are compatible with 1,1,1-trichloroethane and methylene chloride. Replacing aluminum equipment is a limitation for chlorinated solvents. As with any new compliance coating option, some component changes are required.

Cost/Effectiveness

1,1,1-Trichloroethane-based or methylene chloride-based coatings are expected to be slightly higher in cost compared to low solids coatings. They should be cost competitive with low VOC compliance systems, such as high solids coatings. Several factors should be considered when comparing cost of different compliance coating systems. These are:

1. Coating Performance - The coatings formulated with chlorinated solvent are essentially equivalent to the low solid coatings. This is due to formulating with the same resins and pigments. These coatings can achieve desirable film properties, such as good gloss and adhesion. Because of solvency characteristics, it has been noted that these coatings have high dirt tolerance and adhere better to poorly cleaned surfaces.

2. Equipment Costs - The chlorinated solvents usually require no change in fluid handling equipment but, as mentioned earlier, aluminum equipment should not be used. With these coatings, high pressure spray equipment and in-line heaters are not required. Due to evaporation rate, coatings may not require baking ovens to decrease dry time.

3. Energy - The chlorinated solvent-based coatings may be applied and allowed to dry essentially at ambient temperatures, therefore, minimizing the need for heaters. The viscosity adjustment available with this compliance solvent option eliminates the need for in-line heaters, thus an energy savings.

4. Training - Retraining of shop personnel and changes made to application procedures are expected when converting to low VOC coatings. Chlorinated solvent coatings may be applied as low solids coatings were; therefore, minimal retraining is required. Additional information regarding to solvent characteristics and proper handling should be given to all those involved with these solvents.

Certainly other cost considerations may prove important, but these vary from location to location.

Conclusion

1,1,1-Trichloroethane and methylene chloride as paint solvents offer the railroad industry the ability to comply with air quality regulations safely. Resin systems, familiar to the end user, can still be utilized providing a proven performance and fast dry times. There is no need for major procedural changes or extensive retraining of operators on new equipment. The use of 1,1,1-trichloroethane or methylene chloride may be the most feasible and economical means to obtain air quality compliance.

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TABLE 1

STATUS OF 1,1,1-TRICHLOROETHANE AND METHYLENE CHLORIDE IN VOC SIP EXEMPTIONS

(The following data is subject to change)

	1,1,1	Methylene Chloride
Alabama	♦	-
Alaska	0	0
Arizona	0	0
Arkansas	♦	♦
California		
Bay Area	♦	♦
Southcoast	♦	♦
Colorado[a]	-	-
Connecticut[b]	-	-
Delaware	♦	♦
D of Columbia	♦	♦
Florida	♦	♦
Georgia	♦	-
Hawaii	0	0
Idaho	0	0
Illinois	-	-
Indiana	♦	♦
Iowa	0	0
Kansas	♦	♦
Kentucky	-	-
Louisiana	♦	♦
Maine	0	0
Maryland	♦	-
Massachusetts	♦	-
Michigan[c]	-	-
Minnesota	♦	♦
Mississippi	♦	♦
Missouri	♦	-
Montana	♦	-
Nebraska	♦	-
Nevada	0	0
New Hampshire	♦	♦
New Jersey	-	-
New Mexico	0	0
New York	♦	♦
N. Carolina[d]	(♦)	(♦)
N. Dakota	0	0
Ohio	♦	♦
Oklahoma	♦	♦
Oregon	♦	♦
Pennsylvania[e]	♦	♦
Rhode Island	-	-
S. Carolina	♦	♦
S. Dakota	0	0
Tennessee	♦	-
Texas	♦	-
Utah	♦	♦
Vermont	-	-
Virginia	♦	♦
Washington	♦	♦
W. Virginia	0	0
Wisconsin	(♦)	(♦)
Wyoming	0	0

♦ = Exempt
- = Not Exempt
( ) = Tentative
0 = No VOC SIP Necessary

[a]Colorado exempts for degreasing and cleaning only.
[b]Connecticut exempts for metal cleaning only.
[c]Michigan exempts both solvents for new sources only.
[d]N. Carolina's exemption is not across-the-board, but does include coating of miscellaneous metal parts and products, flat wood panaling, and graphic arts.
[e]Pennsylvania exempts for coatings, but not metal cleaning.

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TABLE 2

Current Maximum Exposure Levels of Various Solvents

Solvent	OSHA TWA 8-Hour ppm
1,1,1-Trichloroethane	350
Methylene Chloride	500
n-Hexane	500
Toluene	200
Methyl Ethyl Ketone	200
Acetone	1000
Ethyl Acetate	400

TABLE 3

Flammability Hazards

Solvents	Flash Point Open Cup (°F)	Flash Point Closed Cup (°F)	Flammable Limit In Air 20°C (Volume %) Upper	Lower
Methylene Chloride	NF	NF	None*	
1,1,1-Trichloroethane	NF	NF	10.5	8.0
n-Hexane	--	-7	6.9	1.25
Toluene	45	40	7.0	1.3
Methyl Ethyl Ketone	--	30	11.5	1.81
Methanol	54	50	36.5	6.72
Ethyl Acetate	30	24	11.0	2.25

*12-19% (volume) in air at 30°C

TABLE 4

Physical Properties

Solvents	bp (°C)	Sp. Heat (cal/g/°C)	Heat of Vaporization	Evaporation Rate (n-butyl Acetate) = 1
Methylene Chloride	40.0	0.24	78.7	14.5
1,1,1-Trichloroethane	74.0	0.28	56.7	6.0
n-Hexane	68.7	0.53	82.0	8.1
Toluene	110.6	0.39	86.0	1.5
Methyl Ethyl Ketone	79.6	0.498	106.0	7.7
Methanol	64.8	0.597	262.8	3.5
Ethyl Acetate	77.2	0.470	87.63	4.1

Solubility

Solvents	Solubility Parameter δ	H Bonding (k)	(g) H_2O Soluble in 100g Solvent (25°C)
Methylene Chloride	9.7	2.7	0.20
1,1,1-Trichloroethane	8.6	2.6	0.05
n-Hexane	7.3	2.1	0.01
Toluene	8.9	3.3	0.05
Methyl Ethyl Ketone	9.3	5.0	11.0
Methanol	14.5	6.5	--
Ethyl Acetate	9.1	5.2	3.3

Physical Properties

Solvents	Specific Gravity	Pounds/Gallon 20°C	dyn/cm Surface Tension
Methylene Chloride	1.336	10.98	26.52
1,1,1-Trichloroethane	1.3249	10.99	25.56
n-Hexane	0.6594	5.66	17.91
Toluene	0.866	7.26	30.00
Methyl Ethyl Ketone	0.8051	6.71	24.6
Methanol	0.7924	6.60	22.69
Ethyl Acetate	0.9020	7.51	23.9

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CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-09-2

D-240

878211297

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CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

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CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

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INTRODUCTION

We report here a study of cytogenetic aberrations in rats exposed to methylene chloride (CH_2Cl_2). This investigation was undertaken as part of a 24-month inhalation study of chronic toxicity of methylene chloride in rats and hamsters that is being conducted by the Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, Michigan, and which is scheduled for completion in April, 1978.

Cytogenetic evaluation is a recognized and useful tool for the estimation of genetic risk (Kucerova, 1976). The value of *in vivo* cytogenetic evaluation lies in the fact that effects of various mutagenic agents upon the genetic material--the chromosomes--of the living animal can be directly observed. Since specifically mammalian metabolic processes are taken into account, cytogenetic studies in mammals exposed to high levels of a suspect agent can be particularly useful in arriving at judgments about the dimensions of potential risk to members of the human species exposed to lower concentrations of the substance.

MATERIALS AND METHODS

Four groups of ten Sprague-Dawley albino rats (Spartan sub-strain, SPF-derived) were exposed in inhalation chambers of the

*Toxicology Research Laboratory, Health and Environmental Research, Midland, Michigan

Midland Toxicology Research Laboratory to 3500, 1500, 500, and 0 (control) concentrations of methylene chloride in air for 6 hrs/day, 5 days/week for six months. The animals were group-caged and kept in the chamber with a supply of clean air during the non-exposure intervals. Food was removed during the exposure periods, but was otherwise freely available. Water, via an automatic watering system, remained available at all times. The animals were evaluated daily for signs of toxicity and changes in appearance and behavior. The results of these observations and other findings will be reported separately.

The five rats of each sex to be killed at the end of each of the four six-month exposure periods and used for cytogenetic evaluations were identified before the exposures began.

Following sacrifice, bone marrow cells were collected and cultured, and coded slides were prepared as described in Table 1. Blind analysis of the slides was performed in the Comparative Toxicology Research Laboratory of Occupational Health and Medical Research, Freeport, Texas, by four accredited medical technicians. The cells were evaluated and scored by the criteria of the World Health Organization (Buckton and Evans, 1973). Two hundred cells per animal were scored. The cells were evaluated for chromatid and chromosome breaks, dicentrics, rings, and exchanges; miscellaneous aberrations; severe damage, and the number and percentage of abnormal cells. An abnormal cell is defined as a cell with at least one aberration or abnormality. Findings for each cell, along with

the vernier coordinates, were recorded on a score sheet similar to Figure 3 in Killian et al. (1977). A photograph was made of every abnormal cell scored.

RESULTS

Results of the cytogenetic evaluation are shown in the Tables 2 and 3. Very few aberrations were found in any of the control or test animals. In the total of 8000 cells examined, only 37 (0.46%) abnormal chromosomes were found. Most of the aberrations were chromatid breaks (27) and chromosome breaks (7). One exchange figure was observed at the 1500 ppm dose, and 2 rings were noted at the 3500 ppm dose. Analysis of variance showed that none of the aberration rates in any of the treated groups was significantly different at P≤0.05 from the control group. Also, when all the so-called "stable" aberrations (dicentrics, rings and exchanges) are pooled, the differences are not significant.

SUMMARY

Four groups of 10 Sprague-Dawley albino rats (Spartan sub-strain, SPF-derived) were exposed to 3500, 1500, 500 and 0 (control) ppm of methylene chloride in air for 6 hrs/day, 5 days/week for 6 months. Bone marrow cells from five rats of each sex killed at the end of each of four 6-month exposure periods were collected and cultured for cytogenetic evaluation. Blind analysis of 200 cells per animal was performed by four accredited medical technicians of Occupational Health and Medical Research, Freeport, Texas. The cells were evaluated for chromatid and chromosome breaks; dicentrics, rings, and exchanges; miscellaneous

aberrations; severe damage; and the number and percentage of abnormal cells, an abnormal cell being defined as a cell with at least one aberration or abnormality. It was found that values for the various aberration categories at the 3500, 1500, and 500 ppm dose levels were not significantly different from the control group, and it was concluded that long-term, intermittent, relatively-high concentrations of methylene chloride in air are unlikely to induce increased cytogenetic aberration rates in bone marrow cells of rats of this sub-strain.

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TABLE 1

PROCEDURES FOR CELL COLLECTION AND SLIDE PREPARATION FOR CHROMOSOME ANALYSIS

A. Cell Collection and Preparation

1. The rats were injected intraperitoneally with colchicine (0.4 mg/kg) four hours before being decapitated. The head of the femur was removed and the bone marrow was aspirated using a 10 cc syringe and a 20 gauge needle.

2. The bone marrow cells were mixed in 10 ml Hank's balanced salt solution using a disposable pipette.

3. The cells were centrifuged at 3000 RPM for 10 minutes.

4. The Hank's balanced salt solution was removed and 10 ml of 0.4% KCl was added to the tubes. They were then incubated for 30 minutes in a 37° water bath. (Critical)

5. The cells were then centrifuged for 10 minutes at 3000 RPM.

6. The supernatant was removed and a 3:1 methanol/acetic acid fixative was used to resuspend the cells.

7. The cells were then stored at 4°C for later preparation of slides.

B. Slide Preparation

1. Centrifuge as in A-3 above, decant the fixative and add fresh 3:1 fixative.

2. Remove the slides and place approximately 5 drops of a cell suspension on a slide; pass the slide through a flame. The slides were then dried on a warming plate at 60°.

3. The slides were rinsed gently in distilled H_2O and were placed in Giemsa Stain for 10 minutes. Dehydration was through 2 acetone washes, 2 acetone-xylol changes. The slides were coverslipped from xylol using Harleco Synthetic Resin (HSR) as a mounting medium and were dried in an oven overnight.

TABLE 2. CYTOGENETIC ABERRATIONS DETECTED IN BONE MARROW CELLS FROM RATS EXPOSED TO 3500, 1500, 500 AND 0 PARTS PER MILLION CONCENTRATIONS IN AIR OF METHYLENE CHLORIDE FOR 6 HR/DAY, 5 DAY/WEEK FOR 6 MONTHS

Animal Number	Dose PPM	Chromatid Break w/ Frag-ment	Chromatid Break w/o Frag-ment	Chromosome Break w/ Frag-ment	Chromosome Break w/o Frag-ment	Dicen-trics	Rings	Exchanges	No. of Abnormal Cells
3302	0	1	0	0	0	0	0	0	1
3303	0	0	0	0	0	0	0	0	0
3304	0	0	0	0	0	0	0	0	0
3305	0	3	0	1	0	0	0	0	4
3306	0	2	0	0	0	0	0	0	2
4076	0	1	0	1	0	0	0	0	2
4077	0	0	0	0	0	0	0	0	0
4078	0	1	0	0	0	0	0	0	1
4079	0	0	0	0	0	0	0	0	0
4080	0	1	0	0	0	0	0	0	1
	X =	0.9	0	0.2	0	0	0	0	1.1
	SD =	0.99	0	0.42	0	0	0	0	1.26
3947	500	0	0	0	0	0	0	0	0
3948	500	0	0	1	0	0	0	0	1
3949	500	1	0	1	0	0	0	0	2
3950	500	0	0	0	0	0	0	0	0
3951	500	1	0	0	0	0	0	0	1
4721	500	1	0	0	0	0	0	0	1
4722	500	0	0	0	0	0	0	0	0
4723	500	0	0	0	0	0	0	0	0
4724	500	0	0	0	0	0	0	0	0
4725	500	2	0	0	0	0	0	0	1
	X =	0.5	0.0	0.2	0	0	0	0	0.6
	SD =	0.707	0.0	0.42	0	0	0	0	0.699
3818	1500	0	0	0	0	0	0	0	0
3819	1500	0	0	0	0	0	0	0	0
3820	1500	0	0	0	0	0	0	0	0
3821	1500	0	1	0	0	0	0	0	1
3822	1500	0	0	0	0	0	0	0	0
4392	1500	1	0	0	0	0	0	0	1
4393	1500	0	0	0	0	0	0	0	0
4394	1500	0	0	0	0	0	0	0	0
4395	1500	1	0	0	0	0	0	1	1
4396	1500	3	0	1	0	0	0	0	1
	X =	0.5	0.1	0.1	0	0	0	0.1	0.6
	SD =	0.97	0.316	0.316	0	0	0	0.316	1.229
3688	3500	1	0	0	0	0	1	0	2
3690	3500	1	0	0	0	0	1	0	2
3691	3500	1	0	0	0	0	0	0	1
3692	3500	0	0	0	0	0	0	0	0
3693	3500	1	0	1	0	0	0	0	2
4463	3500	1	0	1	0	0	0	0	2
4464	3500	0	0	0	0	0	0	0	0
4465	3500	1	0	0	0	0	0	0	1
4466	3500	0	0	0	0	0	0	0	0
4467	3500	1	0	0	0	0	0	0	1
	X =	0.7	0	0.2	0	0	0.2	0	1.1
	SD =	0.48	0	0.421	0	0	0.421	0	0.87
	F =	0.33	1.0	0.157			2.29	1.0	0.342
	P =	0.65	0.405	0.921			0.098	0.40	0.660

[a]Both chromatid breaks were in the same cell.

TABLE 3. SUMMARY OF CYTOGENETIC ABERRATIONS DETECTED IN BONE MARROW CELLS FROM RATS EXPOSED TO 3500, 1500, 500, and 0 PARTS PER MILLION CONCENTRATIONS IN AIR OF METHYLENE CHLORIDE FOR 6 HR/DAY, 5 DAY/WEEK FOR SIX MONTHS

				TYPE OF ABERRATION					
	No. of Rats	No. Cells Scored	Dose	Chromatid Breaks	Chromosome Breaks	Dicentrics, Rings, Exchanges, Misc.	Severely Damaged	No. Abnormal Cells	Percent Abnormal Cells
	10	2000	0	9	2	0	0	11	0.55
	10	2000	500	5	2	0	0	6	0.30
	10	2000	1500	6	1	1[a]	0	8	0.40
	10	2000	3500	7	2	2[b]	0	11	0.55
TOTAL	40	8000		27	7	3	0	36	avg. 0.45

[a] Exchange

[b] Rings

000010

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OFFICE OF TOXIC SUBSTANCES — REV. 7/27/82

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Doc Type 22

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Doc Title 23

TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZZARD OF AEROTHENE MM SOLVENT

L.K 5-27-83

Chemical Name (300 per name) 25 | CAS No. (10) 24

DICHLOROMETHANE | 75-09-2

1A

D-247 878211298 OFFICE COPY

THE DOW CHEMICAL COMPANY
BIOCHEMICAL RESEARCH LABORATORY

TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF

AEROTHENE® MM SOLVENT

FILE
K NO. CHG
REF.
SOURCE
SUB. BY
REP'T. BY NLitchfield-JMNorris
CHECKED BY T. R. Torkelson
DATE August 11, 1969

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ADDITIONAL INFORMATION INCLUDING THE EFFECTS OF REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND FORMULATIONS ARE DEVELOPED OR IF PROCESS CHANGES OCCUR.

A sample of Aerothene® MM was submitted to the Biochemical Research Laboratory for toxicological evaluation and definition of industrial handling hazards. Aerothene® MM is a specially inhibited grade of dichloromethane used by the aerosol industry.

The test material has a moderate acute oral toxicity. The single dose oral LD_{50} for female rats is approximately 750 mg/kg. The test material should present no problem from ingestion incidental to industrial handling. It should be emphasized, however, that ingestion of relatively small amounts may result in adverse effects.

Direct contact of the undiluted material with the eye of a rabbit resulted in moderate pain, moderate redness and swelling of the conjunctival membranes, slight transient corneal injury, and slight iritis. Washing of a similarly exposed eye 30 to 60 seconds after contact did not lessen this response. On the 8th day post-instillation, the eye appeared normal.

Minimum eye protection is recommended whenever the likelihood of eye contact exists.

A single prolonged exposure of the undiluted material with the skin of a laboratory rabbit resulted in slight skin redness on the inner surface of the ear and moderate skin redness, slight edema and a slight chemical burn on confined intact and abraded skin. Subsequent prolonged exposures did not cause any increased skin response on the ear. However, the degree of the chemical burn on the confined belly skin increased to moderate. Healing of the burned areas occurred with a moderate scab formation. All exposure sites displayed slight exfoliation. The animal on this test showed a normal body weight gain.

®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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Brief human skin contact with Aerothene MM solvent would not likely result in a skin response. Prolonged exposures, particularly when covered by clothing, shoes or gloves would likely produce skin redness, blistering and a chemical burn. Frequent and prolonged skin exposures would probably cause scaling of the skin. Skin exposures would not result in adverse effects due to absorption of the material through the skin.

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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Doc Type 22
• RI • UP • HEAD 8D SW HS FN

Doc Title 23
THE TOXICITY OF METHYLENE CHLORIDE

Chemical Name (3/0 per page) 25	CAS No. (10) 26
METHYLENE CHLORIDE	75-09-2

D-248

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Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

Subject THE TOXICITY OF METHYLENE CHLORIDE

File
Chg.
Rec'd 5-14-41
Fin'd 7-13-42
Work By E. M. Adams

To File Report Check D. O. Fick 10-11-42 Rept. By E. M. Adams

Acute Oral Toxicity:

The acute oral toxicity was determined for rats. The 100% survival dose was 1.2 g./kg. The 100% fatal dose was 4.0 g./kg.

Further work had been planned, but plans were dropped when it was learned that the National Institute of Health was planning a very extensive investigation.

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BIOCHEMICAL RESEARCH LABORATORY

Material: Methylene chloride

ACUTE ORAL TOXICITY

Animals Rats

Dose (cm/kilo)	Number of animals that died	Number of animals that survived
1.0	0	5
1.2	0	5
1.6	3	2
2.0	6	4
3.0	9	1
4.0	5	0

Biochemical Research Laboratory
THE DOW CHEMICAL COMPANY

Subject: ACUTE VAPOR TOXICITY OF METHYLENE CHLORIDE.

File
Chg.
Rec'd
Fin'd 6-21-44
Work By V. K. Rowe

To File Report Check Edwin E Dunn Rept. By V. K. Rowe
6-21-44

Acute Vapor Toxicity

A few experiments were carried out in which rats were exposed to exceedingly high concentrations of methylene chloride in a 10 L glass jar. The results are as follows:

100 Mg./l.

Animals survived 15, 30 and 60 minute exposures with no apparent ill effects. The symptoms:

Wildness for about 2 minutes
Quiet for up to 15 minutes
Unconsciousness from 15 minutes on
Salivation very prominent in the longer exposures.

Recovery was very rapid upon removal - being complete in about 1 hour.

150 Mg./l.

Animals became markedly unsteady in about 1 minute, were off their feet in 3 minutes, unconscious after 5, gasping for breath after 10, and usually died after 15 to 25 minutes of exposure.

Shorter exposures were not made.

200 Mg./l.

Animals exhibited almost the identical symptoms as mentioned for 150 mg./l. exposure except that 3 animals survived a 15 minute exposure.

Conclusions

These experiments were meager and conclusions from them must be limited. It seems however, that exposures of up to 60 minutes in length which do not cause unconsciousness, probably will not cause death. Furthermore it seems highly improbable

that a persons health would be impaired by any exposure he would voluntarily tolerate for a period not exceeding one hour in length.

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Subject DICHLOROMETHANE (METHYLENE DICHLORIDE).

File
Chg.
Rec'd [illegible]
Fin'd [illegible]
Work By [illegible]

To Check Rept. By H. C. Spencer 9-11-44

COMMENTS ON PROPOSED SAFETY ORDERS

It seems entirely unreasonable to place a limit of 0.1% on dichloromethane in order 1. To begin with, the vapor toxicity of dichloromethane is of an entirely different order than that of yet both are given the same limit in this order. According to recent work by the United States Public Health Service, dichloromethane is by far the least toxic of all the commonly used chlorinated hydrocarbons. Furthermore, as pointed out in the discussion of this order 1 is entirely out of line with the agreement between the United States Public Health Service and the manufacturers of and/or similar volatile chlorinated liquid hydrocarbons which provides for the labelling of all containers in excess of fifteen fluid ounces of mixed solvents containing 24% or over of or similar volatile chlorinated hydrocarbons. If this agreement is used as the basis for labelling, then a limit of 50% or more might be considered reasonable for dichloromethane.

HAZARDS

Since dichloromethane is the least toxic of the commonly used chlorinated aliphatic hydrocarbons, its handling

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and use involves relatively few hazards. The chief hazard involved in its handling and use is probably that of repeated or prolonged exposures to relatively high vapor concentrations (above 500 parts per million), however, such exposures can be prevented with reasonable care. A minor problem is that of dermatitis due to excessive contact of the skin with dichloromethane.

TOXICITY

Single exposure to the vapor of dichloromethane produces anesthesia in laboratory animals and man at concentrations somewhat greater than 5000 parts per million (17 mg./l.). Recovery from anesthesia is, as a rule, uneventful. Liver damage is not commonly produced by single or even repeated exposures to dichloromethane vapor.

In the recent work by the United States Public Health Service, repeated exposures to 5000 parts per million dichloromethane were well tolerated by several species of animals throughout a period of six months. No data are available concerning the effects on human subjects of repeated exposures to dichloromethane, however, from their initial work the United States Public Health Service group tentatively suggests 500 parts per million as the maximum allowable concentration for 8-hour daily exposure for man. Quite probably this suggested limit is too conservative as compared with the "limits" that are widely accepted for the other commonly used chlorinated hydrocarbons, nevertheless, it emphasizes the relatively low

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File
Subject TOXICITY OF DICHLOROMETHANE ("METHYLENE CHLORIDE" OR "METHYLENE BICHLORIDE") -- A LITERATURE SURVEY
Chg.
Rec'd. 10-11-40
Fin'd. 10-10-40
Work By H. C. Spencer

To Check 10-24-40 Rep. By H. C. Spencer

Summary

Acute Oral Toxicity to Dogs:

Dichloromethane	3.0 g. per kg.
Chloroform	2.35 g. per kg.
CCl_4	4.0 g. per kg.

Acute Vapor Toxicity to Laboratory Animals:

Order of increasing acute vapor toxicity for laboratory animals: Dichloromethane < methyl chloride < carbon tetrachloride < chloroform.

Minimal Narcotic Concentration for Mice:

Dichloromethane	0.00041 mol. per l.
CCl_4	0.00035 mol. per l.
$CHCl_3$	0.00017 mol. per l.

Pathological Lesions:

The chief pathological lesion produced by these compounds is fatty degeneration of the liver; however, dichloromethane produces fatty degeneration only under the most severe conditions, while small quantities of chloroform or carbon tetrachloride produce marked degeneration.

vapor toxicity of dichloromethane as compared with the other members of this group.

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Toxicity of Dichloromethane to Man:

Under conditions of good ventilation, dichloromethane is considered to be "practically harmless"(4). At least the vapors of this compound are much less toxic to man than the vapors of CHCl, or CCl, and even possibly somewhat less toxic than the vapors of methyl chloride.

Material

Name: Dichloromethane
Synonyms: "Methylene chloride" or "Methylene dichloride"
Formula:

Formula weight: 84.93
Form: Colorless liquid
Specific gravity: 1.336 20°/4
Melting Point: -96.7° C.
Boiling Point: 40-41° C.
Solubility: Water - 2 parts per hundred at 20° C.
Alcohol - Soluble.
Ether - Soluble.

Conversion factors for a gas, M.W. = 85, at 25° C. and 760 mm. Hg. barometric pressure (1): 1 mg. per liter = 288 ppm. 1 ppm. = 0.00348 mg. per liter.

Fire and Explosive Hazards

"Methylene chloride (dichloromethane) is practically nonflammable and nonexplosive at ordinary temperatures, but at higher temperatures under favorable laboratory test conditions is capable of forming weakly combustible mixtures with air. Formation of combustible mixtures by methylene chloride, however, under practical conditions even at higher temperatures is extremely unlikely, and its fire hazard is therefore very small." (2)

Toxicity of Dichloromethane to Laboratory Animals

Oral Toxicity:

Flühmer (quoted by von Oettingen (3)) found that with oral administration in rabbits 1.1/3 cc. per kilogram gave semi-sleep and light narcosis, whereas 1.6 cc. per kilogram caused deep narcosis but was fatal after 39 hours. Barsoum and Saad (quoted by von Oettingen (3)) found the minimal fatal dose for dogs to be 5.0 g. per kg.

Intravenous Toxicity:

The lethal dose by intravenous injections is 800 mg. per kg. in the dog as compared with 90 mg. per kg. for chloroform (Barsoum and Saad, quoted by Browning (4)).

Subcutaneous Toxicity:

The lethal dose by subcutaneous injection is 2.7 g. per kg. in the rabbit as compared with 0.99 g. per kg. for chloroform (Barsoum and Saad, quoted by Browning (4)).

Comparative toxicity of the chlorinated methane derivatives as determined by Barsoum and Saad (quoted by von Oettingen (3)).

Compound	Intravenous injection dogs, g/kg.	Orally dogs g./kg.	Subcutaneous injections rabbits, g/kg.
Dichloromethane	0.800	5.0	2.7
Chloroform	0.090	2.25	0.9
Carbon tetrachloride	0.125	4.0	--

Vapor Toxicity:

According to Lazarew (quoted by Browning (4)) the lethal concentration on inhalation is 80 mg. per l. (14,500 ppm) in the mouse, as compared with 30 to 40 mg. per l. for chloroform. Unfortunately the length of exposure is not stated. Pantelitsch (quoted by Browning (4)) gives the lethal concentration as 45 mg. per liter for 110 minutes, or 50 mg. per liter for 100 minutes. It is not stated what experimental animal was used.

The results of Flury's experiments on dichloromethane (quoted by von Oettingen (5)) are given in the following tables.

Concent. mg./l.	Concentration ppm	Symptoms
(A) Six hours' exposure of mice		
80	5,800	Side position after 4-1/2 hours, deep narcosis after six hours, recovery after 2-3 hours.
(B) Two hours' exposure of mice		
50-55	8,700-10,000	Side position after 2 hours. 55 mg. per l. necessary to cause deep narcosis.
80	14,500	Was necessary to cause death in 2 hours.
24	7,000	Did not cause side position in 2 hours.
80	14,500	Caused side position in 8-15 minutes.
62-65	18,000	Caused side position in 6-11 minutes. With 1-1/2 hours exposures late death occurred after from 1/2 hour to 5 days.

Nuckols (2) found that dichloromethane in concentrations of the order of 2-1/2 percent by volume for durations of exposure of the order of 8 hours does not cause the death of guinea pigs; however, concentrations of the order of 5 percent by volume for durations of exposure of the order of 1/2 hour are lethal to guinea pigs. Nuckols (2) showed that dichloromethane is decomposed by flame or hot surfaces with the formation of toxic fumes, which are, however, exceedingly irritating and therefore give adequate warning of their presence.

Nuckols (2) gives the following data on the comparative vapor toxicity of the chlorinated methane compounds:

Compound	Kills or seriously injures Guinea pigs: Concentration in Air: % by volume	ppm	Mg./l.	Duration of exp., hours	Order of increasing toxicity
Methyl chloride	2.0-2.5	20,000-25,000	41.5-51.6	8	2
Methylene chloride *	5.1-5.3	51,000-55,000	177-183	0.5	1
Chloroform	2.0-2.2	20,000-22,000	98-108	1	4
Carbon tetrachloride	2.0-2.5	20,000-25,000	139-145	1	3

* Methylene chloride at 2 to 2-1/2 percent by volume does not cause death in guinea pigs exposed for 8 hours.

Comparative vapor toxicity of the chlorinated methane derivatives as determined by Müller and by Lazarew (quoted by von Oettingen (3)).

Compound	(Müller) mol./l.	(Lazarew) mol./l.	Fatal concent. for mice (Lazarew) mol./l.	Fatty deg. of liver (Lazarew)
Dichloromethane	0.00074	0.00041	0.00059	+
Chloroform	0.00033	0.00017	0.00039	++
CCl_4	0.000525	0.00032	0.00044	+++

Response Produced by Dichloromethane in Laboratory Animals

Dichloromethane is a narcotic, but is somewhat weaker than chloroform in its action (4). Hellwig (quoted by Browning (4)) considers it to be 2-1/2 times weaker as a narcotic than chloroform. von Oettingen (3) cites several articles in which different workers have shown that the narcotic action of these compounds increases in the following order:

$$CH_2Cl_2 < CCl_4 < CHCl_3$$

Dichloromethane has been used as an anesthetic, however, several undesirable side-reactions have discouraged its usage. Upon inhalation, dichloromethane produces an alarming excitation stage, with labored breathing, cyanosis, dilated pupils and a rapid weak pulse.

Dichloromethane has been found to be quite toxic to the heart and numerous pharmacological experiments on isolated hearts are quoted by von Oettingen (3). Barcroft and Saad (quoted by Browning (4)) working with the toad heart found:

	Chloroform	Dichloromethane
Lowest concentration that can arrest heart	1:2,000	1:1,000
Strongest concentration with no decided action	1:100,000	1:58,000

Thus the action of chloroform is approximately twice that of dichloromethane.

The hemolytic action as determined by Pleta and quoted by von Oettingen (3) is as follows:

Dichloromethane	0.048 mol. per l.
Chloroform	0.010 mol. per l.
Carbon tetrachloride	0.0016 mol. per l.

Under extremely severe conditions dichloromethane produces fatty degeneration of the liver, however, its activity in this respect is not nearly as marked as is that of chloroform or carbon tetrachloride. In actual animal experiments it has been difficult to produce fatty degeneration of the liver by administration of dichloromethane.

Toxicity of Dichloromethane to Man

Quoting from Browning (4): "The opinion of most authorities on the possibility of industrial poisoning by methylene dichloride, is summed up by

Zernik (1933) in stating that, if free from other products (petroleum distillates, chlorinated hydrocarbons) and under conditions of good ventilation it is practically harmless. With this opinion Collier (1936) seems inclined to disagree; he reports several cases of 'acute intoxication', from its use as a paint remover. The symptoms in the two most severely affected patients who had to leave work were, however, largely subjective, and the evidence was rendered to some extent indefinite by the presence of intercurrent affections, lead poisoning in the one and duodenal ulcer in the other."

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FATE AND EFFECTS OF METHYLENE CHLORIDE IN ACTIVATED SLUDGE

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METHYLENE CHLORIDE | 75-ϕ9-2

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FATE AND EFFECTS OF METHYLENE CHLORIDE IN ACTIVATED SLUDGE

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Date Study Completed: December 9, 1981

ABSTRACT

The fate and effects of methylene chloride in a system simulating a municipal wastewater treatment plant were investigated. Activated sludge, collected at the Midland Municipal Wastewater Treatment Plant, was used to establish a laboratory-scale activated sludge unit. The sludge was fed on a daily "fill and draw" cycle with synthetic sewage and supplemented with 100 mg/L methylene chloride. After an 11-day acclimation period, the continuous release of chloride was detected in the system. Subsequently, the acclimated sludge was used in closed-bottle, respirometer studies. Results of the investigation indicated the disappearance of 100 mg/L of ^{14}C-labeled methylene chloride within 10 hours, with 65% of the parent compound recovered as ^{14}C-labeled carbon dioxide after 50 hours. Experiments conducted at methylene chloride concentrations of 10 mg/L and 1 mg/L, indicated acclimation of the laboratory-scale sludge units after 9 and 11 days, respectively. Respirometer studies demonstrated the disappearance of 10 mg/L of ^{14}C-labeled methylene chloride within 4 hours, with 49% of the parent compound recovered as ^{14}C-labeled carbon dioxide after 50 hours. Respirometer studies conducted at 1 mg/L methylene chloride indicated disappearance of the substrate within 3 hours, with a 66% conversion of the parent compound to carbon dioxide after 50 hours. Rate constants, calculated for the biodegradation of 1, 10, and 100 mg/L of methylene chloride by activated sludge, were 1.42, 1.61, and 0.35 hours^{-1}, respectively.

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Respirometer studies were conducted to determine the ability of activated sludge, previously acclimated to 100 mg/L methylene chloride, to dehalogenate a variety of chlorinated test substrates, including methylene chloride, chloroform, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, and 1,1,2-trichloroethylene. Results of the investigation indicated an apparent strict substrate specificity, as the release of chloride was detected only in the respirometer containing methylene chloride.

Inhibition studies were conducted to investigate the effect of methylene chloride on oxygen consumption and organic carbon degradation by activated sludge. Aliquots of activated sludge were placed into closed-bottle respirometers and exposed to methylene chloride concentrations of 0, 10, 100, and 1000 mg/L. Methylene chloride had no significant effect on oxygen consumption or the removal of total organic carbon by activated sludge over a 24-hour period.

A hypothetical model was developed to examine the significance of volatilization and biodegradation for the removal of methylene chloride from a continuously mixed, activated sludge reactor. Application of the model indicated that the rate of biodegradation was approximately 12 times greater than the rate of volatilization. Furthermore, biodegradation accounted for the removal of 83% of the influent concentration of methylene chloride, whereas only 7% was removed by volatilization from the hypothetical activated sludge reactor. Thus, biodegradation may be the predominant mechanism determining the fate of methylene chloride in activated sludge systems continuously exposed to the compound.

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INTRODUCTION

Simple chlorinated hydrocarbons, such as methylene chloride, are widely used as solvents for cleaning and extraction, and for a variety of other industrial applications. These compounds may enter wastewater treatment systems as a result of spills, or through the routine discharge of waste. The substantial use of chlorinated hydrocarbon solvents raises the question of the fate and effects of these compounds in the environment, and specifically on sewage treatment processes.

For many years, methylene chloride has been generally considered to be nonbiodegradable. Results of the biochemical oxygen demand (BOD) test for methylene chloride indicate that negligible oxygen is consumed over a twenty-day test period (1). However, recent evidence indicates that methylene chloride is in fact biodegradable under both aerobic and anaerobic conditions.

Brunner and Leisinger (2) first reported the isolation of a facultative methylotroph with the ability to utilize methylene chloride as a sole carbon source for growth. The organism was isolated following an extensive chemostat enrichment, and tentatively identified as a Pseudomonas species. The isolate was determined to be a strict aerobe that was capable of growth on methylene chloride, methanol, formate, methylamine, and several other two- and four-carbon compounds. Growth of the organism on the methylene chloride resulted in the release of chloride ion from the substrate. Recent observations of Brunner, Staub and Leisinger (3) indicate the utilization of methylene chloride by a Pseudomonas species is due to the

presence of an inducible enzyme. Furthermore, activity of the enzyme was found to be only partially inhibited when the reaction was carried out under anaerobic conditions. This latter observation suggests that a halidohydrolase, rather than a monooxygenase, is responsible for the initial oxidation of methylene chloride.

Rittmann and McCarty (4) investigated the utilization of methylene chloride by sewage microorganisms. Bacterial cultures were enriched from a seed of primary sewage effluent over a 12-month period. Methylene chloride supported bacterial growth when the mineral salts culture medium was supplemented with sodium bicarbonate. Microscopic examination of the enrichment cultures indicated the presence of motile, gram-negative, rod-shaped bacteria. Batch culture experiments conducted to demonstrate methylene chloride degradation indicated the rate of utilization was dependent on the size of the microbial population. Disappearance of the substrate was not observed in sterile controls. Although the results of the study indicated that methylene chloride supported bacterial growth, it was impossible to determine whether complete mineralization occurred, as neither the formation of intermediates nor carbon dioxide evolution were monitored.

Wood et al. (5) have recently demonstrated the degradation of methylene chloride under anaerobic conditions. Sediment-water samples, obtained from the Florida Everglades, were placed into sealed serum bottles and spiked with 200 µg of methylene chloride. Degradation of the parent compound was found to proceed via methyl chloride, suggesting a mechanism involving reductive dehalogenation of the substrate. Methylene chloride was not

degraded in autoclaved controls. Complete disappearance of methylene chloride in viable sediment-water samples occurred after 18 days, corresponding to a half-life of 10.9 days. The accumulation of methyl chloride in the samples was not observed.

The present study was conducted to investigate the fate and effects of methylene chloride in a system that simulates a municipal wastewater treatment plant. Experiments were conducted to examine the effect of methylene chloride concentration on both the acclimation of activated sludge and on the rate of biodegradation. Additional studies were conducted to determine the effect of methylene chloride on the degradation of readily utilizable test substrates.

MATERIALS AND METHODS

Biodegradation Studies

Activated sludge was obtained from the Midland Municipal Wastewater Treatment Plant, which primarily treats domestic wastes. Approximately 9 liters of activated sludge were adjusted to contain 2500 ± 500 mg/L of mixed liquor suspended solids, and placed into a laboratory-scale, semicontinuous activated sludge system (Figure 1). The unit was constructed from a glass cylinder (6 x 21 in) similar to that described by the Soap and Detergent Association (6). The solids were allowed to settle in the chamber, and the upper 7 liters of waste liquor were removed and replaced with 6.5 liters of deionized water and 65 mL of synthetic sewage stock solution (7). The stock

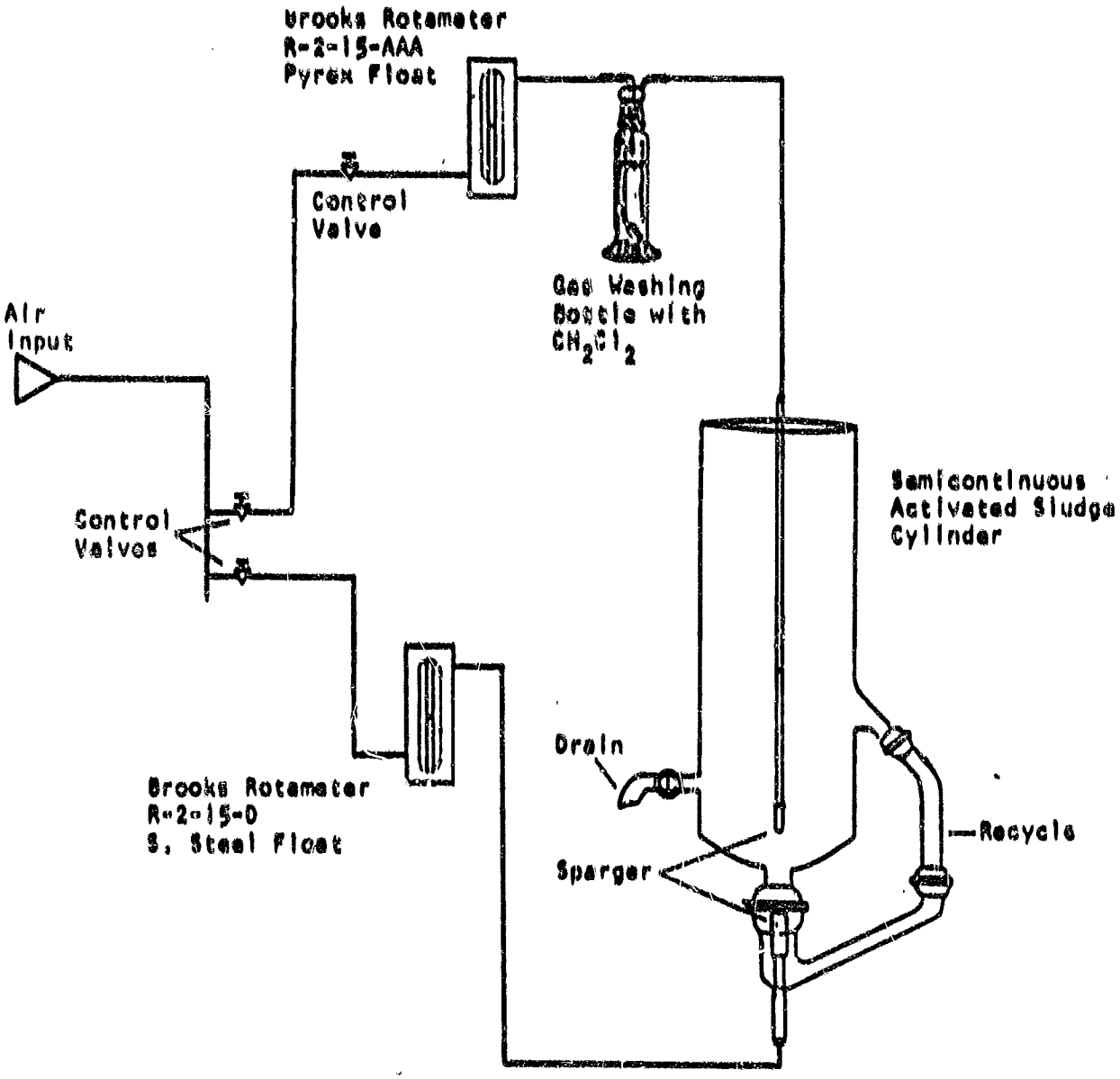

Figure 1. Laboratory-scale Semicontinuous Activated Sludge System

solution was composed of, per liter: glucose, 30 g; Bacto-Nutrient broth, 20 g; K_2HPO_4, 13 g; and $(NH_4)_2SO_4$, 2.5 g. The system was aerated at a rate of 500 mL/min by means of a sparger placed in the bottom of the vessel. The unit was operated on a daily "fill and draw" cycle and incubated at ambient temperature (21°C).

A preliminary study indicated that acclimation of the activated sludge was necessary in order to demonstrate the biodegradation of methylene chloride. Consequently, during incubation the unit was supplemented with an appropriate concentration of methylene chloride. Methylene chloride volatilized from the system was continuously replaced by the addition of an air-methylene chloride mixture (Figure 1). The mixture was prepared by passing air at a slow rate (<5 mL/min) through methylene chloride contained in a gas-washing bottle. The outlet port of the bottle was connected to a sparger that was placed approximately 6 inches above the bottom of the activated sludge unit. Concentration of methylene chloride and chloride ion in the culture was monitored routinely during the acclimation period as described below. The flow rate of methylene chloride saturated air was adjusted to maintain the desired concentration. Acclimation of the activated sludge microorganisms was inferred by detecting the continuous release of chloride ion in the system. Once acclimation was noted, the unit was incubated for several days until the release of chloride in the system reached a constant rate. The pH of the system was monitored periodically, and adjusted to pH 7.2 $\pm$ 0.2 by the addition of 1 N NaOH. Subsequently, the acclimated sludge was used in closed-bottle, respirometer studies to investigate the overall fate of methylene chloride in the system.

Biodegradation studies were conducted using an Oceanography International Corporation model B/BOD® Respirometer System. The respirometer flasks were modified (Figure 2) to facilitate the complete analysis of the transformation process. Each respirometer bottle (1.6 liter) is equipped with a manometric oxygen generating system. As oxygen is consumed by the sample, it is regenerated electrolytically to maintain a constant atmosphere within the bottle. The apparatus is designed to generate 1 mg of oxygen per minute, and a timing circuit records the amount consumed. A stopcock valve, inserted between the oxygen generator and the bottle, allowed isolation of the generator during the removal of samples from the respirometer system. The bottles are also equipped with a CO_2 trap, fitted with a screw-capped, septum port. Carbon dioxide evolved during biodegradation is absorbed by an aliquot (5 mL) of 1 N NaOH in the CO_2 trap. The absorbant can be removed and replaced using a syringe. A long, glass sampling tube and modified glass syringe are utilized for removing samples of mixed liquor for analysis. The respirometer bottles were mixed with a stirring magnet and incubated at ambient temperature (21°C)

Biodegradation of methylene chloride by activated sludge was monitored by observing the rate of disappearance of the parent compound, oxygen consumption, chloride release, and the rate of carbon dioxide evolution. Prior to assembling the respirometers, the acclimated sludge was fed with synthetic sewage and aerated for an additional hour to remove residual methylene chloride. Subsequently, one liter aliquots of activated sludge were dispensed into six respirometer bottles. The units were assembled and allowed to operate for one hour to insure the system was functioning normally. ^{14}C-Labeled methylene chloride

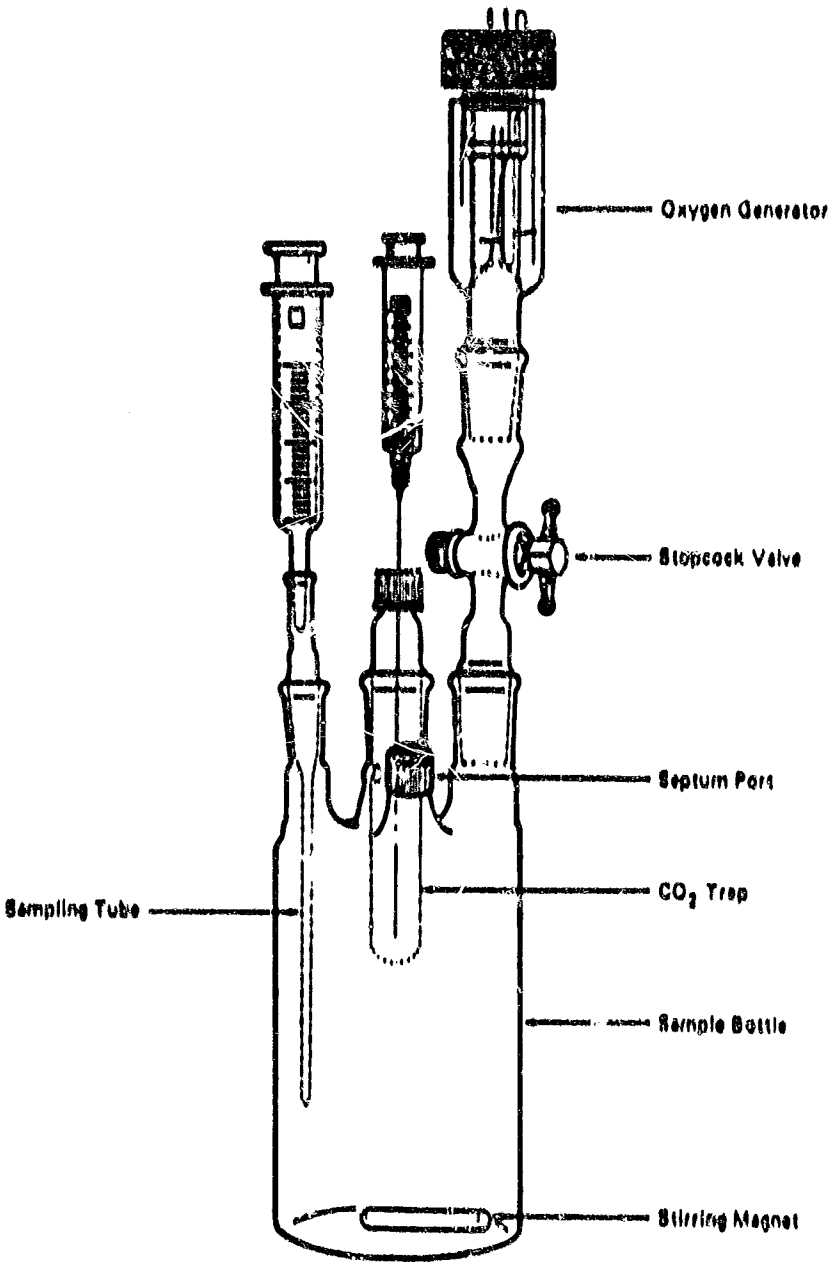

Figure 2. Modified E/BOD® Closed-Bottle Respirometer

was added (neat) to four of the units. Two of the respirometers containing methylene chloride were treated with mercuric chloride (200 mg/L) and served as killed controls for monitoring nonbiological degradation. The remaining two respirometers did not receive methylene chloride, and served as viable controls for monitoring endogeneous metabolism. The respirometers were incubated for 50 hours at 21°C. Samples of mixed liquor were removed periodically from each unit for analysis. Methylene chloride concentration in the mixed liquor was determined by gas chromatography as described below. Oxygen consumption at each interval was read directly from the oxygen recorder. Chloride concentration in mixed liquor samples was analyzed using an ion-specific electrode as described below. ^{14}C-Labeled carbon dioxide evolution was monitored by removing the NaOH solution in the CO_2 trap and replacing it with a fresh aliquot. A remaining sample of activated sludge was analyzed by liquid scintillation counting for a material balance at each time period.

Inhibition Studies

Inhibition studies were conducted to investigate the effect of methylene chloride on oxygen consumption and organic carbon removal by activated sludge when glucose was provided as the carbon source. Activated sludge was equilibrated for 3 days on a "fill and draw" cycle with a defined sewage medium. For these studies, the defined sewage stock solution was composed of, per liter: glucose, 40 g; K_2HPO_4, 13 g; and $(NH_4)_2SO_4$, 15 g. The laboratory-scale activated sludge unit was aerated at a rate of 500 mL/min and incubated at ambient temperature (21°C).

Inhibition studies were conducted in a manner analogous to the respirometer experiments previously described. One liter aliquots of equilibrated sludge were dispensed into four respirometer bottles. Methylene chloride was added (neat) to three of the units at nominal concentrations of 10, 100, and 1000 mg/L. The remaining respirometer served as a control. The systems were incubated for 24 hours at ambient temperature (21°C). Samples of mixed liquor were removed periodically from each respirometer for analysis of total organic carbon as described below.

Analytical Methods

Methylene chloride concentration in activated sludge was monitored by gas chromatography. Two methods were employed for the analyses; the technique utilized for a particular experiment was chosen on the basis of recovery efficiency and sensitivity.

For experiments conducted at an initial methylene chloride concentration of 100 mg/L, the analysis involved an iso-octane extraction procedure similar to the method described by Richard and Junk (8). Samples (5 mL) of activated sludge were extracted with 5 mL of high purity iso-octane (Burdick and Jackson Laboratories, Inc., Muskegon, MI) in 10 mL serum bottles sealed with Teflon-faced silicone septa. Methylene chloride was extracted by shaking the sample on a reciprocating shaker for 30 minutes. A 5 µL aliquot of the iso-octane layer was analyzed by direct injection-gas chromatography as outlined below. Methylene chloride concentration was calibrated using an external standard. The analytical method was previously validated using sewage samples spiked with varying entra-

tions of methylene chloride (see Appendix I). Average recovery efficiencies ranged from 89% at 10 mg/L to 79% at 100 mg/L. Methylene chloride concentrations in activated sludge samples were not corrected for recovery efficiency.

For experiments conducted at initial concentrations of 10 mg/L and 1 mg/L, methylene chloride in activated sludge was analyzed by combined purge and trap-gas chromatography similar to the method of Bellar and Lichtenberg (9). A Hewlett-Packard model 7675A Purge and Trap sampler was utilized for the analysis. The outlet of the sampler was plumbed using 1/16" stainless steel tubing directly to the analytical column via a T-junction fitting. The extra port of the junction was adapted for use as an injector port. Exposed areas of the line were wrapped with a flexible heating tape and maintained at 300°C. Activated sludge samples (5 mL) were placed into 15 mL conical centrifuge tubes and purged with helium at a flow rate of 20 mL/min for 20 minutes at ambient temperature. Methylene chloride volatilized from the sample was trapped on a small column (3 x 1/4 in) packed with Chromosorb 106 (Supelco, Inc., Bellefonte, PA). Adsorbed methylene chloride was injected directly into the gas chromatograph by thermal desorption of the trap column at 250°C for 3 minutes with helium as the carrier gas at a flow rate of 40 mL/min. Methylene chloride concentrations were calibrated using an external standard. The analytical method was previously validated using sewage samples spiked with varying concentrations of methylene chloride. Average recovery efficiencies ranged from 88% at 1 mg/L to 75% at 10 mg/L (see Appendix II). Methylene chloride concentrations in activated sludge samples were not corrected for recovery efficiency.

Gas chromatography was performed using a Hewlett-Packard model 5710A gas chromatograph with a flame ionization detector. The analytical column (glass; 6 ft x 1/4 in) was packed with 1% SP-1000 on 60/80 Carbopak B (Supelco, Inc.). Compounds were eluted from the column isothermally at an oven temperature of 150°C with a helium carrier gas at a flow rate of 40 mL/min. Output of the flame ionization detector was connected to a Hewlett-Packard model 3380S recording integrator. All values were calculated on the basis of peak area measurements. The instrument was calibrated by injecting 5 uL of an external standard of methylene chloride prepared in iso-octane. The detector response was linear over the concentration range of interest (see Appendix III).

Chloride ion concentration in activated sludge was monitored using an Orion model 94-17 solid state chloride electrode, Orion model 90-02 double junction reference electrode and an Orion model 701 specific ion meter. Measurement of chloride concentration in activated sludge samples is affected by the high levels of suspended solids. Consequently, a sample addition method was utilized for the analysis. Samples (10 mL) of activated sludge were transferred into 90 mL of a standard solution containing 10 mg/L of NaCl. Ionic strength of the solution was adjusted by the addition of 2 mL of 5 M $NaNO_3$. The instrument was calibrated using standard solutions of NaCl over the range of 10 to 1000 mg/L. The method was previously validated using samples of activated sludge supplemented with varying concentrations of NaCl (see Appendix IV).

Total organic carbon was determined using a Beckman model 915B total carbon analyzer. Activated sludge samples were filtered through 0.45u membrane filters (Millipore, Corp., Bedford, MA). A 5 mL sample of the filtrate was transferred to a 10 mL

serum bottle, diluted with 5 mL of Milli-Q® continental water (Millipore Continental Water Systems Corp., El Paso, TX), and acidified by the addition of 50 µL of 20% (wt/vol) H_2SO_4. The serum bottle was sealed with a silicone septum, and the sample was purged with air at a rate of 15 mL/min for 30 minutes. Aliquots (50 µL) of the sample were analyzed for total organic carbon as previously described (10). The instrument was calibrated using potassium biphthalate as the standard.

Concentration of mixed liquor suspended solids (MLSS) in activated sludge samples (5 mL) was determined by gravimetric analysis as previously described (11).

Radioactivity associated with various fractions of the respirometer systems was monitored by liquid scintillation counting for a material balance at each period. Radioactivity in the mixed liquor fraction was analyzed by transferring duplicate samples (1.9 mL) of activated sludge to 10 mL of Aquasol® scintillation cocktail (New England Nuclear, Corp., Boston, MA). The level of radioactivity initially associated (time zero) with the mixed liquor was designated as 100% of the original radioactivity. Radioactivity evolved as ^{14}C-labeled carbon dioxide was determined at each period by removing the 1 N NaOH absorbant in the CO_2 trap and replacing it with a fresh aliquot. Samples (50 µL) of the absorbant were transferred in duplicate to 10 mL of Aquasol® scintillation cocktail for analysis.

Radioactivity associated with various fractions of the mixed liquor was monitored in samples prepared for gas chromatographic analysis. For experiments conducted at m...ylene chloride concentrations of 100 mg/L, the levels of radioactivity extractable and nonextractable with iso-octane were analyzed. Samples were

prepared as previously described. Aliquots (1 mL) of both the organic extract and the mixed liquor fraction were transferred in duplicate to 10 mL of Aquasol® scintillation cocktail for analysis. For experiments conducted at methylene chloride concentrations of 10 mg/L and 1 mg/L, the level of nonvolatile radioactivity was monitored by transferring duplicate 1 mL samples of mixed liquor, after purge and trap analysis, to 10 mL of Aquasol® scintillation cocktail.

Blank samples for liquid scintillation counting were prepared in duplicate by adding the appropriate type and amount of a sample from respirometers not exposed to the tracer, to 10 mL of Aquasol® scintillation cocktail. Standards were prepared in duplicate by adding 0.1 mL of a ^{14}C-labeled toluene standard (4.5×10^5 dpm/mL; New England Nuclear, Corp.) to appropriate blank samples.

All samples were analyzed using a Packard model 2425 liquid scintillation counter. Samples were counted for 4 minute intervals for 3 cycles of the counter. Radioassay data were analyzed using an IBM model 370 computer. Program GCSR was utilized for data analysis (12). The computer was programmed to subtract blank counts and to calculate the counting efficiency from the analysis of representative standards. The information was used to calculate the radioactivity in each sample (dpm). Radioactivity in the samples was corrected for volume, and the distribution of radioactivity in various fractions of the respirometer system was calculated with respect to the total activity initially present in the mixed liquor.

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Chemicals

Radioactively labeled (^{14}C) methylene chloride was purchased from the New England Nuclear Corp., Boston, MA. The radioactive material (specific activity; 4.85 uCi/mmole) was diluted with unlabeled methylene chloride (Distilled in Glass® Grade, Burdick and Jackson Laboratories, Inc., Muskegon, MI) prior to use (see Appendix V). The radiotracer was determined to be 98% radiochemically pure (13).

High-purity (Distilled in Glass® Grade) methylene chloride, chloroform, 1,1,2-trichloroethylene, and iso-octane were obtained from Burdick and Jackson Laboratories, Inc. 1,1-Dichloroethane was from Aldrich Chemical Co., Milwaukee, WI. 1,2-Dichloroethane and 1,1,1-trichloroethane were obtained from the J. T. Baker Chemical Co., Phillipsburg, NJ. Bacto-Nutrient Broth was from Difco Laboratories, Inc., Detroit, MI. All other materials were of the highest purity commercially available and were used without further purification (see Appendix VI).

RESULTS

Acclimation of Activated Sludge to Methylene Chloride

Preliminary studies indicated that acclimation of activated sludge microorganisms was necessary in order to demonstrate the biodegradation of methylene chloride. Consequently, laboratory-scale activated sludge units were operated for two weeks on a "fill and draw" cycle and continuously supplemented with methylene chloride as previously described.

Three activated sludge reactors were established and acclimated to nominal concentrations of 100 mg/L, 10 mg/L, and 1 mg/L of methylene chloride. Concentrations of methylene chloride and chloride ion were monitored periodically in the systems. Acclimation of the units was inferred by detecting the continuous release of chloride ion in the cultures (Figure 3). Note that the daily drop in chloride concentration was due to replacement of the culture medium.

Unit A was established on February 2, 1981, and continuously supplemented with methylene chloride at a nominal concentration of 100 mg/L. Over the 15-day acclimation period, the system was exposed to an average methylene chloride concentration of 85.5 mg/L, with a range of 47.3 mg/L to 114.6 mg/L. After 11 days, the continuous release of chloride was detected in the culture (Figure 3A). The maximum rate of chloride release in the system on day 14 was 3.6 mg of Cl^- released hr^{-1} g $MLSS^{-1}$.

Unit B was established on April 6, 1981. Over the 12-day acclimation period, the system was exposed to an average methylene chloride concentration of 9.3 mg/L, with a range of 0.8 mg/L to 18.1 mg/L. After 9 days, the continuous release of chloride was detected in the culture (Figure 3B). The maximum rate of chloride release in the system on day 10 was 3.1 mg Cl^- released hr^{-1} g $MLSS^{-1}$.

Unit C was established on April 21, 1981. Over the 15-day acclimation period, the system was exposed to an average methylene chloride concentration of 0.53 mg/L, with a range of 0.0 mg/L to 1.2 mg/L. After 11 days, the continuous release of chloride was detected in the culture (Figure 3C). The maximum rate of chloride release in the system on day 13 was 1.3 mg Cl^- released hr^{-1} g $MLSS^{-1}$.

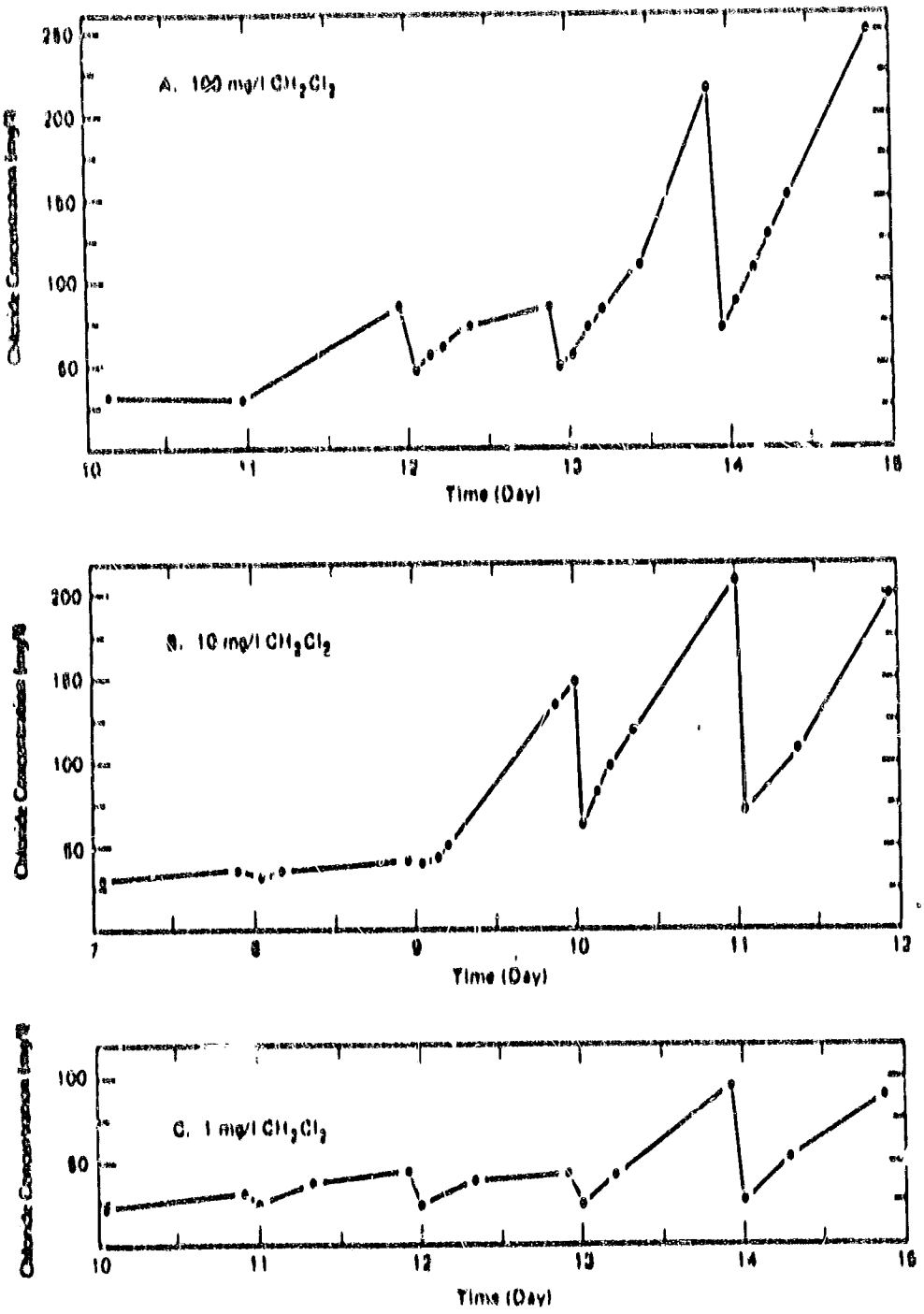

Figure 3. Chloride Release During Acclimation of Activated Sludge to Methylene Chloride

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Fate of Methylene Chloride in Activated Sludge

Closed-bottle, respirometer studies were conducted using acclimated sludge to investigate the overall fate of methylene chloride in the system. Biodegradation of ^{14}C-labeled methylene chloride was monitored by observing the rate of disappearance of the parent compound, oxygen consumption, chloride release, and the rate of evolution of ^{14}C-labeled carbon dioxide.

Respirometer studies were conducted to investigate the fate of 100 mg/L of methylene chloride in activated sludge. Prior to assembling the respirometers, the acclimated sludge was fed with synthetic sewage and aerated for an additional hour to remove residual methylene chloride. Samples (1 liter) of the culture (2.24 g MLSS per liter) were subsequently placed into six respirometer bottles. ^{14}C-Labeled methylene chloride (0.91 uCi/mmole) was added to four of the bottles at a nominal concentration of 100 mg/L. Two of the respirometers containing methylene chloride were treated with mercuric chloride (200 mg/L) and served as killed controls. The remaining two respirometers served as viable controls for monitoring endogenous metabolism. The respirometers were incubated for 50 hours at 21°C. Results of the investigation are shown in Figures 4 and 5.

Disappearance of 100 mg/L of methylene chloride was observed in the experimental samples within 10 hours, with a concomitant release of chloride (Figure 4A). Note that the value for methylene chloride concentration at zero time represents the nominal concentration added to the respirometer. The rate of biodegradation was calculated to be 7.4 mg CH_2Cl_2 consumed hr^{-1} g $MLSS^{-1}$. The rate constant calculated for the disappearance of methylene chloride was 0.35 $hour^{-1}$. The degradation of methylene chloride was not observed in the killed control samples, nor was the release of chloride detected upon incubation of viable control samples (Figure 4B).

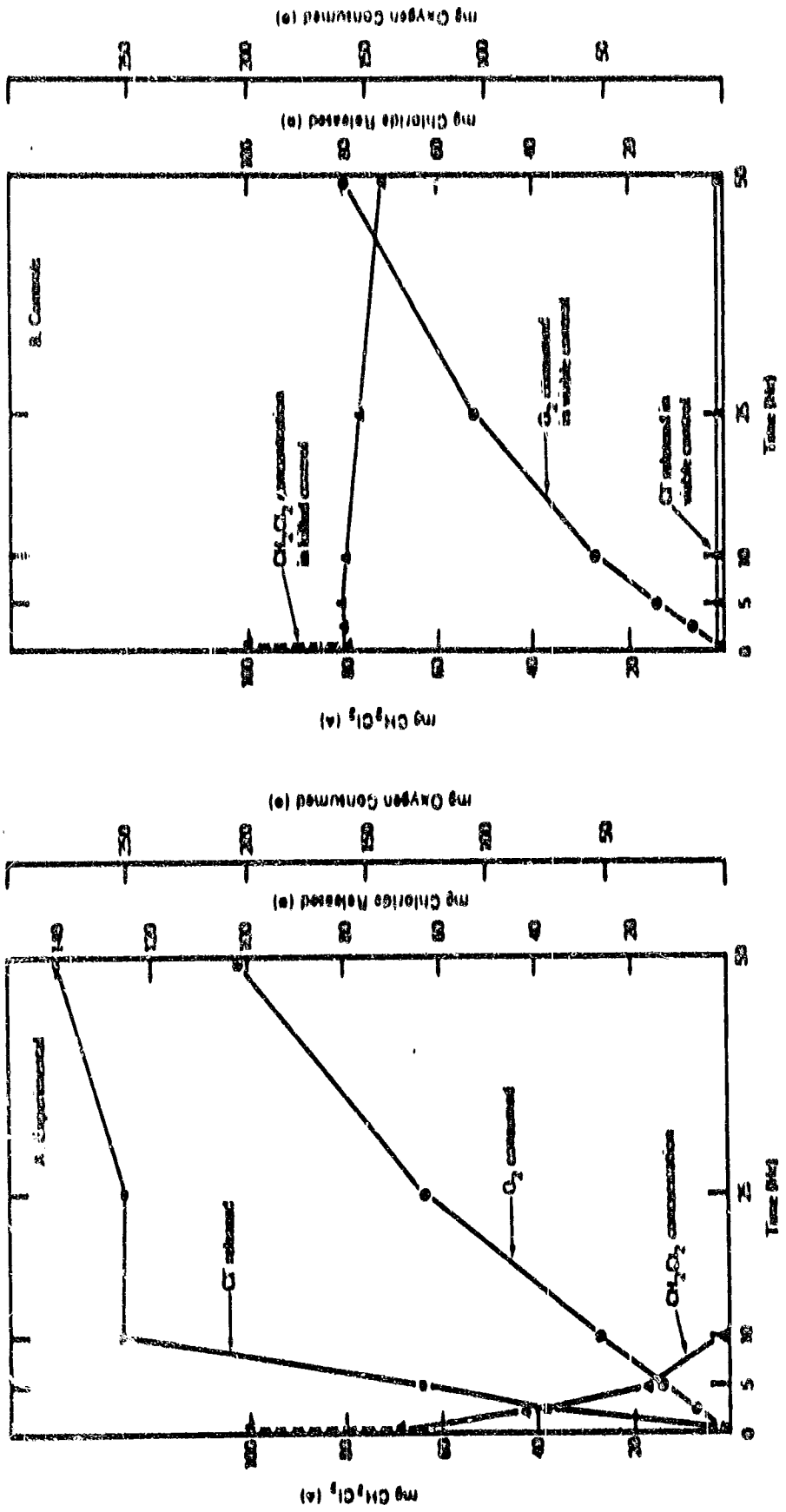

Figure 1. Biodegradation of Methylene Chloride (100 mg/L) by Activated Sludge

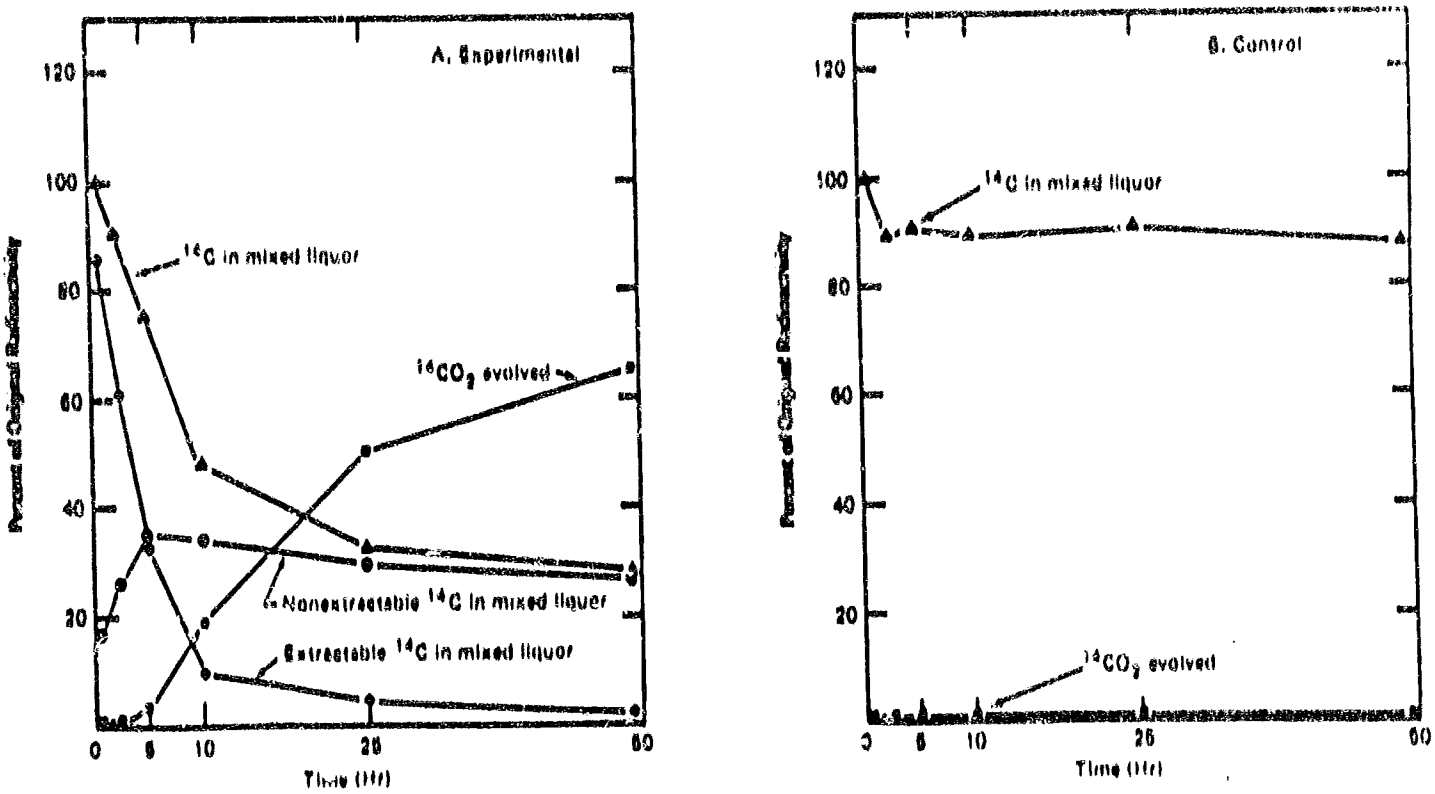

Figure 5. Fate of ^{14}C-Labeled Methylene Chloride (100 mg/L) in Activated Sludge

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Disappearance of ^{14}C-labeled substrate from the mixed liquor was observed in experimental samples with the simultaneous evolution of ^{14}C-labeled carbon dioxide (Figure 5A). After 50 hours incubation, 65% of the parent compound was recovered as carbon dioxide. Disappearance of the substrate was not observed in killed control samples, nor was the evolution of ^{14}C-labeled carbon dioxide detected (Figure 5B). Overall material balances for the experimental and killed control samples were 93.2% and 89.0%, respectively.

Experiments were conducted in an identical manner to investigate the biodegradation of methylene chloride by activated sludge that had been previously acclimated to concentrations of 10 mg/L and 1 mg/L. Results of the investigations are shown in Figures 6, 7, 8, and 9.

Respirometer studies, conducted to investigate the fate of 10 mg/L of methylene chloride in activated sludge (2.06 g MLSS per liter), indicated disappearance of the substrate in experimental samples within 4 hours with a concomitant release of chloride (Figure 6A). Note that the value for methylene chloride concentration at zero time represents the nominal concentration added to the respirometer. The rate of degradation was calculated to be 2.3 mg CH_2Cl_2 consumed hr^{-1} g $MLSS^{-1}$. The first-order rate constant calculated for the disappearance of methylene chloride was 1.61 $hour^{-1}$. Disappearance of methylene chloride was not observed in killed control samples, nor was the release of the chloride detected in the viable controls (Figure 6B). Biodegradation of ^{14}C-labeled methylene chloride (7.6 μCi/mmole) was observed in the experimental samples with the simultaneous evolution of ^{14}C-labeled carbon dioxide (Figure 7A). The evolution of carbon dioxide was not detected in the killed controls (Figure 7B). Overall material balances for the experimental and killed control samples were 91.7% and 92.2%, respectively.

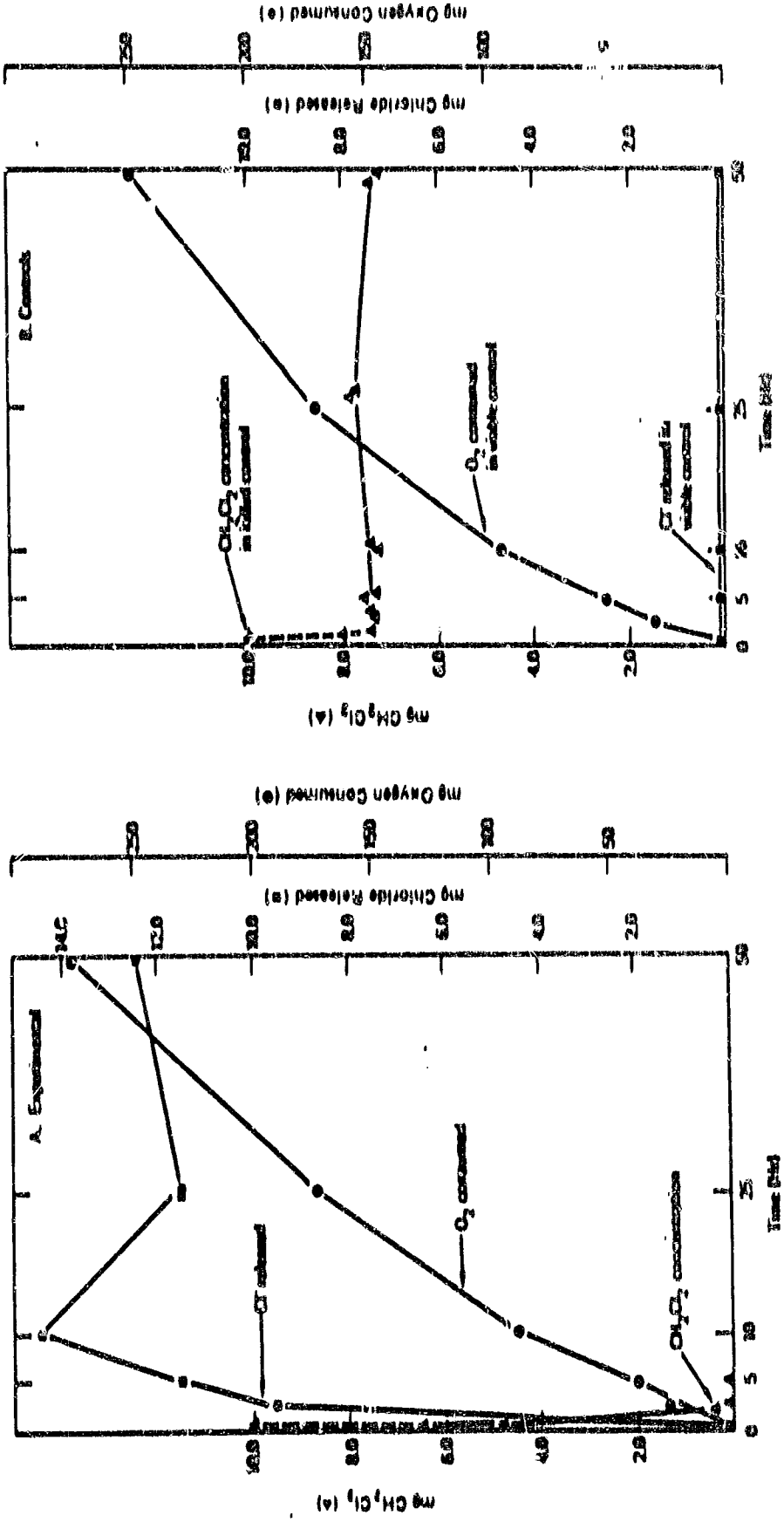

Figure 6. Biodegradation of Methylene Chloride (10 mg/L) by Activated Sludge

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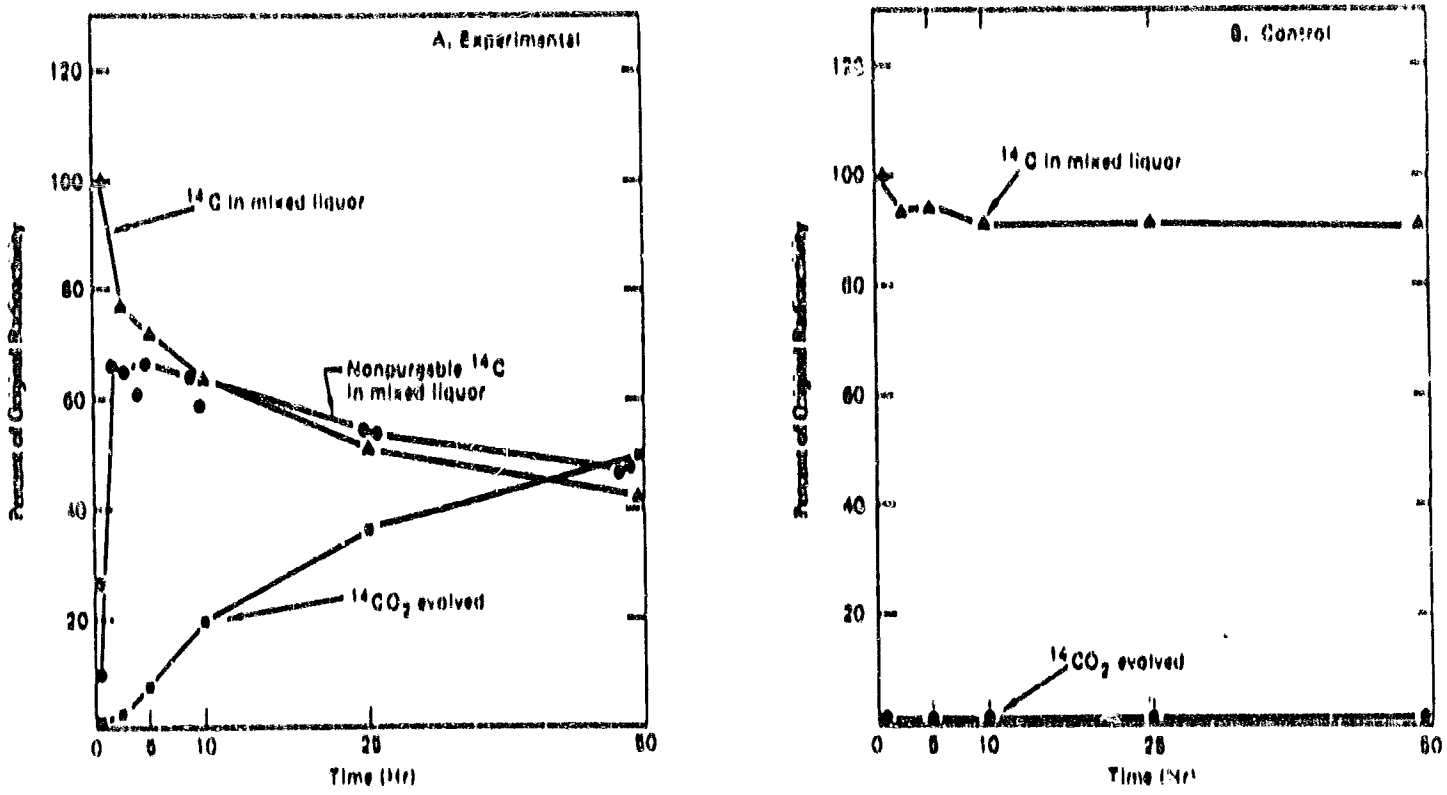

Figure 7. Fate of ^{14}C-Labeled Methylene Chloride (10 mg/L) in Activated Sludge

Results of the biodegradation of 1.0 mg/L of ^{14}C-labeled methylene chloride (7.6 uCi/mmole) by activated sludge (2.59 g MLSS per liter), indicated the complete disappearance of the parent compound within 3 hours (Figure 8A). Note that the value for methylene chloride concentration at zero time represents the nominal concentration added to the respirometer. The rate of degradation was calculated to be 0.14 mg CH_2Cl_2 consumed hr^{-1} g $MLSS^{-1}$. The first-order rate constant calculated for the disappearance of methylene chloride was 1.42 $hour^{-1}$. After 50 hours, 66% of the substrate was recovered as ^{14}C-labeled carbon dioxide (Figure 9A). Disappearance of methylene chloride was not observed in killed control samples (Figure 8B), nor was the evolution of ^{14}C-labeled carbon dioxide detected (Figure 9B). Overall material balances for the experimental and killed control samples were 96.6% and 99.5%, respectively.

Substrate Specificity of Methylene Chloride Acclimated Sludge

Respirometer studies were conducted to determine the ability of activated sludge, previously acclimated to methylene chloride, to dehalogenate a variety of chlorinated test substrates.

Municipal activated sludge was acclimated over a 13-day period to 100 mg/L of methylene chloride. Prior to assembling the respirometers, the acclimated sludge was fed with synthetic sewage and aerated for an additional hour to remove residual methylene chloride. Subsequently, aliquots (1 liter) of the culture (2.24 g MLSS per liter) were placed into six respirometer bottles. Nominal concentrations of 100 mg/L of the following test substrates were added to the respirometers: methylene chloride, chloroform, 1,2-dichloroethane, 1,1-dichloroethane, 1,1,1-trichloroethane, and 1,1,2-trichloroethylene. Chloride concentration in mixed liquor samples from each

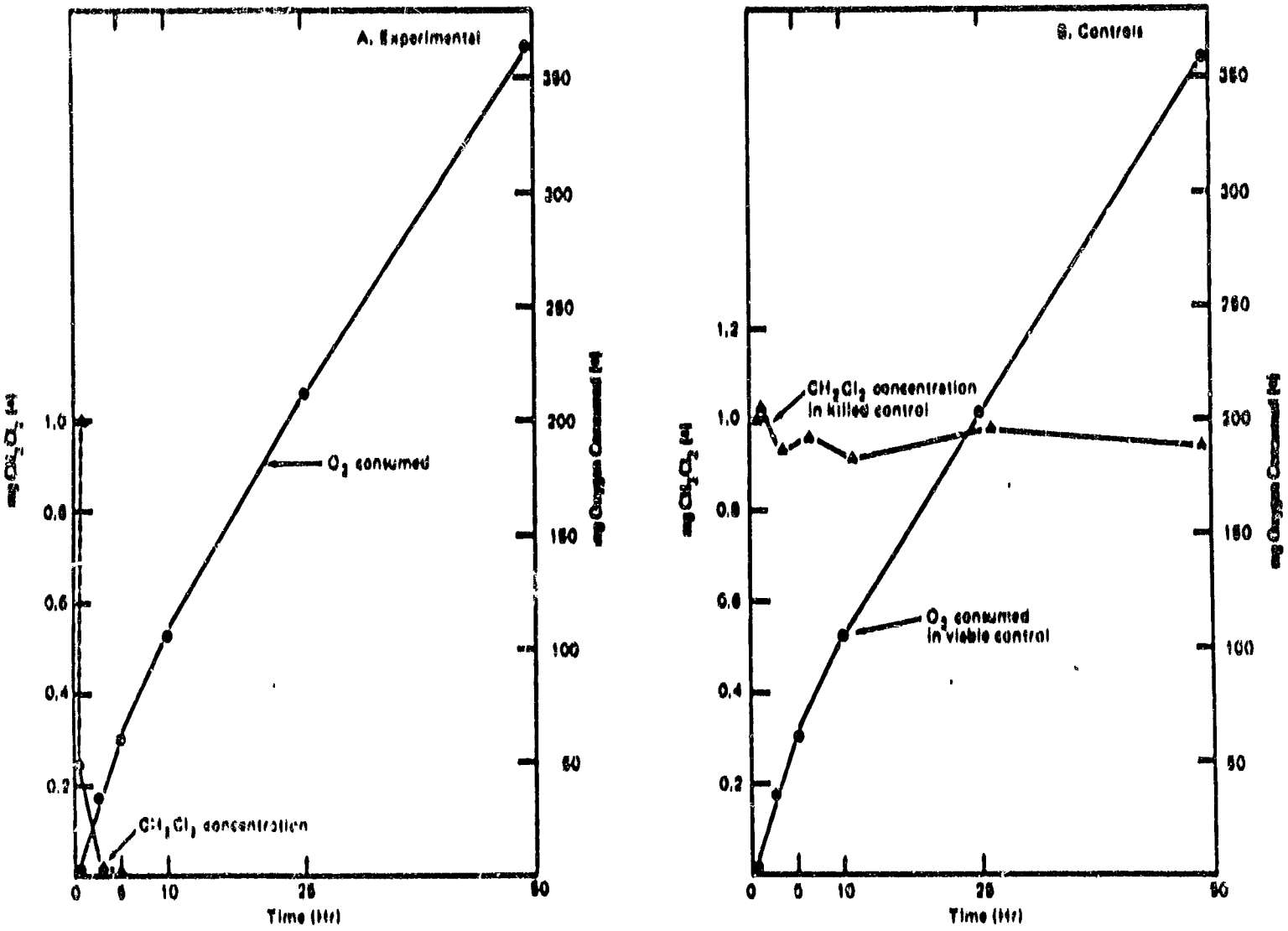

Figure 8. Biodegradation of Methylene Chloride (1 mg/L) by Activated Sludge

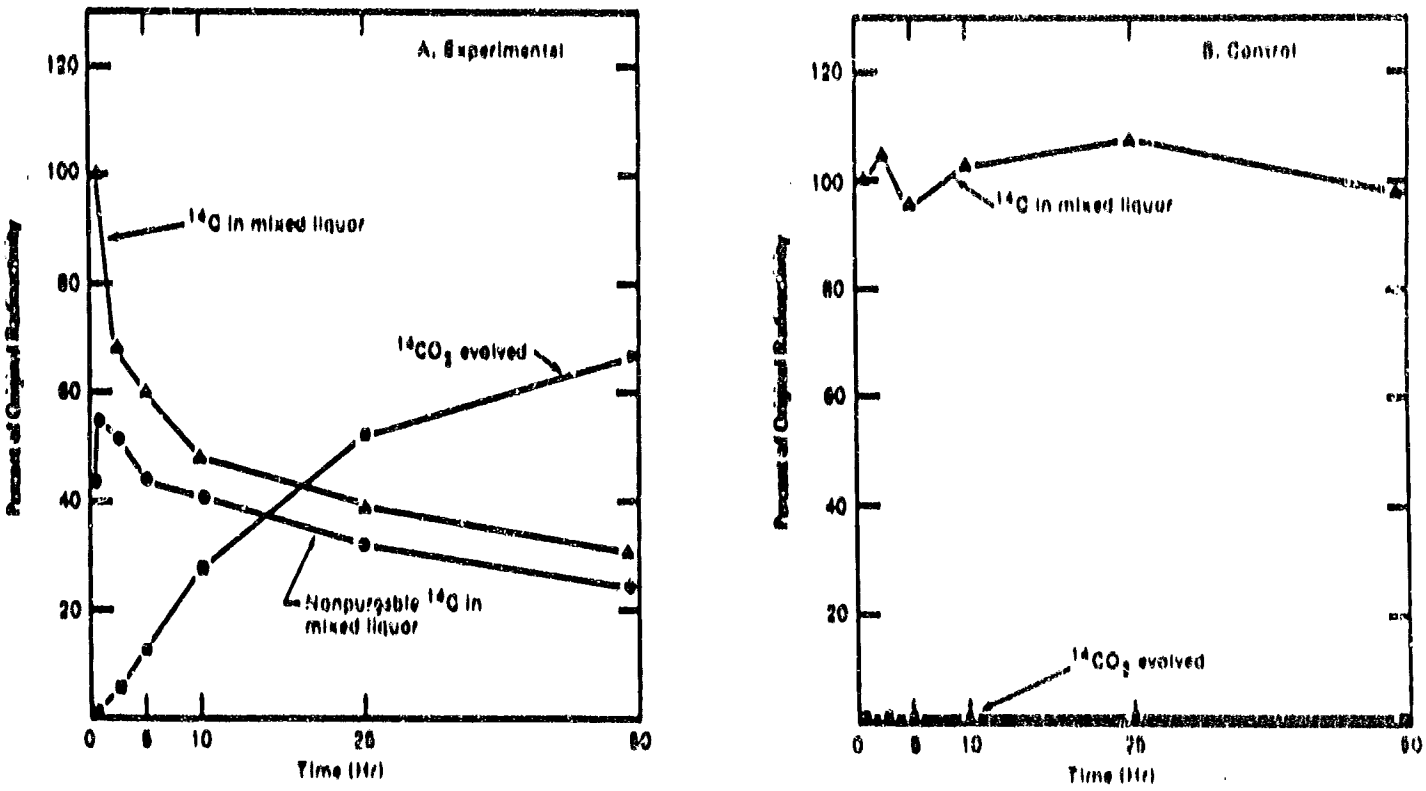

Figure 9. Fate of ^{14}C-Labeled Methylene Chloride (1 mg/L) in Activated Sludge

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bottle was monitored periodically. In addition, the concentration of chlorinated substrate in each respirometer was monitored using an iso-octane extraction procedure as previously described. The respirometers were incubated for 50 hours at 21°C.

Results of the investigation, summarized in Table I, indicate that acclimated sludge exhibits an apparent strict substrate specificity, as the release of chloride was detected only in the respirometer containing methylene chloride. Although a slight decrease in concentration of the test substrate was noted in several of the respirometers, a concomitant release of chloride was not observed.

Effect of Methylene Chloride on Activated Sludge

Inhibition studies were conducted to investigate the effect of methylene chloride on oxygen consumption and organic carbon degradation by activated sludge when glucose was provided as the carbon source.

Activated sludge was initially equilibrated for 3 days on defined sewage in the absence of methylene chloride. Aliquots (1 liter) of the culture (2.54 g MLSS per liter) were placed into respirometer bottles and exposed to methylene chloride at nominal concentrations of 10, 100, and 1000 mg/L. An additional respirometer served as a control. Oxygen consumption and the concentration of nonvolatile organic carbon in the systems was monitored for 24 hours.

Results of the investigation indicated that methylene chloride had no significant effect on oxygen consumption (Figure 10) or total organic carbon removal (Figure 11) by activated sludge over a 24-hour period. Identical results were obtained in an investigation conducted using synthetic sewage as the carbon source.

Table I. Investigation of Substrate Specificity of Methylene Chloride Acclimated Sludge

Test Substrate	Parameter Monitored in Mixed Liquor	Concentration in Mixed Liquor (mg/L)			
		Time (Hr)			
		0	5	10	50
Methylene Chloride	CH_2Cl_2	76.0	43.8	12.1	0.0
	Cl^-	122.0	175.0	235.0	237.0
Chloroform	$CHCl_3$	84.4	87.6	88.6	85.2
	Cl^-	122.0	125.0	126.0	125.0
1,2-Dichloroethane	CH_2ClCH_2Cl	99.5	95.5	95.0	87.8
	Cl^-	122.0	126.0	127.0	126.0
1,1-Dichloroethane	$CHCl_2CH_3$	81.7	84.9	83.1	74.5
	Cl^-	122.0	126.0	127.0	118.0
1,1,1-Trichloroethane	CCl_3CH_3	85.5	77.0	74.0	73.0
	Cl^-	122.0	120.0	126.0	125.0
1,1,2-Trichloroethylene	$CCl_2=CHCl$	73.5	79.0	79.1	74.6
	Cl^-	122.0	122.0	123.0	126.0

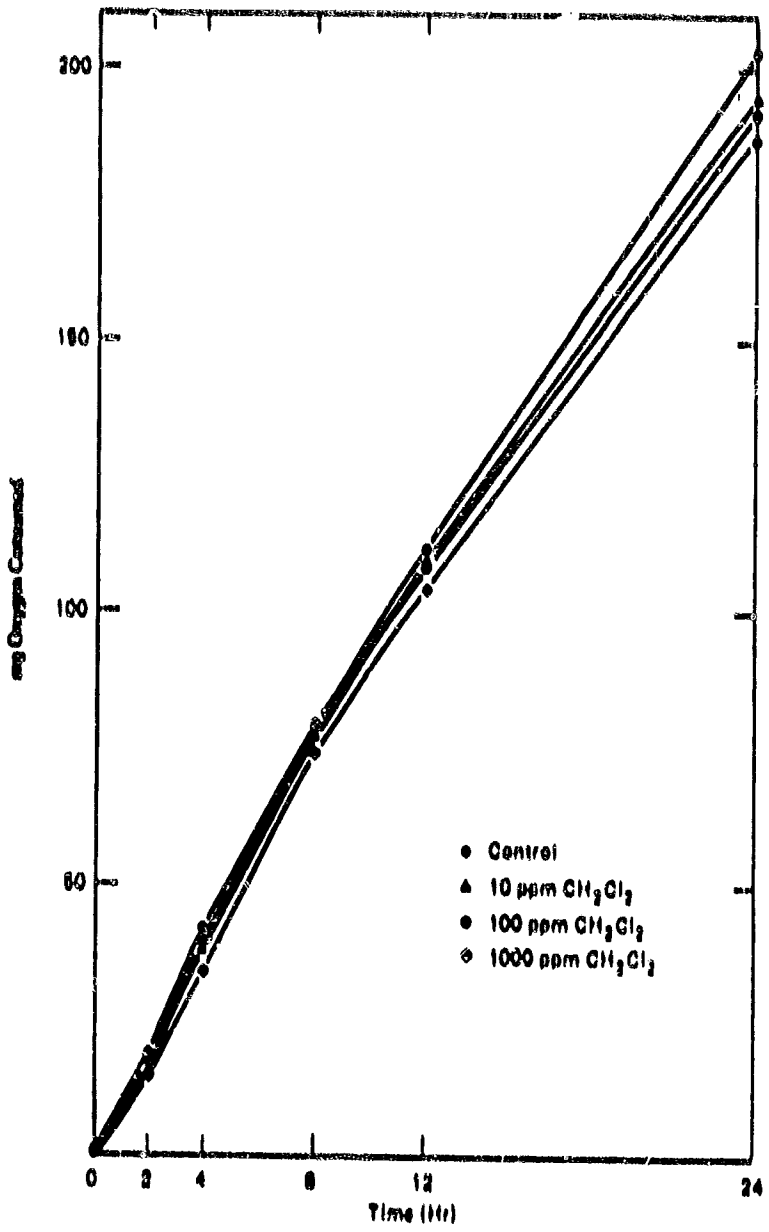

Figure 10. Effect of Methylene Chloride on Oxygen Consumption by Activated Sludge

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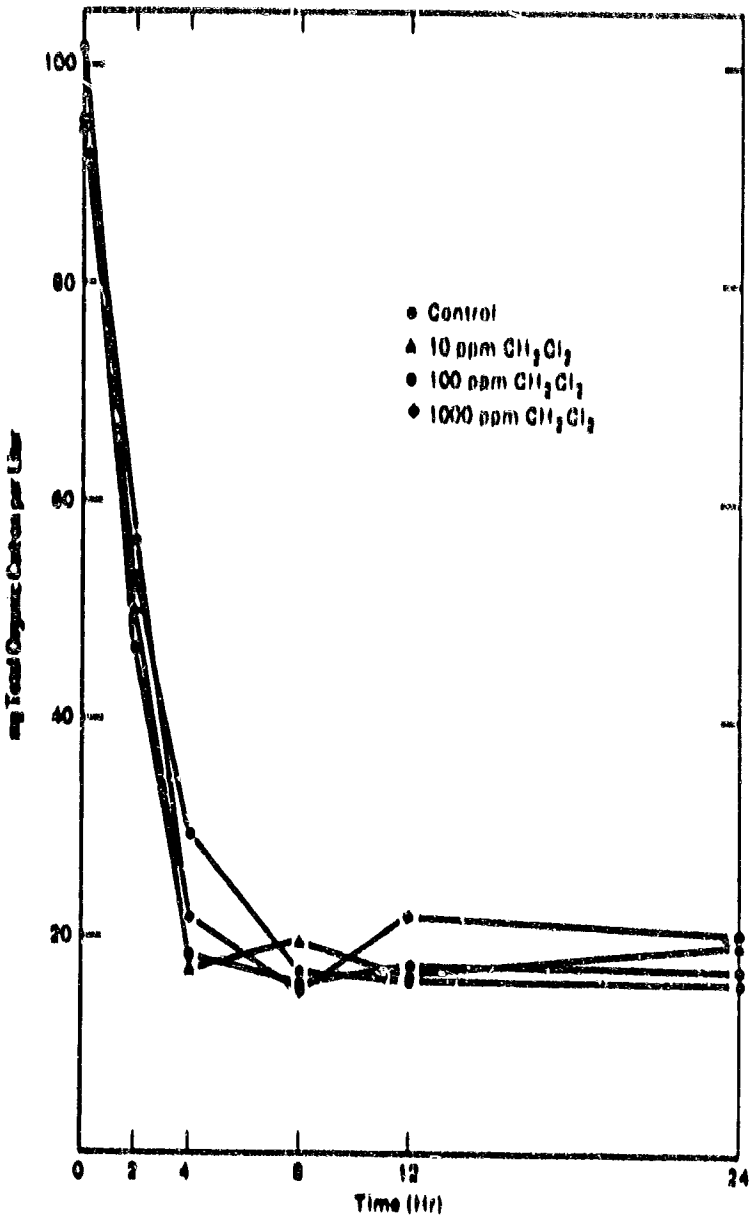

Figure 11. Effect of Methylene Chloride on Glucose Metabolism by Activated Sludge

DISCUSSION

Microorganisms enriched from municipal activated sludge are capable of the mineralization of methylene chloride to carbon dioxide and chloride ions. The biodegradation of methylene chloride by activated sludge is consistent with the previous reports of methylene chloride utilizing bacteria (2,3,4), and indicates the possibility for biodegradation in the environment.

The biodegradation of methylene chloride by activated sludge requires acclimation of the microbial population by exposure to the compound for a period of time. The requirement for acclimation is consistent with the failure of the compound to degrade in tests of "ready biodegradability," such as the biochemical oxygen demand test, in which acclimation may not occur. Acclimation of a microbial population is thought to involve one or more of the following: (a) induction or derepression of specific enzymes present at low or nonexistent levels in the population before exposure, (b) selection of new metabolic capabilities as a result of genetic changes, and (c) an increase in the numbers of organisms capable of a particular transformation (14). Other factors, including the concentration of the substrate and the availability of alternate carbon sources have been reported to influence the acclimation of a microbial population (15). The present study has demonstrated the ability of microorganisms present in activated sludge to acclimate when exposed to methylene chloride concentrations between 0.5 mg/L and 114.6 mg/L. The exact mechanisms involved in this process are unclear.

Acclimated cultures were shown to rapidly degrade methylene chloride under aerobic conditions. Rate constants for the disappearance of substrate were calculated to be 0.35, 1.61, and 1.42 hours^{-1}, from respirometer experiments conducted at nominal concentrations of 100, 10, and 1 mg/L of methylene chloride, respectively. The lower rate constant observed in the experiment conducted at 100 mg/L methylene chloride may suggest that this level is near saturation. Brunner et al. (3) have reported that the maximum growth rate for a Pseudomonas species on methylene chloride was observed at a concentration of approximately 170 mg/L.

Methylene chloride acclimated sludge exhibits a relatively strict substrate specificity. Of a variety of chlorinated aliphatic compounds tested, the disappearance of substrate with a concomitant release of chloride was detected only in activated sludge samples containing methylene chloride. Rittmann et al. (16) have also reported the inability of bacterial cultures, previously enriched on methylene chloride, to degrade a variety of halogenated test substrates, including chloroform, 1,1,1-trichloroethane, and tetrachloroethylene. In addition, Brunner et al. (3) reported that 1,1-dichloroethane was not attacked by resting cells of a Pseudomonas species previously grown with methylene chloride.

Shock loads of methylene chloride would not be expected to adversely affect the microbial population of an aerobic wastewater treatment plant. Concentrations of methylene chloride between 10 mg/L and 1000 mg/L had no significant effect on oxygen consumption or total organic carbon metabolism by activated sludge when glucose was provided as the growth substrate. Identical results were also observed when synthetic

sewage was provided as the test substrate. Preliminary studies conducted to investigate the effect of methylene chloride on nitrogen metabolism were unclear.

The mechanism involved in the biodegradation of methylene chloride by activated sludge is as yet unclear. Attempts in our laboratory to demonstrate an intermediate product have been unsuccessful. Brunner et al. (3) have proposed that a halidohydrolase is responsible for the oxidation of methylene chloride by a Pseudomonas species. This reaction has been previously reported by Hoppel and Porterfield (17) for the dehalogenation of methylene chloride by rat liver homogenates. Substitution of a hydroxyl group for one chlorine substituent of methylene chloride would lead to the formation of monochloromethanol. This intermediate would spontaneously decompose to formaldehyde, releasing the second chlorine substituent. The product of the initial reaction, formaldehyde, would be readily consumed during microbial growth, either by incorporation into cellular constituents, or by further oxidation to carbon dioxide. Alternatively, Kubic and Anders (18) have demonstrated that a monooxygenase, present in the microsomal fraction of rat liver, oxidizes methylene chloride via the unstable intermediate, formyl chloride, to carbon monoxide. A similar mechanism has been suggested for the oxidation of methylene chloride by the methane monooxygenase from Methylococcus capsulatus (19). Thus, the biodegradation of methylene chloride to carbon dioxide by activated sludge could conceivably involve either an initial attack by a monooxygenase, with the subsequent oxidation of carbon monoxide to carbon dioxide, or an initial attack by a halidohydrolase, with the further oxidation of formaldehyde to carbon dioxide. In view of the high yields of carbon dioxide recovered, it is likely that the latter mechanism is predominant in the system. Further studies would be necessary to clarify this point.

The relative importance of two processes, volatilization (air-stripping) and biodegradation, expected to be the major factors determining the fate of methylene chloride in an activated sludge reactor, was examined with a hypothetical model (Figure 1). The model consisted of a completely mixed reactor, with a volume (V) of 1.6×10^7 liters, operating at steady state conditions. The hydraulic flow rate (Qw) was assigned a value of 2.3×10^6 liters per hour, and thus the hydraulic residence time of the system was approximately 7 hours. The system was aerated at a flow rate (Qa) of 1.7×10^7 liters per hour, based on a standard wastewater treatment design factor (air flow/hydraulic flow) of 7.5 (20). The model assumes that the rates of each process controlling the fate of methylene chloride in the reactor can be treated independently, and that the results of laboratory experiments can be extrapolated to describe the process in the complete system. Thus, to a first approximation, a mass balance for the reactor would be described by the equation:

$$V \frac{dC}{dt} = Rate_{input} - Rate_{output} - Rate_{volatilization} - Rate_{biodegradation}$$

The rate of methylene chloride input (K_1) to the system would be a function of the hydraulic flow rate (Qw) and the influent concentration (Co). Correspondingly, the output rate (K_2) would be a function of flow rate (Qw) and the average concentration of methylene chloride in the system (C). From the report of Mackay et al. (21), the rate of volatilization (air-stripping) can be written as a function of the aeration rate (Qa), the concentration of methylene chloride in the reactor (C), and a coefficient (P) describing the partitioning of methylene chloride between the air and water phases. The partition coefficient (P) is a dimensionless term, and is related to the Henry's law constant by the equation,

$$P = \frac{H}{RT}$$

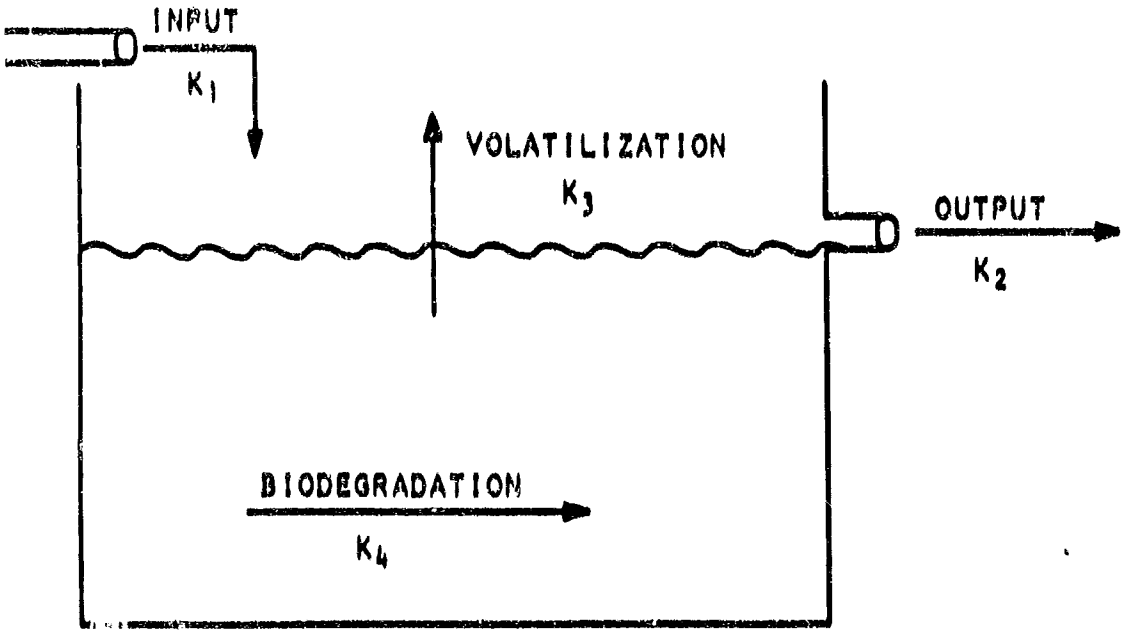

Figure 12. Hypothetical Model for Describing the Fate of Methylene Chloride in an Activated Sludge Reactor

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where H is the Henry's law constant (m^3 atmos. $mole^{-1}$), R is the gas constant (m^3 atmos. $mole^{-1}$ K^{-1}), and T is the temperature (K). Dilling (22) has reported a value of 0.11 for the partition coefficient of methylene chloride at 25°C. Thus, the rate of volatilization (K_3) of methylene chloride from the activated sludge reactor is described by the equation:

$$K_3 = PQaC$$

Assuming the degradation of the methylene chloride by activated sludge is first-order, the rate of biodegradation is a function of the volume of the system (V), the concentration of methylene chloride in the reactor (C) and a constant (k_b) describing the biodegradation rate. Thus, the rate of biodegradation (K_4) would be expressed by the equation:

$$K_4 = k_b VC$$

Therefore, the overall mass balance for the activated sludge reactor can be written as follows:

$$V \frac{dC}{dt} = QwCo - QwC - PQaC - k_b VC$$

If the concentration of methylene chloride in the reactor is assigned a value of 1 mg/L, then the rate of volatilization from the system would be approximately 2×10^6 mg/hour. Alternatively, the rate of methylene chloride biodegradation, by an acclimated culture, would be approximately 23×10^6 mg/hour, based on the experimentally derived rate constant of 1.42 $hours^{-1}$. Solving the mass balance equation for the influent concentration of methylene chloride yields a value of approximately 12 mg/L. Thus, biodegradation would be expected to remove 83% of the influent concentration of methylene chloride, whereas only 7%

would be removed by volatilization from the hypothetical activated sludge reactor. In view of the fact that the rate of biodegradation is approximately 12 times greater than the rate of volatilization, it is suggested that biodegradation may be the predominant mechanism determining the fate of methylene chloride in activated sludge systems continuously exposed to the compound.

CONCLUSIONS

Activated sludge obtained from a municipal wastewater treatment plant was acclimated to concentrations of 1 mg/L to 100 mg/L methylene chloride by continuous exposure to the compound for 9 to 11 days. Acclimated cultures were shown to mineralize methylene chloride to carbon dioxide and chloride ions. Rate constants, calculated for the biodegradation of 1, 10, and 100 mg/L of methylene chloride by activated sludge, were 1.42, 1.61, and 0.35 hours^{-1}, respectively. Methylene chloride acclimated sludge exhibits a strict substrate specificity, and does not attack chloroform, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, or 1,1,2-trichloroethylene. Concentrations of methylene chloride between 10 mg/L and 1000 mg/L had no significant effect on oxygen consumption or carbon metabolism by activated sludge. A hypothetical model was developed to examine the significance of volatilization (air-stripping) and biodegradation for the removal of methylene chloride from an activated sludge reactor. Application of the model indicated that the rate of biodegradation was approximately 12 times greater than the rate of volatilization. Thus, biodegradation may be the predominant process determining the fate of methylene chloride in activated sludge systems continuously exposed to the compound.

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APPENDIX I

Validation of Iso-Octane Extraction Procedure for Analysis of Methylene Chloride in Activated Sludge

Procedure: Aliquots (5 mL) of activated sludge, previously treated with mercuric chloride (200 mg/L), were dispensed into 10 mL serum bottles and spiked with varying concentrations of methylene chloride by the addition of known amounts of a methylene chloride/iso-octane standard solution. Iso-octane (5 mL) was added to the samples. The activated sludge was extracted for 30 minutes on a reciprocating shaker. Aliquots (5 µL) of the extract were analyzed by gas chromatography as described in Materials and Methods. A typical gas chromatogram is shown in Figure 13. The instrument was calibrated by injecting 5 µL of an external standard of methylene chloride prepared in iso-octane. Results of the investigation are summarized in Table II.

Calculations:

CH_2Cl_2 Concentration in Sample (µg/mL) =

$$\frac{\text{Peak Area for 5 µL Sample Extract}}{\text{Concentration Factor}} \times 200$$

Concentration Factor =

$$\frac{\text{Peak Area for 5 µL External Standard}}{\text{Standard Concentration (µg)}}$$

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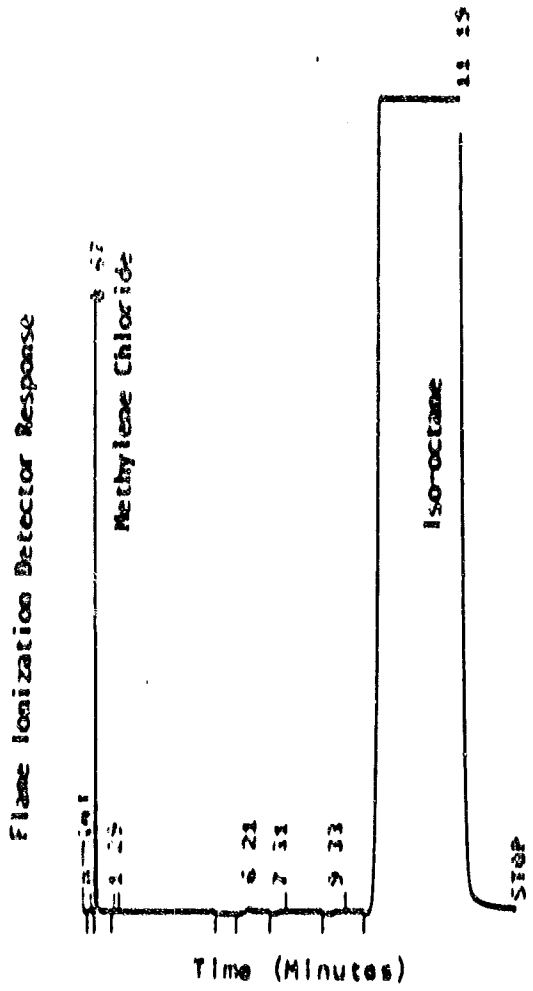

Operator	Date
Column Glass	Detector FID
Length 6 ft	Voltage
Dia. 1/4 in	Sensit. Range = 10
Liquid Phase SP-1000	Flow Rates, ml/min
Wt. % 1%	Hydrogen 30 Air 240
Support Carbopak B	Scavenge
Mesh 60/80	Split
Carrier Gas Helium	Temperature, °C
Rotometer	Det. 300 Inj. 300
Inlet Press. 50 psig	Column Initial 150
Rate 40 ml/min	Final Isothermal
CHART SPEED	Rate
SAMPLE CH_2Cl_2	Solvent Iso-octane
Size	Concn.

Figure 13. Gas Chromatographic Analysis of Methylene Chloride/Iso-octane Extracts

Table II. Validation of Iso-octane Extraction Procedure for Methylene Chloride Analysis

CH_2Cl_2 in Sample (mg/L)	CH_2Cl_2 Extracted (mg/L)	Average	% Recovery
98.0	77.3	77.1	78.7
98.0	76.9		
68.6	54.8	54.4	79.3
68.6	54.0		
49.0	41.1	40.6	82.9
49.0	40.1		
29.4	26.3	26.2	89.1
29.4	26.1		
9.8	8.8	8.7	88.8
9.8	8.6		
1.0	1.1	1.0	100.0
1.0	0.9		

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APPENDIX II

Validation of Purge and Trap Procedure for Analysis of Methylene Chloride in Activated Sludge

Procedure: Aliquots (1 liter) of activated sludge, previously treated with mercuric chloride (200 mg/L), were dispensed into respirometer bottles and spiked with methylene chloride (neat) at concentrations of 1, 5, and 10 mg/L. Samples were removed from the bottles periodically and transferred to graduated conical centrifuge tubes. The samples were analyzed for methylene chloride using a purge and trap procedure as described in Materials and Methods. A typical gas chromatogram is shown in Figure 14. The gas chromatograph was calibrated by injecting 5 µL of an external standard of methylene chloride prepared in iso-octane. Results of the investigation are summarized in Table III.

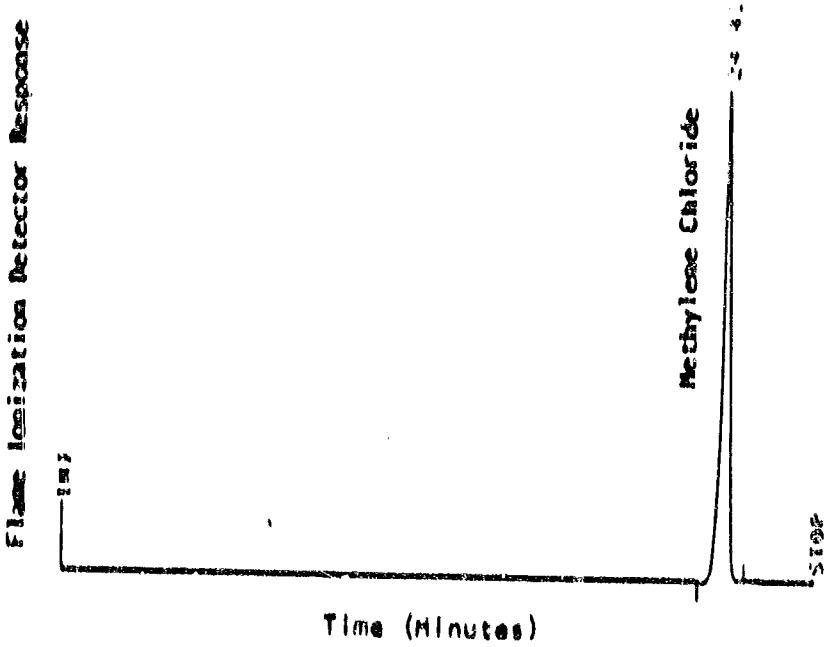
Calculations:

CH_2Cl_2 Concentration in Sample (µg/mL) =

$$\frac{\text{Peak Area for Sample}}{\text{Concentration Factor}} + \text{Sample Volume}$$

Concentration Factor =

$$\frac{\text{Peak Area for 5 µL External Standard}}{\text{Standard Concentration (µg)}}$$



Purge and Trap Conditions

Column S. Steel
Length 3 in
Dia. 1/4 in
Packing Chromosorb 106
Purge Gas Helium
Rate 20 ml/min
Time 20 min
Temperature Ambient
Desorb Mode
Time 5 min
Temperature 250°C
Carrier Gas Helium
Rate 40 ml/min

Operator	Date
Column Glass	Detector FID
Length 6 ft	Voltage Range=10
Dia. 1/4 in	Sensit.
Liquid Phase SP-1000	Flow Rates, ml/min
Wt. % 1%	Hydrogen 30 Air 240
Support Carbopak B	Scavenge
Mesh 60/80	Split
Carrier Gas Helium	Temperature, °C
Rotameter	Det. 300 Inj. 200
Inlet Press 50 psig	Column Initial 150
Rate 40 ml/min	Final Isothermal
CHART SPEED	Rate
SAMPLE CH_2Cl_2	Solvent
Size	Concn.

Figure 14. Gas Chromatographic Analysis of Methylene Chloride by Purge and Trap Technique

Table III. Validation of Purge and Trap Procedure for Methylene Chloride Analysis

CH_2Cl_2 in Sludge Sample (mg/L)	CH_2Cl_2 Analyzed (mg/mL)	% Recovery
10.0	7.49	74.9
10.0	7.41	74.1
10.0	7.46	74.6
10.0	7.72	77.2
	Average	75.2
5.0	3.82	76.5
5.0	3.82	76.5
5.0	3.87	77.5
5.0	4.04	80.8
	Average	76.8
1.0	0.86	86.0
1.0	0.88	88.0
1.0	0.87	87.0
1.0	0.89	89.0
	Average	87.5

APPENDIX III

Linearity of Flame-Ionization Detector Response for Analysis of Methylene Chloride

Procedure: Standard solutions of methylene chloride were prepared in iso-octane over the concentration range of interest (0.1 mg/mL to 10.0 mg/mL). Aliquots of the standard solutions were analyzed by gas chromatography as described in Materials and Methods. Linearity of the detector response (peak area measurement) with respect to the amount of methylene chloride injected is shown in Figure 15. The concentration ranges examined in the linearity study are applicable for determining the concentration of methylene chloride in activated sludge samples when analyzed by the appropriate method.

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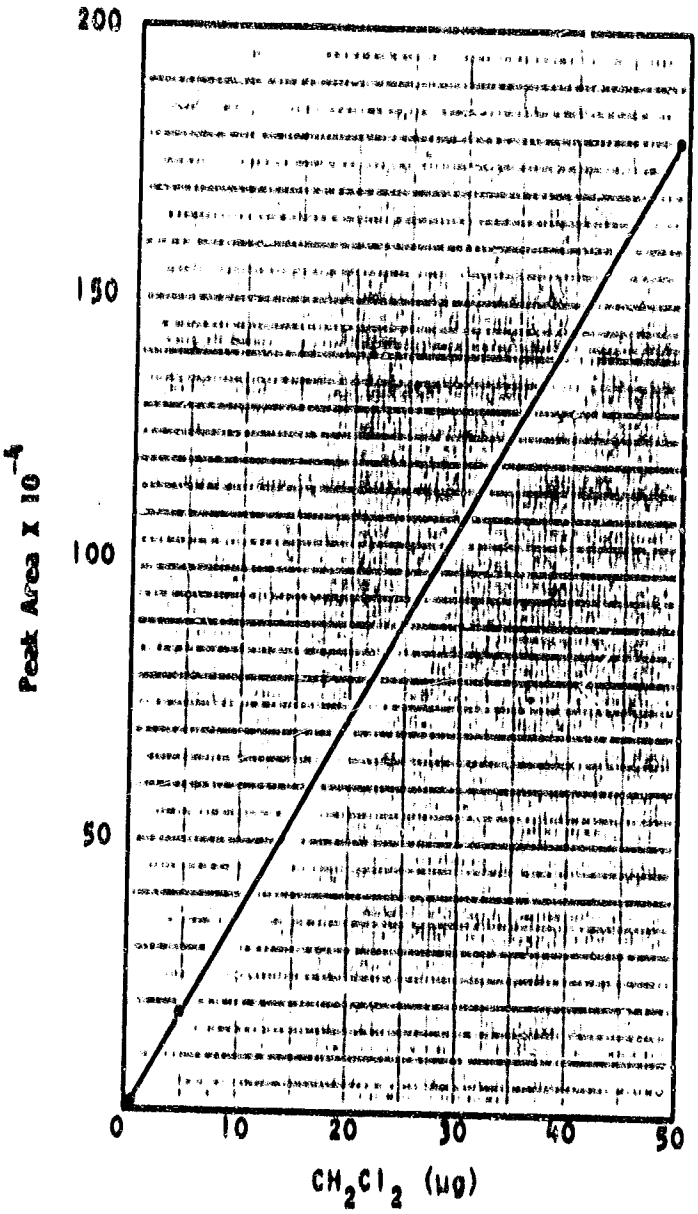

Figure 15. Linearity of Detector Response for Methylene Chloride Analysis

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APPENDIX IV

Validation of Sample Addition Method for Chloride Analysis in Activated Sludge

Procedure: Measurement of the chloride concentration in activated sludge samples is affected by the high level of suspended solids. Consequently, a sample addition method was employed. Samples (100 mL) of activated sludge were spiked with varying concentrations of chloride ion by the addition of known amounts of an aqueous standard of NaCl. Aliquots (10 mL) of the solutions were transferred to 90 mL of a standard solution containing 10 mg/L of NaCl. The ionic strength of the solution was adjusted by the addition of 2 mL of 5 M $NaNO_3$. The sample was analyzed using a chloride-specific electrode, and the relative millivolts observed was recorded. The electrode was calibrated using standard solutions of NaCl over the range of 10 to 1000 mg/L, and a standard curve was prepared (Figure 16). Results of the investigation are summarized in Table IV.

Calculations:

Cl^- Concentration in Sample (mg/L) =

10 (Observed concentration in mg/L) - 90

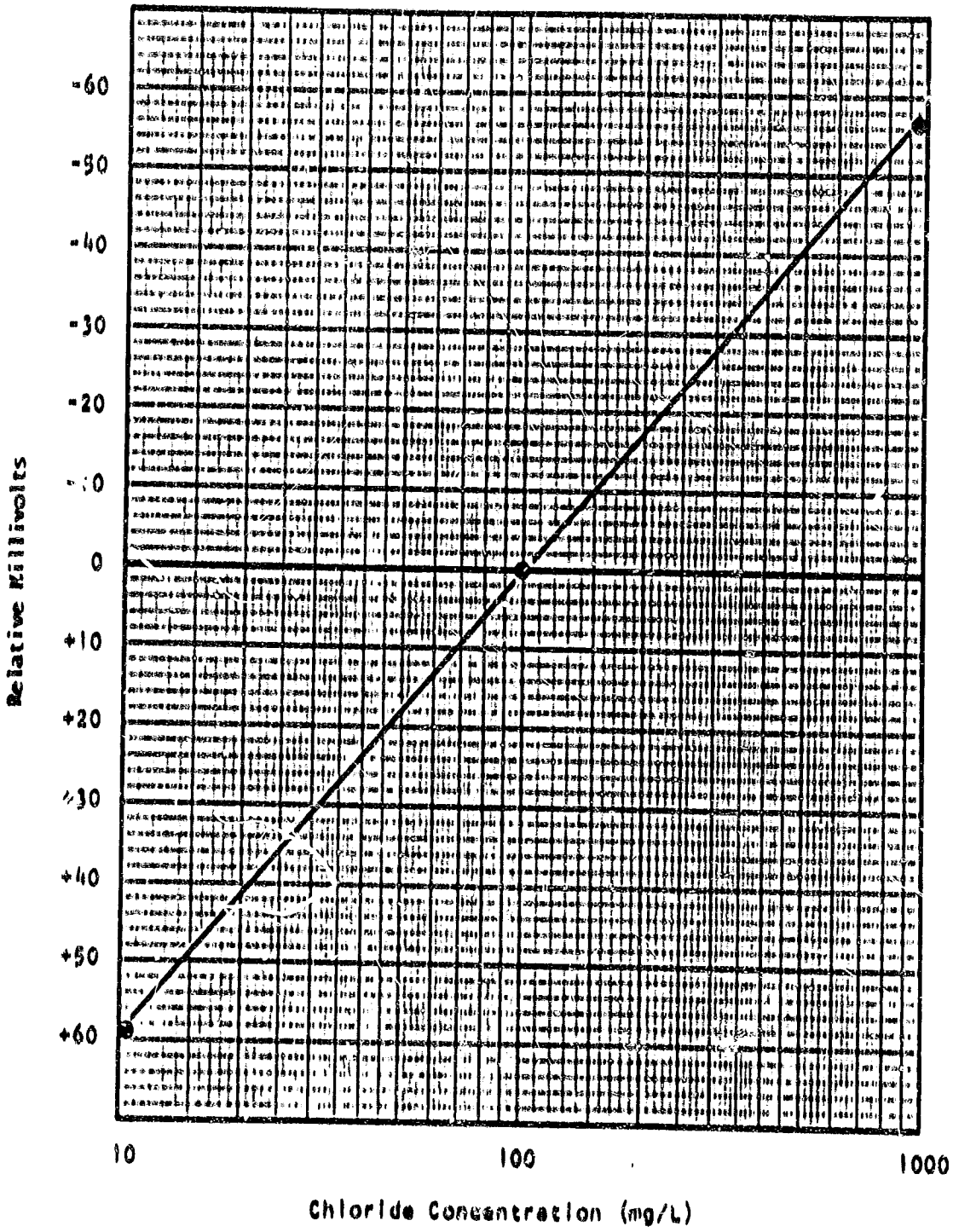

Figure 16. Standard Curve for Chloride Ion Electrode

Table IV. Validation of Sample Addition Method for Chloride Analysis

Chloride Added to Sample (mg/L)	Measured Concentration (mg/L)	Corrected Concentration (mg/L)[1]
0.0	55.0	0.0
10.0	75.0	20.0
20.0	80.0	25.0
50.0	110.0	55.0
100.0	160.0	105.0
120.0	165.0	110.0
150.0	215.0	160.0
170.0	225.0	170.0
200.0	260.0	205.0
250.0	320.0	265.0

[1] Chloride concentration was corrected for the level of chloride initially present in the sample (55 mg/L).

APPENDIX V

Radioactive Materials

$^{14}CH_2Cl_2$

New England Nuclear Corp. Lot NEN 1291-205
S.A. 4.85 mCi/mmole

For 100 ppm Experiment

Diluted with unlabeled CH_2Cl_2 (Burdick Jackson Lot AE887)
ESR Reference: ES-ITT-334-34a
Activity: 3.15×10^4 dpm/μL
S.A. 0.91 μCi/mmole

For 10 ppm Experiment and 1 ppm Experiment

Diluted with unlabeled CH_2Cl_2 (Burdick Jackson Lot AE887)
ESR Reference: ES-ITT-334-19a
Activity: 2.63×10^5 dpm/μL
S.A. 7.60 μCi/mmole

^{14}C-Toluene (Standard)

New England Nuclear Corp. Lot NEN 697-242
Activity (2-15-79) 4.5×10^5 dpm/mL

APPENDIX VI

Chemicals Utilized

Compound	Source	Grade	Lot Number(s)
Glucose	Baker	Reagent	427554; 945001500
Nutrient Broth	Difco	Bacto	608003
K_2HPO_4 (anh)	Baker	Reagent	702106
$(NH_4)_2SO_4$ (anh)	Baker	Reagent	605454
NaCl	Baker	Reagent	947334
$NaNO_3$	Baker	Reagent	9263756
Potassium Biphthalate	Baker	"Primary Reagent"	818110
1 N NaOH	Blanco Reagents	CO_2 free H_2O	F9-05

Solvent	Source	Grade	Lot Number(s)
CH_2Cl_2	Burdick-Jackson	Distilled in Glass®	AH784; AP270
$CHCl_3$	Burdick-Jackson	Distilled in Glass®	AH281
$CHCl_2CH_3$	Aldrich	Reagent	091147
CH_2ClCH_2Cl	Baker	"Photrox Reagent"	904354
CCl_3CH_3	Baker	Reagent	011355
$CHCl=CCl_2$	Burdick-Jackson	Distilled in Glass®	AD940
Iso-Octane	Burdick-Jackson	Distilled in Glass®	AD585

878211300

TITLE OF STUDY: FATE AND EFFECTS OF METHYLENE CHLORIDE IN ACTIVATED SLUDGE

In compliance with Good Laboratory Practice Regulations, this study was inspected by the Quality Assurance Unit, and the results of these inspections reported to Management and the Study Director on 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 reports are located at the submitting laboratory.

Study Started:	09/02/80	Report Issued Date:	01/15/82
Dates of Inspection:	02/19/81	Date of Report:	03/09/81
	06/08/81		06/08/81
	12/09/81		12/09/81

W. M. McCarty 1/14/82
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0 0357

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Author(s)

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MID No. (7) | D & B NO. (11)

Contractor

Doc Type: •RI• UP• HEASD 8D SU HS FN

Doc Title: TOXICITY OF METHYLENE CHLORIDE TO SAPHNIDS

Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-Ø9-2

LK
5 VPI

D-341

8782/1301

TOXICITY OF METHYLENE CHLORIDE TO DAPHNIDS

August 16, 1979

by

W. M. McCarty
Environmental Sciences Research Laboratory
Dow Chemical U.S.A.
Midland, Michigan 48640

Date Test Started: November 1, 1978

Date Test Completed: February 14, 1979

ABSTRACT

Methylene chloride was evaluated for acute toxicity to an aquatic invertebrate species, Daphnia magna Straus, in a static water test.

The 48-hour LC50 value for the daphnids was 27 (21-32)* mg/L.

*95% confidence interval

INTRODUCTION

Methylene chloride was evaluated for acute toxicity to an aquatic invertebrate species, Daphnia magna Straus, in a static water test. The acute daphnid toxicity was run to secure environmental data.

MATERIALS AND METHODS

Sample

The sample of methylene chloride used for this acute toxicity test was identified as Lot 03266 DY 001. The test solution was prepared in methanol.

Water

Water used in the acute toxicity test was dechlorinated Lake Huron water. This water exhibits chemical characteristics in the following ranges: dissolved oxygen (D.O.)[a], 7.7 mg/L; pH 7.6[b]; total alkalinity, 85 mg/L as $CaCO_3$; total hardness, 100 mg/L as $CaCO_3$; specific conductivity, 177 µmhos/cm; and resistivity[c], ~570 ohms/cm. Other water data are in Table I[1].

---

[a]Yellow Springs Instruments Model 54 - Oxygen Meter

[b]Sargent-Welch pH Meter - Model LS

The static water acute toxicity test follows the test method described in the EPA publication, "Methods for Acute Toxicity Test with Fish, Macroinvertebrates, and Amphibians"[2].

Daphnids

The static acute toxicity test method consisted of exposing first instar daphnids reared in our laboratory to various concentrations of the material being tested in dechlorinated Lake Huron water at 20°C ± 1°C.

A stock solution was prepared in methanol. The concentration of the stock solution was 400 mg/ml. Stocks older than 48 hours were discarded.

The required amount of stock solution was combined with sufficient dechlorinated Lake Huron water to make a final volume of 200 ml in the 250 ml test beakers. A control was set using dechlorinated Lake Huron water. Because methanol was used in the stock solution, a second series of beakers were exposed containing the largest amount of solvent used in any concentration to act as a solvent control. The concentration of solvent in any beaker did not exceed 0.5 ml/L. Ten first instar daphnids were then added to each beaker and the beakers

---

[6]Yellow Springs Instruments Model 31 - Conductivity Bridge

TABLE I

Raw Lake Huron Water Analyses

Turbidity as ppm SiO_2	2.3
Color, APHA	<5
Alkyl benzene sulfonate, ppm	<0.1
Arsenic, ppm	<0.005
Barium, ppm	*(1.0)
Cadmium, ppm	*(0.01)
Chlorine, ppm	7.0
Chromium, ppm	*(0.05)
Copper, ppm	0.03
Cyanide, ppm	*(0.01)
Fluoride, ppm	0.07
Iron, ppm	0.1
Lead, ppm	*(0.05)
Magnesium, ppm	7.0
Manganese, ppm	*(0.05)
Nitrate, ppm	*(0.1)
Phenols, ppm	*(0.001)
Selenium, ppm	*(0.01)
Silver, ppm	*(0.05)
Sulfate, ppm	12
Total dissolved solids, ppm	110
Zinc, ppm	*(0.3)
PCB's, ppm	*(0.02×10^{-3})
Mercury, ppb	*(5)

*Parameter was below the limits of detection which are included in parentheses.

set in a constant temperature incubator. Three beakers were used for each exposure concentration and each control. The standard exposure time was 48 hours. Mortality data were recorded at 24 and 48 hours. Death was defined as no response to a gentle prodding. Dead daphnids were not removed from any beaker during the test.

Statistical Calculations

For each set of mortality data, the LC values and their 95% confidence intervals are given. The LC50 is the estimated concentration of the test material at which 50% of the test organisms are dead at a specified time interval. This value is determined using Finney's probit analysis method[3] via computer program.

RESULTS

Seven daphnid acute toxicity tests were run to secure statistical data. The first six test runs were considered invalid because of a >10% mortality of the control test organisms. The 24- and 48-hour LC50 values and their 95% confidence intervals were determined on Daphnia magna. Methylene chloride exhibited a 48-hour LC50 value of 27 (21-32) mg/L to the daphnids. Concentrations tested and mortality data are shown in Table II. The control daphnids exhibited no mortality during the test.

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

W. M. McCarty

W. M. McCarty
Research Chemist
Study Director

APPROVED BY:

H. C. Alexander 8-16-79

H. C. Alexander
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TABLE II

TOXICITY OF METHYLENE CHLORIDE TO DAPHNIDS AND STATISTICAL DATA

ORIGINAL DATA, CONCENTRATIONS AND PERCENT KILL

180.00	100.00	56.00	32.00	18.00	0.00
93 %	97 %	77 %	43 %	23 %	0 %

METHYL[illegible] CHLORIDE STATIC ACUTE DAPHNIA MAGNA 20C 2-14-79
24 HOURS. THE FOLLOWING DATA IS USED FOR CALCULATIONS

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB(PERCENT)
180	30	28	93.3333	.433996E-4
100	30	29	96.6667	.28871E-5
56	30	23	76.6667	.261144
32	30	13	43.3333	29.2332
18	30	7	23.3333	.261144

THE BEST INFORMATION THAT THE BINOMIAL TEST CAN GIVE IS THAT YOU ARE MORE THAN 95 PERCENT CONFIDENT THAT THE LC50 IS BETWEEN 18 AND 56

****** MOVING AVERAGE METHOD ******

SPAN	C	95 PERCENT CONFIDENCE LIMITS		
3	.272618E-1	33.2753	29.4812	37.0542

****** PROBIT METHOD ******

CALCULATED CHI-SQUARE= 4.32987 CRITICAL VALUE(P<.05)= 7.81

SLOPE AND 95% LIMITS ARE 2.7055 (1.88509 , 3.52591)

LC50 AND 95% LIMITS ARE 33.2173 (25.7029 , 40.8262)

LC10 AND LC90 ARE 11.1587 AND 98.8811

TABLE II (Continued)

TOXICITY OF METHYLENE CHLORIDE

TO DAPHNIDS AND STATISTICAL DATA

ORIGINAL DATA, CONCENTRATIONS AND PERCENT KILL

180.00	100.00	56.00	32.00	18.00	0.00
100 %	100 %	87 %	50 %	33 %	0 %

METHYLENE CHLORIDE STATIC ACUTE DAPHNIA MAGNA 20C 2-14-79
48 HOURS. THE FOLLOWING DATA IS USED FOR CALCULATIONS

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB(PERCENT)
100	30	30	100	.931323E-7
56	30	26	86.6667	.297381E-2
32	30	15	50	57.2232
18	30	10	33.3333	4.93496

THE BEST INFORMATION THAT THE BINOMIAL TEST CAN GIVE IS THAT YOU ARE MORE THAN 95 PERCENT CONFIDENT THAT THE LC50 IS BETWEEN 18 AND 56

****** MOVING AVERAGE METHOD ******

SPAN	C	95 PERCENT CONFIDENCE LIMITS		
2	.268356E-1	28.354	25.9867	30.7379

****** PROBIT METHOD ******

CALCULATED CHI-SQUARE= 2.6611 CRITICAL VALUE(P<.05)= 5.99

SLOPE AND 95% LIMITS ARE 3.51172 (2.31979 , 4.70364)

LC50 AND 95% LIMITS ARE 27.0035 (21.4011 , 32.3214)

LC10 AND LC90 ARE 11.6529 AND 62.5758

878211301

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2. Committee on Methods for Toxicity with Aquatic Organisms. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-660/3-75-009.

3. Finney, D. J. 1952. Statistical Methods in Biological Assay. Cambridge University Press.

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Doc Type • RI • WP • HEAD 8D SW HS FN 22

Doc Title 23

DOT TEST FOR CORROSIVENESS

ON METHYLENE CHLORIDE

U.S.P. GRADE

Chemical Name (300 per name) 25	CAS No. (10) 26
METHYLENE CHLORIDE	75-09-2

1A

D-395 878211304

BIOMEDICAL RESEARCH
Texas Division Toxicology

SUBMITTED BY	CHARGE	DATE	
		February 4, 1975	

DOT TEST FOR CORROSIVENESS ON METHYLENE CHLORIDE - URETHANE GRADE

REPORTED BY T. G. Pullin, B. A. Hodge, R. L. Schwebel R. V. Johnston, D.V.M.

INFORMATIVE SUMMARY WITH CONCLUSION - BASED ON THE SAMPLE RECEIVED AND [illegible] INCLUDING THE EFFECTS OF REPEATED EXPOSURE MAY BE REQUIRED AS [illegible] CHANGES OCCUR.

A sample of Methylene Chloride - Urethane Grade was submitted to the Comparative Toxicology Research Laboratory for evaluation of corrosiveness to skin in accordance with the Department of Transportation Hazardous Materials Regulation of the Code of Federal Regulations, Title 49, Section 173.240.

A corrosive material is one that causes irreversible change or destruction to the intact skin of an albino rabbit after an exposure period of four hours. The test procedure to be used is described in Paragraph 1500.41 of 16 CFR, Part 1500.

Methylene Chloride - Urethane Grade is non-corrosive.

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Contractor 21

Doc Type 22

• RI • UP • HEASD 8D SUHS EN

Doc Title 23

CARBOXYHEMOGLOBIN METHYLENE CHLORIDE LEVELS IN HUMAN BLOOD MISUSING A PAIN STRIPPER CONTAINING METHYLENE CHLORIDE

Chemical Name (300 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75 Ø9 2

1A

D·399 878211305

R & D REPORT

DOW CHEMICAL U.S.A.

[illegible]

[illegible]

Carboxyhemoglobin and Methylene [illegible]

Misusing a Paint Stripper Containing Methylene Chloride

[illegible]

[illegible] Braun, E. L. Garfield, and M. B. Chenoweth

[illegible] L. J. Garfield M. B. Chenoweth

[illegible]

B A Schwartz

This report is: [ ] Interim [ ] Final and multiple [ ] New [ ] Revise

DATA REFERENCES (book and page):

(Refer also to earlier related reports and publications.)

PATENT STATUS: [ ] disclosure submitted [ ] case filed [ ] no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

NOV 0 3 1976

CLEARANCE

Summary

During and after an exposure to methylene chloride (MC) resulting from the incorrect use (inadequate ventilation) of a commercial paint stripper containing mainly MC blood samples were obtained from the exposed men. The levels of MC in the plasma and carboxyhemoglobin (COHb) in the blood were determined. The inhalation exposure resulted in a MC plasma concentration maximum of 7 ppm and a % COHb blood maximum of 10%. The clearance half-life ($t_{1/2}$) of MC from plasma was estimated at 0.8 to 1.8 hrs. The $t_{1/2}$ of COHb was 10.5 hrs which is significantly longer than the 5 hr $t_{1/2}$ of COHb resulting from a CO exposure.

The present threshold limit value (TLV) for CO is 50 ppm and results in an equilibrium maximum concentration of 8% COHb. Exposure to CO at the present TLV results in lower COHb levels than those encountered by heavily inhaling cigarette smokers (12% COHb) or a non-smoker misusing a methylene chloride-containing paint stripper (10% COHb).

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METHODS

Methylene Chloride Plasma Levels. The MC plasma levels were determined by gas chromatography - mass spectrometry according to the method of Hermann and Braun (1976).

Carboxyhemoglobin Levels. The COHb levels were determined spectrophotometrically on a IL CO-oximeter (Instrument Laboratories, Lexington, Mass.). A 0.4 ml blood sample was injected into the instrument (previously calibrated for human blood), and the percent COHb was read directly.

Methylene Chloride Exposure Levels. The MC concentration in the room air and in the breathing zones of the subjects were determined (gas chromatography) by D. F. Himmelsbach (Industrial Hygiene, Health and Environmental Research).

Subjects. Three healthy males 22 to 35 years of age weighing 68 to 104 kg were exposed to a commercial paint stripper containing methylene chloride for 3 hrs before lunch; after 1.5 hrs for lunch, they were exposed for an additional hour. Blood samples were obtained at 0.5, 1.0 and 2.0 hrs during the first exposure, at the end of the second exposure, and 1.9, 5.0, 19.0 and 25.0 hrs after the last exposure. (See Table 1). Subjects "A" and "B" were slightly obese. They did the actual work while the lean subject "H" was inactive in his role as monitor.

INTRODUCTION

Methylene chloride (MC), considered the safest of the chlorinated hydrocarbons (Documentation of Threshold Limit Values, 1971), is used as a paint stripper, degreaser, and general purpose solvent. The present Threshold Limit Value (TLV) is 500 ppm according to the American Conference of Government Industrial Hygienists (1971). Stewart et al. (1972), have shown that after exposure to MC there is a measurable increase in carboxyhemoglobin (COHb) levels. Subsequently, Ratney et al. (1974), measured CO concentrations in alveolar air of individuals exposed to known concentrations of MC and, from these alveolar CO levels, calculated the theoretical COHb levels. Based on these calculations, exposure to MC at the TLV would result in a level of COHb (1%) which are higher than those resulting from exposure to CO at the present TLV (0% COHb).

The objective of this study was to determine MC plasma levels and COHb blood levels after a known exposure to MC resulting from the incorrect use (inadequate ventilation) of a commercial paint stripper. A heavily painted cabinet was stripped in a non-ventilated basement workshop of 900 cubic feet volume.

RESULTS AND DISCUSSION

Figure 1 shows the MC plasma concentrations of the subjects at various times during and after the exposure. The MC concentrations fluctuated considerably even within the same individual during the exposure. MC plasma concentrations evidently equilibrate rapidly with changes in alveolar air concentrations of methylene chloride. On the basis of *in vitro* tonometer experiments (Braun., 1976) using blood, 10 minutes is required to reach steady state with MC in the head space gas; however, once the MC flow into the tonometer carrier gas is stopped, the MC in the blood drops below detection limits in less than 5 minutes. The rapid disappearance of MC from plasma *in vitro* after the cessation of the exposure agrees with observations *in vivo*. By 5 hrs post exposure in the present study, all MC plasma concentrations were below the 0.5 ppm detection limit. The half-time ($t_{1/2}$) of MC plasma clearance was estimated to be 0.6 to 1.0 hrs.** This $t_{1/2}$ estimate agrees with the 1.0 hr $t_{1/2}$ calculated from the data reported by DiVincenzo *et al.* (1973).

** With the tonometer volume and carrier gas flow rate, the MC half-time in the tonometer head space was calculated to be 1.8 seconds.

Plasma MC values were determined using measured concentrations after the cessation of exposure. A value of 0.25 ppm (1/2 the detection limit) at the time interval at which the concentration was first below the 0.5 ppm detection limit.

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The MC plasma concentrations correlate with the percent COHb (Figure 2) determined during and after the exposure. Subject "H" showed consistently the lowest MC plasma concentration and also the lowest percent COHb. Subject "A" had both the highest MC and COHb levels. The $t_{1/2}$ of COHb clearance from the blood resulting from a MC exposure was calculated to be 10.5 hrs which is dramatically different from the 3 hr $t_{1/2}$ of COHb (Stewart et al., 1972) resulting from a CO exposure. One possible explanation for this increased $t_{1/2}$ of COHb is a continuing supply of CO in the blood due to metabolism of accumulated MC in the body. Stewart et al. (1972) reported that the biological half-life of COHb was greatly prolonged ($t_{1/2}$ = 11-15 hrs) in individuals exposed to MC over those exposed to CO.

Table 1 shows the MC concentration in the room air and breathing zones of the individual subjects during the exposure. Note that subjects "A" and "B" had exposures of approximately 900 ppm and comparable MC plasma levels (~7 ppm); "H" was exposed to approximately 600 ppm during the initial 3-hr exposure period which resulted in proportionally lower plasma levels (4.4 ppm). During the final 1-hr exposure, subjects "A" and "B" received higher exposures than subject "H". Although there was a qualitative relationship between the MC exposure and MC plasma concentrations, this relationship was not quantitatively proportional. The exposure should not be used as an index of the actual amount of MC absorbed, since it has already been demonstrated by DiVincenzo (1972) that

the amount of physical exertion during exposure has a significant influence on HC plasma levels.

Figure 3 is a plot of theoretical COHb blood levels for four consecutive daily exposures to methylene chloride (using daily regimen as described earlier). COHb blood levels were simulated by the method of Wagner (1971). Knowing the elimination rate constant (k_e), the time to a given fraction of steady state can be calculated from the following equation:

$$t_a = \frac{-1}{k_e} \ln (1-a)$$

where, t = time to a given fraction of steady state; and
a = fraction of steady state.

Ninety-eight percent of steady state COHb blood levels is attained after four exposures. The maximum COHb blood level at but steady state for 625 ppm exposure of HC is approximately 15%. The aforementioned COHb levels are all higher than the 5% COHb level resulting from a TLV exposure (50 ppm) of CO. A COHb blood level of 15% is comparable to that observed in a two-pack-a-day cigarette smoker (12% COHb) or a heavily inhaling-pipe smoker (20% COHb) according to data supplied by Goldsmith and Landow (1975).

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During this study, a mild level of intoxication was produced in the subjects who also complained about the quality of the atmosphere. No other overt clinical observations were noted.

CONCLUSIONS

The present threshold limit value (TLV) for CO is 50 ppm and results in an equilibrium maximum concentration of 8% COHb. The HC exposure in this study resulted in COHb levels higher than the COHb level resulting from an exposure of CO at the present TLV. However, a heavily inhaling cigarette smoker and the paint strippers in this study would have comparable COHb levels.

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Figure 1

MO PLASMA CONCENTRATION IN PPM'S DURING AND AFTER EXPOSURE

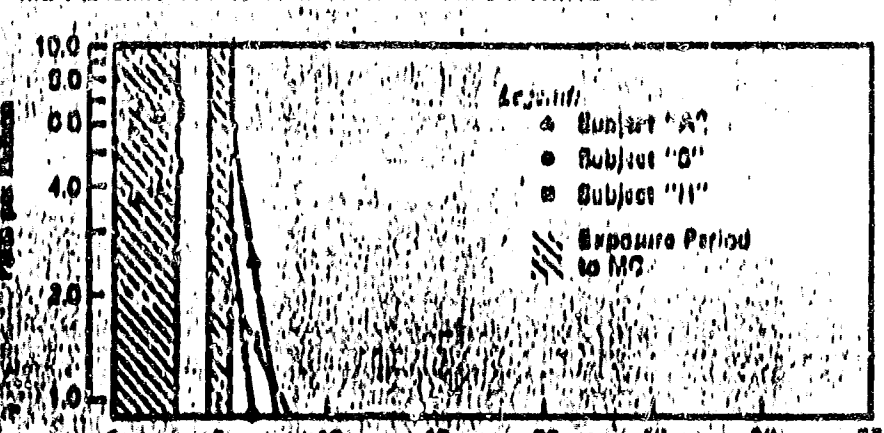

Figure 2

CARBOXYHEMOGLOBIN (%) IN BLOOD DURING AND AFTER EXPOSURE

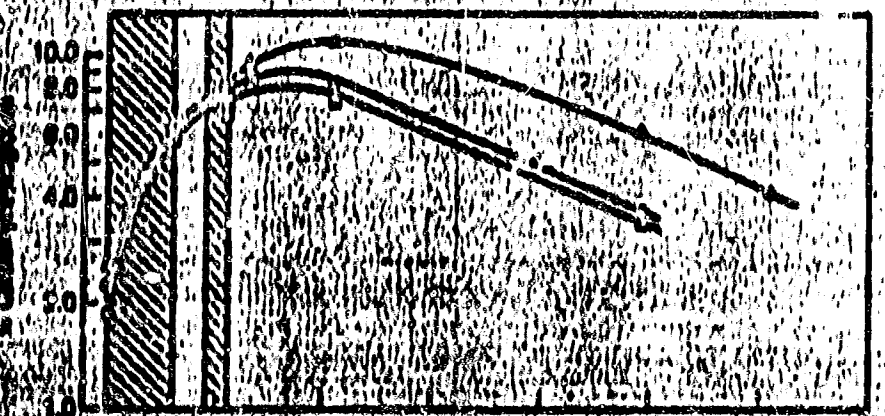

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TABLE 1

SUMMARY OF EXPOSURE DURATION AND CONCENTRATIONS, AND BLOOD SAMPLING TIMES

Course of Experiment	Exposure	Methylene Chloride Conc. Room Air (ppm)	Personnel Dosimeter A	B	C	Blood Sample Times
0 to 3 hrs	yes	680	920	930	620	0.5, 1, 2, and 3 hrs
3 to 4.5 hrs	no	420	60	21	17	none
4.5 to 8.5 hrs	yes	350	580	510	350	5.5 hrs
8.5 to 30 hrs	no	210[a]	-[b]	-[b]	-[b]	6.5, 10.5, 24.5 and 30.5[c]

[a] Between 8.5 and 24.5 hrs.

[b] Not measured.

[c] Only "A" was sampled at 30.5 hrs.

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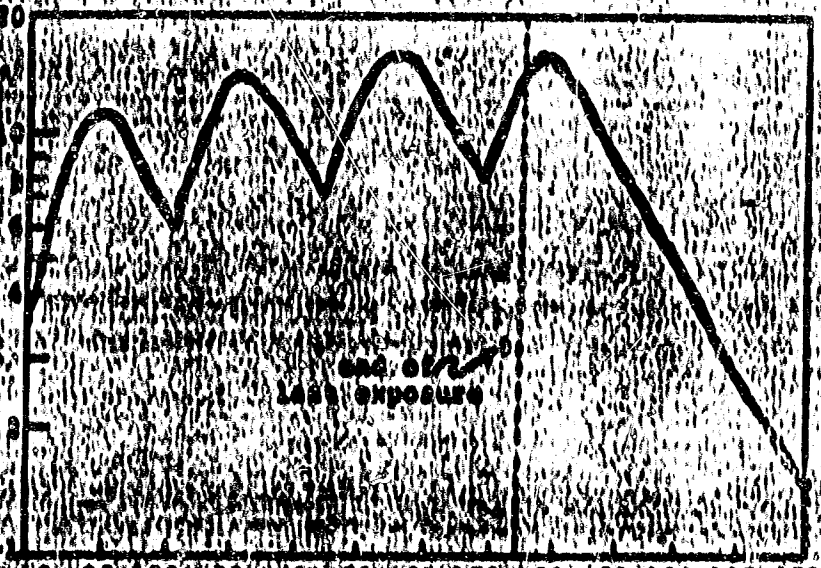
FIGURE 2

APPROACH OF COHb TO A STEADY-STATE LEVEL AFTER MULTIPLE EXPOSURE TO METHYLENE CHLORIDE (630 ppm)



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Doc Title: METHYLENE CHLORIDE DISTRIBUTION AND MACROMOLECULAR INTERACTION OF INHALED METHYLENE CHLORIDE IN VARIOUS TISSUES OF THE MALE SPRAGUE - DAWLEY RAT

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METHYLENE CHLORIDE	75-09-2

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METHYLENE CHLORIDE: DISTRIBUTION AND MACROMOLECULAR INTERACTIONS OF INHALED METHYLENE CHLORIDE IN VARIOUS TISSUES OF THE MALE SPRAGUE-DAWLEY RAT

By

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ABSTRACT

A significant increase in the number of sarcomas in the ventral neck region in and around the salivary glands of male (but not female) Sprague-Dawley rats has recently been observed in our laboratory following inhalation of 3500 ppm of methylene chloride ($MeCl_2$) 6 hours/day, 5 days/week for two years. In an attempt to understand the toxicological basis for this unusual response, the distribution of $MeCl_2$ or a potential reactive intermediate was evaluated in male Sprague-Dawley rat salivary tissue (submaxillary, sublingual, parotid glands) relative to other target (liver) and nontarget tissues (kidney, abdominal muscle and/or fat) immediately following a 6-hr exposure to 3500 ppm of ^{14}C-$MeCl_2$. As indicated by whole tissue ^{14}C activity, the highest quantity of radioactivity per gram of tissue was found in the fat, with approximately 10-fold less found per gram of kidney, muscle, and submaxillary and sublingual glands. The concentration of ^{14}C activity in the parotid gland was approximately 3-5 fold greater than that of the submaxillary and sublingual gland, and most probably represented that due to associated adipose and lymphoid tissue which could not be totally dissected away from the diffuse parenchymal tissue of the parotid gland. As an indicator of possible reactive intermediate formation, the extent of irreversible binding of ^{14}C-activity to tissue macromolecules was also evaluated. The greatest extent of apparent macromolecular binding was found in the liver, followed in decreasing order by the sublingual gland, kidney, submaxillary gland, parotid gland and muscle. Thus, $MeCl_2$ appears not to be preferentially concentrated, nor preferentially biotransformed to a reactive intermediate(s), in salivary tissue relative to the other tissues evaluated. In an attempt to determine whether acute exposure to $MeCl_2$ might produce tissue alterations such as hypertrophy or hyperplasia which are not observable after long-term exposure, the DNA content and DNA synthesis in salivary tissue, liver and kidney were evaluated following exposure to either 0 or 3500 ppm of $MeCl_2$, 6 hr/day for 3 days. No significant differences were detected between the exposed and control groups in the DNA synthesis or content of any of the tissues evaluated. The results of the present study have shown that the apparent sensitivity of the salivary tissue of male Sprague-Dawley rats chronically exposed to high levels of $MeCl_2$ does not appear to be related to preferential concentration of $MeCl_2$ (or a reactive intermediate) in that tissue or

to early salivary tissue injury not seen at later times. Thus, the toxicological basis for the sarcomas observed in the long-term study of $NaCl_2$ remains obscure.

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INTRODUCTION

Methylene chloride (CH_2Cl_2; dichloromethane; $MeCl_2$) is a volatile solvent used in a wide variety of industrial and commercial applications. Because of its volatility, inhalation of $MeCl_2$ vapors constitutes the primary route of human exposure. A long-term chronic toxicity and oncogenicity study of inhaled $MeCl_2$ has recently been completed in our laboratory (Burek et al., 1980). In this study, male and female Sprague-Dawley rats and Syrian Golden hamsters were exposed to 0, 500, 1500, and 3500 ppm of $MeCl_2$ 6 hr/day, 5 days/week for 24 months. $MeCl_2$ did not exhibit any definite signs of toxicity in either sex of hamsters. In fact, hamsters had less extensive spontaneous geriatric changes and an exposure-related decreased mortality (females). In contrast to the hamster, the liver appeared to be the primary target organ in the rat with slight exposure-related effects observed in both sexes at all exposure concentrations.

An increased number of benign mammary tumors per tumor-bearing rat was observed in the female rats at 500, 1500 and 3500 ppm and to a lesser extent in the male rats at 1500 and 3500 ppm. The total number of rats with benign mammary tumors was however not statistically increased nor was there any observable progression from benign to malignant tumors. It is hypothesized that since Sprague-Dawley rats spontaneously develop benign mammary tumors in ~ 80% of the females and ~ 10% of the males, (i) stress related to the high $MeCl_2$ exposure concentrations or (ii) possible altered normal steroid hormone metabolism resulting from the slight hepatic toxicity may have enhanced the normal spontaneous benign mammary tumor response in these animals (Burek et al., 1980).

The most unusual response in the chronic study was an increased number of sarcomas (malignant mesenchymal tumors) in the ventral neck region, in and around the salivary glands of male rats (but not females) exposed to 1500 or 3500 ppm of $MeCl_2$. The incidence of sarcomas (no. of sarcomas/no. of treated rats) and corresponding exposure concentrations of $MeCl_2$ were

as follows: 0 ppm (1/124), 500 ppm (0/124), 1500 ppm (5/124) and 3500 ppm (11/124). This effect was only statistically significant at 3500 ppm with a trend for an increase at 1500 ppm. The toxicological significance of this finding, if any, is not entirely clear since (i) only male rats were affected and pharmacokinetic and toxicologic studies conducted to date have not revealed appreciable sex differences in the response to $MeCl_2$; (ii) while the origin of the tumors (sarcomas) was uncertain, they appeared to arise from mesenchymal tissue rather than from the epithelial components of the glands; (iii) the rats in this study had been infected early in the treatment interval by the viral disease sialodacryoadenitis which primarily affects the salivary glands; and (iv) $MeCl_2$ failed to produce a similar salivary tumor response in Fischer 344 rats, based on the preliminary results of a long-term high dose gavage study sponsored by the National Cancer Institute (1981).

In an attempt to gain insight into possible underlying mechanisms for this unusual salivary tumor response, the distribution and macromolecular interactions of $MeCl_2$ were evaluated in male Sprague-Dawley rats. Specifically, it was the objective of the present study to determine (i) whether exposure to ^{14}C-$MeCl_2$ results in any preferential distribution of $MeCl_2$, or a potential reactive intermediate, in salivary tissue relative to other target or nontarget tissue, and (ii) whether alterations in salivary tissue, liver or kidney DNA content or synthesis occurs after acute exposure to $MeCl_2$.

METHODS

Chemicals. Technical grade $MeCl_2$ (Lot #7-100) was obtained from The Dow Chemical Company, Plaquemine, LA and used throughout the study. The purity of the $MeCl_2$ was found to be >99.9% by gas chromatographic analysis. ^{14}C-$MeCl_2$ (Lot #1291-205) was purchased from the New England Nuclear Corporation (Boston, MA) at a specific activity of 4.85 mCi/mmole. The radiochemical purity of the ^{14}C-$MeCl_2$ was verified as ≥98% by mass spectrometry/radio-gas chromatography with a Finnigan 3200 mass spectrometer (chemical ionization mode) interfaced with a Panax beta detector (Hermann, 1980).

Animals. Male Sprague-Dawley rats (Spartan Research Laboratories, Haslett, MI) weighing 290-360g were used in the present study. This strain of rat was chosen because of its use in the long-term chronic toxicity and oncogenicity study of $MeCl_2$ conducted in our laboratory (Burek et al., 1980). The rats were uniquely identified with a metal ear tag and acclimated to our laboratory for at least 7 days in a temperature (~72°F) and humidity (~45%) controlled room with a 12 hour lighting cycle. Water and food (PURINA CERTIFIED RODENT CHOW, Ralston Purina, St. Louis, MO) were provided *ad libitum* except during exposure. The rats were selected for study using a computer randomization routine.

Vapor Generation and Analysis. Exposure to 3500 ppm of $MeCl_2$ vapor took place in a 30-liter glass chamber operated under dynamic airflow conditions (10 liters of air/min). $MeCl_2$ vapor was generated by metering liquid $MeCl_2$ at a controlled rate into a vaporization flask using an FMI pump (Fluid Metering, Inc., Oyster Bay, NY) and mixing with the chamber airflow. For exposure to ^{14}C-$MeCl_2$, sufficient ^{14}C-radiotracer was added to the liquid $MeCl_2$ to provide a concentration of ~500 dpm/ml of chamber atmosphere.

The analytical concentration of $MeCl_2$ in the chamber atmosphere was determined at 30 min intervals by gas chromatography (Varian, Model 2400) using flame ionization detection. One ml of chamber atmosphere was directly injected onto a 3' x 1/8" (o.d.) stainless steel column packed with 80/100 mesh Porapak QS (Waters Assoc., Inc., Milford, MA). A column oven temperature of 150°C and a detector temperature of 210°C were used. Gas flow rates for these analyses were: air, 250-300 ml/min; hydrogen and helium (carrier gas), 30 ml/min. For the ^{14}C-$MeCl_2$ exposure, the ^{14}C activity of the chamber atmosphere was determined at hourly intervals by bubbling a 1-ml aliquot of chamber atmosphere from a syringe into a liquid scintillation vial containing a mixture of Conaifluor (Mallinckrodt Chemical Company, St. Louis, MO): 2-methoxyethanol:toluene (6:11:83). Throughout the course of the study, radioactivity was quantified using either a Mark III (Searle Analytic, Inc., Chicago, IL) or LS-9000 (Beckman Instruments, Inc., Irvine, CA) liquid scintillation spectrometer.

EXPERIMENTAL PROCEDURE

Tissue Concentration and Macromolecular Binding. Ten rats were exposed to 3500 ppm of ^{14}C-$MeCl_2$ vapor for 6 hr to assess the concentration of $MeCl_2$ and macromolecular binding of ^{14}C activity in selected tissues. Immediately upon termination of the exposure, the rats were sacrificed by decapitation. The submaxillary, sublingual and parotid salivary glands, liver, kidneys, and samples of abdominal muscle and fat were then removed from five of the animals and frozen in liquid nitrogen. The ^{14}C activity in these tissues was determined as follows. The frozen tissue was pulverized at liquid nitrogen temperature using a SPEX freezer mill (Spex Industries, Inc., Metuchen, NJ). Aliquots of tissue were placed in tared liquid scintillation vials containing 20 ml of ACS liquid scintillation fluid (Amersham Corp., Arlington Heights, IL). The vials were then reweighed to determine the weight of the pulverized tissue added, counted by liquid scintillation spectrometry, and reported as μmole equivalents of $MeCl_2$/g of tissue.

For the macromolecular binding studies, the tissues from the five remaining rats were frozen on dry ice and stored at -70°C until analyzed. Approximately 25-50% (w/v) homogenates of liver, kidney, muscle, parotid and submaxillary glands and 5-10% (w/v) homogenates of the sublingual gland were prepared in distilled water. The irreversible binding of ^{14}C activity to the tissue macromolecules was determined as reported by Schumann et al. (1980). Briefly, the solvent extracted trichloroacetic acid precipitable tissue pellet was solubilized in 1.0 M KOH, neutralized with 1.0 M HCl and the ^{14}C activity of 1-2 ml aliquots quantitated by liquid scintillation spectrometry. Aliquots (20 μl) of the 1 M KOH solution containing the solubilized tissue macromolecules were assayed for protein by the method of Lowry et al. (1951). The results are expressed as μmole equivalents of $MeCl_2$ bound/g of tissue protein.

Tissue DNA Content and DNA Synthesis. Ten rats were lightly anesthetized with methoxyfluorane and an ALZET capsule (Alza Corp., Palo Alto, CA) containing 200 μCi of ^3H-thymidine (^3H-TdR) (New England Nuclear,

Boston, MA; 21.6 Ci/mmole) implanted subcutaneously in the dorsal neck region of each rat. Beginning the following morning, five of the rats were exposed to 3500 ppm of $MeCl_2$ for 6 hr on each of three consecutive days. The other five rats were exposed in an identical manner to chamber air only. On the day following the last exposure, the rats were sacrificed by decapitation and the salivary tissue (parotid, sublingual and submaxillary glands), liver and kidneys were removed, weighed, frozen on dry ice and stored at -70°C until analyzed.

The salivary tissue was homogenized in 3.25 ml of 0.04 M EDTA/0.0 1 M NaCl (pH 8.0) and DNA isolated by slight modification of the method described by Reitz et al. (1980). After homogenization, the cells were immediately lysed by the addition of 0.75 ml of 16.5% sodium dodecyl sulfate (SDS) and mixed with 1 ml of 5 M $NaClO_4$. The mixture was heated to 60°C for 10 min, cooled, and extracted in 5 ml of 4% isoamyl alcohol in chloroform (1 hr shaking at 20-25°C). After extraction, the aqueous layer was carefully removed and DNA spooled on a glass rod after addition of 2 vol of cold 95% ethanol. DNA was isolated in a similar manner from approximately 1 gram quantities of liver and kidney which had been homogenized in 10 ml of the 0.04 M EDTA/0.015 M NaCl solution. In this case 3 ml of 16.5% SDS, 5 ml of 5 M $NaClO_4$ and 15 ml of 4% isoamyl-alcohol in chloroform were used.

The isolated DNA was blotted dry and then incubated in 2 ml (salivary tissue and kidneys) or 3 ml (liver) of DNAse(0.1 mg/ml enzyme in 0.05 M Tris buffer + 0.01 M $MgCl_2$, pH 7.6) at 37°C for 3-3.5 hr. DNA was quantified in 20 (liver), 50 (parotid, submaxillary glands and kidney) or 300 µl (sublingual gland) aliquots of the hydrolyzed DNA solution by the diphenylamine reaction (Burton, 1956). Radioactivity was quantified in 1.0 ml (liver-kidney) or 1.5 ml (salivary tissue) aliquots of the hydrolyzed DNA solution by liquid scintillation spectrometry. Tissue DNA content and DNA synthesis are reported as mg DNA/g tissue and dpm ^{3}H-TdR/µg DNA, respectively.

Statistical Analysis. The DNA content and DNA synthesis data were analyzed using a computer program that performed an F-test for homogeneity of variance and Student's t-test. The level of statistical significance chosen for establishing a difference between the control and treated groups was $p<0.05$.

RESULTS

The mean analytical concentration and specific activity of $MeCl_2$ in the inhalation chamber atmosphere for the 6-hr exposure to 3500 ppm of ^{14}C-$MeCl_2$ were determined to be 3420±220 (SD) ppm and 3737±493 (SD) dpm/μmole of $MeCl_2$, respectively. The mean daily analytical concentration for the 6 hr/day, 3-day exposure to 3500 ppm of $MeCl_2$ was 3473±30 (SD) ppm.

Table 1 shows the distribution of ^{14}C activity in the various tissues of male Sprague-Dawley rats immediately after exposure to 3500 ppm of ^{14}C-$MeCl_2$. The highest tissue concentration of ^{14}C activity (μmole equivalents of $MeCl_2$/g of tissue) was found in the fat. Approximately 10-fold less ^{14}C activity per g of tissue was observed in the liver, kidney, muscle, submaxillary and sublingual glands. The concentration of ^{14}C activity found in the parotid gland was intermediate between these tissues and that of the fat. Radioactivity was observed irreversibly bound to the greatest extent to liver macromolecules (μmole equivalents of $MeCl_2$ bound/g of tissue protein), followed by the sublingual gland, kidney, submaxillary and parotid glands. The lowest extent of binding was observed in muscle. Exposure to 3500 ppm of $MeCl_2$, 6 hr/day for 3 days, did not significantly alter the DNA content (mg DNA/g tissue) or DNA synthesis (dpm of ^3H-TdR incorporated/μg DNA) from control values in any of the tissues evaluated (Table 2).

DISCUSSION

The present study was conducted in an attempt to determine whether the sarcomas observed in the ventral neck region of male Sprague-Dawley rats exposed to 3500 ppm of $MeCl_2$ for 2 years (Burek et al., 1980) might be

related to preferential distribution of $MeCl_2$ or a reactive intermediate(s) in salivary tissue relative to other target (liver) or nontarget tissue (fat and/or kidney and muscle). In addition, the DNA content of these tissues as well as tissue DNA synthesis were evaluated as possible indicators of early tissue change following high level exposure to $MeCl_2$.

Since the rats were sacrificed immediately after exposure and the tissues were rapidly frozen and processed at liquid nitrogen temperature, it was assumed that the total ^{14}C content of the tissue reflected that due predominantly to $MeCl_2$. In view of its known lipid solubility (oil:water partition coefficient = 21; Sato and Nakajima, 1979), it was not surprising that the highest concentration of $MeCl_2$ was observed in the fat immediately after exposure. There did not appear to be any preferential concentration of $MeCl_2$ in salivary tissue (sublingual and submaxillary glands) relative to liver, kidney or muscle. The higher concentration of $MeCl_2$ observed in the parotid gland most probably represented that due to accompanying adipose and lymphoid tissue which could not be completely dissected away from the diffuse glandular tissue.

The irreversible binding of ^{14}C activity to tissue macromolecules was evaluated as a gauge of potential reactive intermediate(s) formation. The greatest extent of irreversible macromolecular binding of ^{14}C activity was found in the liver. This observation is consistent with the liver being the primary site for xenobiotic biotransformation and with the work of Anders et al. (1977) which suggests that metabolism of ^{14}C-$MeCl_2$ is required for the irreversible binding of radioactivity to microsomal protein and lipid. In the present study, radioactivity was not found to be preferentially bound to salivary tissue macromolecules compared to that in the liver or kidney. The slightly higher mean binding value and the greater variation observed in the sublingual gland may have been related to the small amount of tissue which could be obtained for analysis per rat.

Irreversible binding of ^{14}C activity to hepatic and renal macromolecules

of rats has been reported by other investigators following administration of ^{14}C-$MeCl_2$ (Reynolds and Yee, 1967; and DiVincenzo and Hamilton, 1975). Since $^{14}CO_2$ and ^{14}CO have been reported as the primary biotransformation products in rats following exposure to ^{14}C-$MeCl_2$ (DiVincenzo and Hamilton, 1975; McKenna et al., 1979; and McKenna and Zempel, 1981), the extent to which the binding observed in the present study represents incorporation of single ^{14}carbon units in normal macromolecular biosynthesis, versus alkylation, is uncertain.

Exposure of rats to 3500 ppm of $MeCl_2$, 6 hr/day for 3 days, did not alter salivary tissue, liver or kidney DNA synthesis or DNA content, suggesting the lack of a hypertrophic or hyperplastic response in these tissues. This finding is in agreement with the results of a recent study by Landry et al. (1981) in which no exposure-related histopathological alterations were observed in salivary, liver or kidney tissue after 1 or 3 days of exposure to 3500 ppm of $MeCl_2$. Landry et al. (1981) did observe swelling of the external nares, edema in 2 of 6 male rats in the area near the salivary glands and a decrease in the size of the acini of the parotid salivary glands in rats exposed to 8000 ppm of $MeCl_2$ for 1 or 3 days. It was however concluded that these effects were apparently not causally related with the occurrence of sarcomas in the ventral neck region of the chronically exposed male rats since (1) the primary area of edema and inflammation was near the external nares, and not the ventral neck region, (2) edema and inflammation were observed in both males and females, (3) edema and inflammation did not occur at 3500 ppm, and (4) the acute effect observed in the parotid salivary gland (decreased acinar size) was thought to be a reversible nonspecific alteration.

In summary, the apparent sensitivity of the salivary tissue of male Sprague-Dawley rats chronically exposed to high levels of $MeCl_2$ does not appear to be related to preferential concentration of $MeCl_2$ (or a reactive intermediate) in that tissue or to early salivary tissue injury (based on tissue DNA content and synthesis). Thus the toxicological basis for the sarcomas observed in the long-term study of $MeCl_2$ remains obscure.

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TITLE OF STUDY: Methylene Chloride: Distribution and Macromolecular Interactions of Inhaled Methylene Chloride in Various Tissues of the Male Sprague-Dawley Rat. MET-K-001712-(21)

In compliance with Good Laboratory Practice Regulations, the study phases were inspected by the Quality Assurance Unit and the results of these inspections reported to Management and the Study Director on 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 reports are located at the submitting laboratory.

Study Started:	12-17-80	Report Issued Date:	11-30-81
Dates of Inspection:	12-11-80	Date of Report:	12-11-80
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	10-23-81		10-26-81

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TABLE I

METHYLENE CHLORIDE: DISTRIBUTION AND MACROMOLECULAR INTERACTIONS OF INHALED METHYLENE CHLORIDE ($MeCl_2$) IN VARIOUS TISSUES OF THE MALE SPRAGUE-DAWLEY RAT

Tissue Concentration and Irreversible Macromolecular Binding of ^{14}C Activity Immediately After Termination of a 6-Hour Exposure to 3500 ppm of ^{14}C-Methylene Chloride[a]

Tissue	Tissue Concentration (μmole Eq. of $MeCl_2$/g tissue)	Macromolecular Binding (μmole Eq. $MeCl_2$ bound/g protein)
Liver	1.59±0.08	4.61±0.88
Kidney	1.76±0.18	2.50±0.40
Muscle	1.61±1.21	0.48±0.17[b]
Fat	17.7±1.61	-[c]
Parotid	5.22±0.98	1.67±0.21
Submaxillary	1.75±0.46	1.86±0.21
Sublingual	0.96±0.41	3.27±1.06[d]

[a]The data are presented as mean ± SD (n=5)

[b]n=4

[c]Not determined

[d]n=3

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TABLE 2

METHYLENE CHLORIDE: DISTRIBUTION AND MACROMOLECULAR INTERACTIONS OF INHALED METHYLENE CHLORIDE ($MeCl_2$) IN VARIOUS TISSUES OF THE MALE SPRAGUE-DAWLEY RAT

Tissue DNA Content and DNA Synthesis Following Three 6-Hr Exposures to 3500 ppm of Methylene Chloride[a]

Tissue	DNA Content (mg DNA/g tissue)		DNA Synthesis (dpm ^{3}H-TdR/µg DNA)	
	Control	Treated	Control	Treated
Liver	1.89±0.24	1.83±0.11	8.29±2.66	10.8±2.52
Kidney	3.22±0.20	3.03±0.22	12.2±2.69	14.2±4.69
Parotid	3.16±0.56	3.45±1.08	12.4±3.90	12.7±4.10
Submaxillary	3.40±0.29	3.41±0.51	11.8±2.05	10.6±3.03
Sublingual	2.41±0.69	2.32±0.29	7.19±1.37	6.71±1.69

[a]The data are expressed as mean ± SD (n=5). The rats were sacrificed one day following the last 6-hr exposure. There were no statistically significant differences between corresponding control and exposed groups at $p < 0.05$.

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Chemical Name (300 per name)	CAS No. (10)
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DOW CHEMICAL U.S.A.

October 7, 1981

TOXICOLOGY RESEARCH LABORATORY

METHYLENE CHLORIDE - URETHANE GRADE: RAT ACUTE ORAL TOXICITY

9 PAGES IN FULL REPORT

A. E. Carreon

P. G. Watanabe and K. S. Rao

This report is: INTERIM / FINAL and mainly: NEW / REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

A sample of Methylene Chloride - Urethane Grade identified as Lot #SI 6384776 was submitted to the Toxicology Research Laboratory on June 26, 1981 for assessment of acute oral toxicity. The source of this clear liquid was the Inorganic Chemicals Laboratory, Midland, Michigan.

Acute oral toxicity tests were conducted on male CD" (Fischer 344-derived) rats (Charles River Breeding Laboratories, Inc., Portage, Michigan) weighing 181 15 g.

The acute oral toxicity of this material was low. The single-dose oral LD_{50} for male rats was 1792 mg/kg (1189-2692 mg/kg, 95% confidence interval) when calculated by the moving average method of analysis. Rats were administered 630, 1300, 2500 or 5000 mg/kg of the undiluted test material by single-dose gavage. The following in-life signs of toxicity were observed in test animals: lack of physical coordination, lethargy, rapid shallow breathing, body tremors, palpebral closure, rough hair-coats and a blackening of the tip of the tail (1/4 of distal portion) along with some scaling and/or sloughing of the tail. Rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats 2 weeks post-treatment, there was some evidence of gastrointestinal irritation, characterized by roughened areas and multifocal erosions within the stomach wall. However, no problem is anticipated from human ingestion of this material encountered incidental to industrial handling. Systemic injury may occur from accidental or deliberate ingestion of appreciably larger amounts.

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METHYLENE CHLORIDE - URETHANE GRADE

RAT ACUTE ORAL TOXICITY

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SUMMARY

The single dose oral LD_{50} for Methylene Chloride - Urethane Grade for male rats was 1792 mg/kg (1189-2692 mg/kg, 95% confidence interval). Rats were administered 630, 1300, 2500 or 5000 mg/kg of the undiluted test material. The following in-life signs of toxicity were observed in test animals (dose groups affected are in parentheses): lack of physical coordination, lethargy, rapid shallow breathing, body tremors (all dose levels) rough hair coats (1300 mg/kg), palpebral closure and blackening of the tip of the tail often with secondary sloughing of up to 1/4 of the distal end of the tail (1300 and 2500 mg/kg). Rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats 2 weeks post-treatment, there was some evidence of gastrointestinal irritation, characterized by roughened areas and multifocal erosions within the stomach wall.

INTRODUCTION

A sample of Methylene Chloride - Urethane Grade identified as Lot #SI 6304776 was submitted to the Toxicology Research Laboratory for assessment of acute oral toxicity. The source of this clear liquid was the Inorganic Chemicals Laboratory.

To assist the reader in evaluating the acute toxicological properties of this material, copies of the original laboratory work sheets are appended to this report.

TEST ANIMALS

Acute oral toxicity tests of this material were conducted on male CDF (Fischer 344-derived) rats (Charles River Breeding Laboratories, Portage, Michigan), weighing 181-225 g. All animals were maintained on a 12 hour light and dark cycle. They were supplied with commercial certified laboratory chow (Ralston Purina Company, St. Louis, Missouri) and water ad libitum. All animals were acclimated to the laboratory environment at least 1 week prior to testing, and were housed 2 or 3 per cage. Each animal was uniquely identified by an individual metal eartag.

ACUTE ORAL TOXICITY

Method

Four groups of male rats, each consisting of 6 rats/dose level, were fasted overnight prior to dosing. The following morning, each group received 630, 1300, 2500 or 5000 mg/kg of the test material by single-dose gavage. Each rat was weighed immediately prior to treatment, the day after treatment and weekly thereafter for a 2 week post-treatment observation period. The rats were observed periodically during this time for signs of toxicity. All surviving rats were submitted for a

gross necropsy examination 2 weeks post-treatment. The acute oral median lethal dose was calculated by the moving average method (Thompson and Weil, 1952). The "slope" was calculated as $\sigma/\log(LD_{84}/LD_{16})$.

Results

Results of acute oral toxicity tests are given in Table 1. The single-dose oral LD_{50} for male rats was 1792 mg/kg (1109-2602 mg/kg, 95% confidence interval), with an approximate slope of 4.1.

Results of animal observation during the 2 week post-treatment observation period are given in Table 2. In-life signs of toxicity observed in test animals included: lack of physical coordination, lethargy, rapid shallow breathing, body tremors, palpebral closure and a blackening of the tip of the tail often with secondary sloughing of up to 1/4 of the distal end of the tail. Rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats 2 weeks post-treatment, there was some evidence of gastrointestinal irritation, characterized by roughened areas (630 and 1300 mg/kg) and multifocal erosions (1300 and 2500 mg/kg) within the stomach wall.

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TABLE 1

METHYLENE CHLORIDE - URETHANE GRADE
ACUTE TOXICOLOGICAL PROPERTIES

MORTALITY OF MALE RATS DOSED ORALLY WITH THE TEST MATERIAL

Dose (ml/kg)	#Dead/#Treated	Time of Death
630	0/6	
1300	1/6	1 rat - 2 hours
2500	5/6	1 rat - 8.5 hours 3 rats - Day 2 1 rat - Day 3
5000	6/6	1 rat - 2 hours 5 rats - 8.5 hours

TABLE 2

METHYLENE CHLORIDE - URETHANE GRADE: ACUTE TOXICOLOGICAL PROPERTIES

OBSERVATIONS OF MALE RATS DOSED ORALLY WITH THE TEST MATERIAL

Dose (mg/kg)	Signs of Toxicity	#Affected/#Treated	Approximate Time of Onset
630	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
1300	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
	Palpebral closure	5/5[a]	5 rats - 5.5 hours
	Rough hair coat	5/5[a]	5 rats - 2 hours
	Blackened distal 1/4 of tail	5/5[a]	3 rats - Day 2 2 rats - Day 3
	Sloughing of distal 1/4 tail	5/5[a]	5 rats - Day 14
1500	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
	Rough hair coat	5/5[a]	5 rats - 5.5 hours
	Palpebral closure	5/5[a]	5 rats - 5.5 hours
	Blackened distal 1/4 of tail	1/1[a]	1 rat - Day 3
	Sloughing of distal 1/4 tail	1/1[a]	1 rat - Day 4
5000	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes

[a]Data presented as number affected/number surviving

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TOXICOLOGY RESEARCH LABORATORY
THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN

ACUTE ORAL TOXICITY

METHYLENE CHLORIDE
Urethane Grade

SPECIES: ☒ RAT ☐ GUNY ☐ RABBIT ☐ MOUSE

SEX: ☐ ☐

SYRINGE: [illegible]
EMPTY SYRINGE: [illegible]
[illegible] CC: [illegible]
SP. GRAVITY: [illegible]

☐ SOLUTION ☐ SUSPENSION
[illegible] IN [illegible] CC [illegible]
☐ WATER ☐ CORN OIL
☐ OTHER

CAGE	DATE FED	ANIMAL MARKING	WEIGHT GM.	DOSE GM./KG.	DOSE CC.	ANIMAL WEIGHT (GM.) [illegible]	[illegible]	[illegible]	DATE
230- 11	[illegible]	7517	196	.62	0.09	207	230	246	
		7518	210		0.10	228	243	264	
		7519	224		0.11	225	255	272	
12		7520	192		0.09	205	227	241	
		7521	208		0.10	216	235	253	
		7522	222	V	0.11	234	256	272	
13		7523	200	1.2	0.20	209	226	240	
		7524	220		0.22	223	250	270	
		7525	214		0.21	220	246	266	
14		7526	200		0.20	197	216	226	
		7527	185		0.18	177	201	210	
		7528	181	V	0.18	[illegible]	[illegible]	[illegible]	
15		7529	216	2.5	0.41	[illegible]	[illegible]	[illegible]	
		7530	200		0.38	196	[illegible]	7/16/81	[illegible]
		7531	225		0.42	223	254	261	
16		7532	198		0.36	[illegible]	[illegible]	[illegible]	
		7533	208		0.40	[illegible]	[illegible]	[illegible]	
		7534	205	V	0.39	[illegible]	[illegible]	[illegible]	
17		7535	189	5.0	0.72	[illegible]	[illegible]	[illegible]	
		7536	210		0.80	[illegible]	[illegible]	[illegible]	
		7537	210		0.80				
18		7538	217		0.82	[illegible]	[illegible]	[illegible]	
		7539	211		0.80				
RESERVATION OF	[illegible]	7576	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

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METHODOLOGY

Animals used for acute oral toxicity tests are fed by single-dose gavage using a calibrated syringe and a blunt feeding needle (rats, mice, guinea pigs) or catheter (rabbits). The animals are fasted overnight (except rabbits) and are weighed before dosing, the day after dosing, and at 1 and 2 weeks after dosing. The animals are observed closely the day of treatment, then periodically for the 2-week observation period. Unless otherwise noted, 6-8 week old Sprague-Dawley rats, 6-8 week old CF₁ mice, 6-8 week old Hartley guinea pigs, or 3-3 1/2 month old New Zealand albino rabbits are used. For range studies, 2 or 3 animals of one sex per dose level for 2 to 8 levels are used; the third animal (if used) is sent to pathology for a gross examination at 24 hours after treatment. For LD50 studies, 5 animals per sex per dose level for 2 to 6 levels are used; all survivors are submitted for a gross pathological examination after the 2-week weighing. Non-corrosive liquids are dosed as received, corrosive liquids are diluted in water or corn oil. Pastes and solids are prepared as solutions or suspensions in water, corn oil, or 0.5% methocel.

DATE	COMMENTS
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7/13/81	Aug 13, 1981 [illegible]
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METHYLENE CHLORIDE - A FOUR WEEK INHALATION TOXICITY STUDY IN RATS HAMSTERS AND MICE

LK 8.31.83

Chemical Name (300 per name) 25	CAS No. (10) 26
METHYLENE CHLORIDE	75-09-2

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METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

By

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INTRODUCTION

Methylene chloride (dichloromethane, CH_2Cl_2), is used extensively as a solvent in several industrial and consumer markets.

Several intermittent inhalation toxicity studies of CH_2Cl_2 have been conducted in laboratory animals. Heppel, et al. (1944) exposed dogs, rabbits, guinea pigs and rats to 5000 ppm CH_2Cl_2 7 hrs/day, 5 days/week for 6 months. Adverse effects were only observed in guinea pigs, and included reduced weight gain, decreased food intake and some deaths. Pneumonia and hepatic centrilobular fatty degeneration were seen in the guinea pigs which died. Heppel, et al. (1944) also exposed dogs, monkeys, rabbits, guinea pigs and rats to 10,000 ppm CH_2Cl_2 4 hrs/day, 5 days/week for 8 weeks. Dogs were injured by "energetic and purposeless movements", perhaps the excitement stage of anesthesia, and were removed from the study after a few exposures. The other species showed central nervous system (CNS) effects of varying degrees, consisting of reduced activity, somnolence and/or incoordination. Possible liver effects were seen in 2 of 4 dogs and 4 of 6 guinea pigs. No significant lesions were found in rats. Heppel and Neal (1944) showed reduced motor activity in rats which were exposed to 5000 ppm CH_2Cl_2 for 90 minutes every other day.

Thomas (1971), Weinstein (1972), Bullock (1971), Haun (1971), Haun (1972), and MacEwen (1972) have shown that continuous CH_2Cl_2 exposure produces adverse effects at markedly lower levels than in intermittent exposures. Mice, rats, dogs and monkeys were exposed to 1000 or 5000 ppm methylene chloride for 24 hours/day for up to 14 weeks. A slight increase in mortality was observed in mice inhaling 1000 ppm. Dogs inhaling 1000 ppm died or were sacrificed prior to termination of the exposure. Rats and monkeys showed decreased weight gains upon exposure to 1000 ppm CH_2Cl_2. An increase in serum enzyme levels was noted in dogs inhaling 1000 ppm methylene chloride for 4 weeks. Exposures of animals to 5000 ppm methylene chloride were terminated early. Mice and dogs exhibited a high incidence of mortality. Histopathological effects observed consisted primarily of a fat accumulation in mice, rats and dogs inhaling 1000 or 5000 ppm methylene chloride.

Some work on metabolism of CH_2Cl_2 has been reported. Stewart (1972) reported CH_2Cl_2 vapor inhalation produced increased carboxyhemoglobin

ABSTRACT

CD-1 mice, Golden Syrian hamsters, Sprague-Dawley and COF (F-344) rats were exposed to 0, 2500, 5000, or 8000 ppm methylene chloride vapor 6 hr/day, 5 days/week for a total of 19, 18, 19 or 7 exposures, respectively, in 21-28 days. Body weight data was obtained throughout the study. Clinical chemistry parameters were measured. All animals underwent gross pathological examination at the termination of the experiment. The weights of the liver and kidney were recorded for the 0, 2500 and 8000 ppm groups, and organ/body weight ratios were calculated.

Animals exposed to 8000 ppm methylene chloride exhibited anesthetic effects and a decrease in body weights in rats. The animals exposed to 8000 ppm showed slight anesthesia, decreased body weight in male rats, increased SGPT values in female mice and Sprague-Dawley rats and increased liver weights in female mice, hamsters and rats. Animals exposed to 2500 ppm methylene chloride exhibited more scratching activity than controls but showed no other effect attributable to exposure.

levels in man. Kubic et al. (1974), Kubic and Anders (1975), and Ratney (1974) have demonstrated metabolism of CH_2Cl_2 to CO which accounts for the observed increase in carboxyhemoglobin levels after CH_2Cl_2 exposure. DiVincenzo (1975) has reported on the fate and distribution of $^{14}CH_2Cl_2$ in rats after ip injection. At 24 hours, 91.5% of the dose was eliminated in the expired air unchanged, 2% was eliminated as CO, 3% as CO_2, 1.5% as an uncharacterized metabolite. One percent was excreted in the urine and 2% remained in the carcass. McKenna et al. (1979) have examined the pharmacokinetics and metabolism of inhaled methylene chloride. The major metabolites were carbon monoxide and carbon dioxide. The metabolism of methylene chloride was shown to be a saturable process.

The current TLV for methylene chloride as set by The American Conference of Governmental Industrial Hygienists is 200 ppm, with an intended change to 100 ppm in 1981.

The results of a 4 week study of intermittently inhaled methylene chloride in mice, hamsters, and rats which are reported here were used to determine appropriate levels for a subsequent two year chronic toxicity and oncogenicity study.

METHODS

General Study Design. Mice, hamsters and rats were exposed to 0, 2500, 5000 or 8000 ppm methylene chloride for up to 4 weeks. Due to the effects observed in animals inhaling 8000 ppm methylene chloride these animals were only exposed for 7 exposures in three weeks. The animals were weighed throughout the study period. Clinical chemistry parameters were measured at the scheduled necropsy. Absolute and relative organ weights of the liver and kidney were recorded at necropsy.

Material. Three grades of methylene chloride were used in the probe due to the unavailability of urethane grade methylene chloride. Technical grade methylene chloride was used on the first day of exposure to 5000 and 8000 ppm methylene chloride. Aerothene grade CH_2Cl_2 was used on days 2-9 of exposure and urethane grade CH_2Cl_2 was used on days 10-29 of exposure. No analysis was made of the methylene chloride used in this study.

Chambers, Vapor Generation and Analysis. The chambers used were rooms 8' x 8' x 8' in size (14.5 cubic meters). They were equipped with stainless steel ceilings in the shape of a regular quadrangular pyramid. The walls and floors of the chambers were coated with epoxy resin. Temperature and humidity in the chambers were controlled by a system designed to maintain temperature and relative humidity at approximately 21°C and 50%, respectively.

CH_2Cl_2 vapor was generated by metering the liquid at a constant rate with a precision pump into a warmed vaporization flask (approximately 200°C). The vapor was swept with filtered air into the main chamber airflow of about 1950-3000 liters per minute (70-107 cfm, 8-12 air changes per hour).

Air standards were prepared by mixing known amounts of methylene chloride with 100 L of filtered air in a SARAN* bag. These standards were then analyzed by a MIRAN 1A infrared spectrometer at a wavelength of 7.87 μ. The concentration of CH_2Cl_2 was measured at least four times/day for each chamber with the infrared spectrometer and a time weighted average for each chamber was calculated.

Animals. Animals used in this study were CD-1 mice (Charles River Laboratory, Wilmington, MA) and Golden Syrian hamsters (Engles Laboratory Animals, Farmersburg, IN), 4-6 weeks of age, Sprague-Dawley (Spartan Research, Haslett, MI) and CDF (Fischer 344 derived, Charles River Laboratory, Wilmington, MA) albino rats, 6-8 weeks of age. All animals were identified by an ear notch and were randomly assigned to four

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groups with 10 animals/species (strain)/sex in each group. These groups were exposed to 0 (filtered air), 2500, 5000, or 8000 ppm of CH_2Cl_2 vapor 6 hr/day, 5 days/week for a total of 19, 18, 19, or 7 exposures, respectively in 21-28 days. The 8000 ppm exposure group was exposed on days 1, 14, 15, 16, 17, 20 and 21 of the study and were weighed on days 0, 13, 17, and 20 of the study. The 8000 ppm exposure was terminated after 7 exposures due to the anesthetic effects observed. The animals inhaling 2500 ppm methylene chloride were initially exposed one day later than the remaining groups and were added due to the effects observed in animals inhaling 8000 ppm. Consequently, the actual experiment days vary between the 2500 ppm and the 0 or 5000 ppm groups. The mice and hamsters inhaling 0, 2500 or 5000 ppm methylene chloride were exposed for an additional day to methylene chloride. Food was removed during exposure but was present ad libitum at all other times. Water was present ad libitum at all times to rats; water was not available to mice and hamsters during exposure but was present ad libitum at all other times. The animals inhaling 0, 2500 or 5000 ppm methylene chloride were weighed at least twice each week and observed daily for signs of toxicity. Although the experiment days between the various groups vary by one day, the body weights were statistically analyzed together.

Clinical Studies. Blood urea nitrogen (BUN) and serum glutamic pyruvic transaminase (SGPT) values were determined from mice at all concentrations. Blood urea nitrogen and SGPT activity values were measured from hamsters exposed to 0 or 8000 ppm; SGPT activity and alkaline phosphatase (AP) values were measured from hamsters exposed to 0, 2500 or 5000 ppm methylene chloride. Clinical chemistry determinations of BUN, SGPT activity, and total bilirubin were performed on serum collected from rats exposed to 0 or 8000 ppm methylene chloride. Serum from the rats inhaling 0, 2500, or 5000 ppm $MeCl_2$ was used for determining SGPT activity, BUN, and AP values.

Pathology. After the final exposure to 0, 2500 or 4000 ppm, rats and hamsters, but not mice, were fasted overnight prior to necropsy. At the time of necropsy all animals were anesthetized with methoxyflurane prior to clamping of the trachea and subsequent decapitation. Each animal was given a complete gross pathological examination. Liver, kidney, and body weights were recorded at time of necropsy on all animals. Samples of liver and kidney from five animals per sex per species (strain) and any other tissues deemed appropriate at necropsy were preserved in 10% buffered formalin for histologic examination.

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Due to the anesthetic effects observed in animals exposed to 6000 ppm methylene chloride (discussed in Results Section-Animal Observation), this group was terminated after 7 exposures in 21 days. Gross pathology was performed on these animals on the same days as the last exposure. To provide a sufficient sample size for clinical chemistry and organ weight determinations, the extra animals received in the shipments for this study were used as a control group and necropsied with the 6000 ppm group. Consequently, due to the varying number of animals remaining in each group, the number of extra animals necropsied varied from 3 to 10 animals/sex (strain).

Statistical Evaluation. Clinical chemistry, organ weights, and body weight data were evaluated using an analysis of variance and Dunnett's test (Steel and Torrie, 1960). The level of significance chosen for all cases was $p < 0.05$.

RESULTS

Chamber Analysis. A summary of chamber air analyses is shown in Table 1. Since the analytical concentrations were close to the target concentrations, the desired concentrations of 0, 2500, 5000 and 8000 ppm will be used throughout the remainder of the report.

Animal Observation. The animals exhibited anesthetic symptoms due to methylene chloride vapors in the following order: Sprague-Dawley rats (most severe), CDF rats, mice and hamsters (least severe). Mice and hamsters exhibited lesser degrees of anesthesia as characterized by continuous movement with poor coordination and scratching. Rats progressed from lesser degrees of anesthesia to nearly complete anesthesia during the exposure to 8000 ppm CH_2Cl_2. All animals exposed to 8000 ppm methylene chloride showed slight symptoms of anesthesia and included scratching, groggy appearance, and difficulty in walking. All animals exposed to 2500 ppm methylene chloride exhibited more scratching activity than controls but exhibited no other adverse effect.

The Sprague-Dawley rats also exhibited symptoms consistent with infectious sialodacryoadenitis in the control and all exposure groups throughout the duration of the study.

Body Weights. No treatment-related effect was observed on body weights of CD-1 mice or Syrian hamsters inhaling concentrations as high as 8000 ppm methylene chloride for 7 exposures or 5000 ppm for 20 exposures (Tables 2-5). Male CDF rats showed statistically significantly decreased body weight at 5000 and 8000 ppm but only slight weight loss at 2500 ppm methylene chloride (Table 6). Female CDF and Sprague-Dawley rats showed statistically significantly decreased mean body weight at 8000 ppm but not at 5000 ppm methylene chloride (Tables 7 and 9). Male Sprague-Dawley rats showed statistically significantly decreased body weight at 8000 ppm and occasionally at 5000 ppm (Table 8).

Clinical Chemistry and Organ Weights. Clinical chemistry and organ weight values for animals exposed to methylene chloride are listed in Tables 10-17. Those effects considered of toxicologic significance included an increase in SGPT activity and absolute and relative liver weight of CD-1 female mice inhaling 5000 ppm methylene chloride (Table 11), an increase in relative liver weight of female Syrian hamsters and female CDF rats inhaling 5000 ppm methylene chloride (Tables 13 and 15),

an increase in SGPT activity in male Sprague-Dawley rats inhaling 5000 ppm methylene chloride (Table 16), and an increase in SGPT activity and absolute and relative liver weights of female Sprague-Dawley rats inhaling 5000 ppm CH_2Cl_2 (Table 17).

Other statistically significant effects were observed but were not considered to be toxicologically significant. These include a decrease in BUN and relative kidney weights in male CD-1 mice inhaling 5000 ppm CH_2Cl_2 (Table 10), an increase in the body weights of female CD-1 mice inhaling 5000 ppm (Table 11), a decrease in SGPT activity of male Syrian hamsters inhaling 8000 ppm methylene chloride (Table 12), a decrease in BUN levels of CDF rats inhaling 8000 ppm (Tables 14-15), a decrease in absolute kidney weights of male CDF rats inhaling 2500 or 5000 ppm CH_2Cl_2 (Table 14), and a decrease in AP levels of female CDF rats inhaling 2500 or 5000 ppm methylene chloride (Table 15).

Gross Pathology-8000 ppm. Due to the clinically detected anesthetic effects observed in the animals inhaling 8000 ppm methylene chloride, this group was terminated approximately 1 week before the terminal necropsy of the remaining groups after receiving 7 exposures in 21 days.

Mice. (Table 18). In the mice exposed to 8000 ppm, lesions of questionable significance consisting of equivocal focal areas of pulmonary congestion and slight to equivocal paleness of the liver were observed. There were no other lesions noted in any of the control or exposed mice.

Hamsters. (Table 18). Several of the female hamsters exposed to 8000 ppm of methylene chloride exhibited corneal cloudiness upon eye examination. No other lesions were noted in the hamsters.

Rats. (Table 19). CDF rats exhibited several effects considered to be directly or indirectly associated with exposure to 8000 ppm methylene chloride. Externally, these included (1) traumatic lesions to the tail which were assumed to be associated with the anesthetic effects, and (2) a decrease in overall size of the carcass of one male rat. Internally, treatment-related lesions were limited to slight decreases in adipose reserves and a focal gastric ulceration.

Sprague-Dawley rats inhaling 8000 ppm exhibited several effects considered to be directly or indirectly associated with the exposure to methylene chloride. Externally, these included (1) decreased overall size of the carcass, (2) traumatic lesions and blood on facial regions,

(3) unkempt appearance of the hair coat, (4) traumatic lesions to tail, and (5) secretions lateral to the external nares. Internally, this included (1) decreased amounts of adipose reserves, (2) lumen of stomach distended with gas and/or ingesta having a fluid appearance and containing hemolyzed blood, (3) increased number of darkened foci in lungs, (4) pale anemic appearance secondary to hemorrhage from tail lesions, (5) focal ulcerations and erosions of gastric mucosa and (6) darkened contents of distal intestines consistent with hemolyzed blood. Most of the observations in Sprague-Dawley rats were due to the anesthetic effects of exposure to methylene chloride.

Gross Pathology-0, 2500 or 5000 ppm.

Gross necropsy examination of mice, hamsters and rats inhaling 0, 2500, or 5000 ppm methylene chloride revealed various lesions and observations (Table 20). These lesions and observations occurred with similar frequency and severity in both the control and exposed groups, or in a scattered pattern that did not suggest an exposure-related effect.

CONCLUSIONS

As indicated previously in this report, the intent of this study was to ascertain the maximum tolerated concentration of methylene chloride for a subsequent two year toxicity/oncogenicity study. Animals exposed to 8000 ppm methylene chloride showed anesthetic effects, and decreased body weight in rats. Animals inhaling 5000 ppm methylene chloride showed slight anesthesia, decreased body weight in male rats but not female rats, increased SGPT values in female mice and Sprague-Dawley rats and increased liver weights in female mice, hamsters and rats. The elevated SGPT values and liver weights are indicative of possible liver damage. Animals exposed to 2500 ppm methylene chloride exhibited more scratching activity than controls but exhibited no other effect attributable to exposure.

In this study, effects were observed at the same concentration in which Heppel (1944) did not see any adverse effects in rats and mice. The liver was apparently the target organ in this study, as with previous studies. Because of the results obtained, 8000 and 5000 ppm methylene chloride were considered to be excessive exposure levels for the 2-year study, and 2500 ppm did not appear to affect the animals over the 30 day period. Therefore, concentrations of 0, 500, 1500 and 3500 ppm methylene chloride were chosen for a chronic inhalation toxicity/oncogenicity study.

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METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE
HET-K-1712-13A

QUALITY ASSURANCE STATEMENT

This report represents data generated prior to the enactment of the FDA Good Laboratory Practice Regulations. The study was conducted according to standards used in this laboratory at that time. The report accurately reflects all of the data generated. All data and reports are located at the submitting laboratory.

STUDY STARTED:	5 November 1979	REPORT ISSUED:	6 August 1981
PROTOCOL AUDITED:		REPORTED:	
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FINAL REPORT AUDITED:	29 May 1981	REPORTED:	29 May 1981

W. F. Heuvel 6 August 1981
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TABLE 1

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Chamber Concentrations of Methylene Chloride

Control	2500 ppm	5000 ppm	8000 ppm
Desired Concentration			
Number of Chamber Operation Days	20	19	20	7
Analytical Concentration, ppm X ± S.D. of daily time weighted average		2406±118	4913±107	7659±312
Analytical Coefficient of Variation		4.6%	3.8%	4.1%
Range of Daily Analytical Concentrations		2242-2670	4690-5230	7046-8100

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TABLE 2

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weights of Male CD-1 Mice

Experiment Days	Control	2500 ppm	5000 ppm	8000 ppm[b]
0	28±2[a]	30±2	28±2	28±2
3	32±2	30±2	30±2*	N.D.
6	30±3	32±1	34±2*	N.D.
8	33±2	32±2	33±1	N.D.
10	34±2	33±2	34±1	N.D.
13	33±2	34±2	36±1	33±2
17	35±3	34±2	35±2	35±2
20	34±3	34±3	35±2	35±2
24	35±3	35±2	32±3	Sac.
28	39±3	36±3*	37±2	Sac.

[a]Mean ± Standard Deviation, grams.

[b]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

*Significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 3

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weights of Female CD-1 Mice

Experiment Days	Control	2000 ppm	5000 ppm	0000 ppm[b]
0	23±1[a]	25±1*	24±2	24±1
3	26±2	25±1	26±2	N.D.
6	24±1	27±1*	27±3*	N.D.
8	27±1	27±2	27±1	N.D.
10	27±1	27±2	30±1*	N.D.
13	27±1	28±2	28±1	27±1
17	30±1	30±2	30±2	27±2*
20	29±2	29±2	30±1	28±1
24	30±2	30±2	30±2	Sac.
28	30±2	31±2	32±2	Sac.

[a]Mean ± Standard Deviation, grams

[b]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

*Significantly different from control mean using Dunnett's test, $p < 0.05$.

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TABLE 4

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weights of Male Syrian Hamsters

Experiment Days	Control	2500 ppm	5000 ppm	8000 ppm[b]
0	68±5[a]	72±5	68±6	67±5
3	71±5	71±5	70±5	N.D.
6	76±6	79±5	78±5	N.D.
8	80±7	80±6	77±6	N.D.
10	82±7	79±6	79±6	N.D.
13	82±8	86±6	83±6	88±6
17	86±9	86±7	83±6	91±6
20	90±9	91±8	88±6	95±7
24	95±10	97±7	90±8	Sac.
28	97±10	101±8	95±9	Sac.

[a]Mean ± Standard Deviation, grams

[b]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

No values were significantly different from control mean using Dunnett's test, $p<0.05$.

TABLE 8

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weights of Female Syrian Hamsters

Experiment Days	Control	2500 ppm	5000 ppm	8000 ppm[b]
0	64±7[a]	68±7	68±6	63±8
3	67±8	69±7	71±5	N.D.
6	74±8	77±8	79±5	N.D.
8	77±9	77±8	79±5	N.D.
10	80±10	78±8	82±5	N.D.
13	81±10	85±9	85±5	89±9
17	84±11	87±9	85±6	91±9
20	90±12	91±10	90±6	96±9
24	93±12	95±10	87±7	Sac.
28	96±14	100±11	97±7	Sac.

[a]Mean ± Standard Deviation, grams

[b]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

No values were significantly different from control mean using Dunnett's test, $p < 0.05$.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

TABLE 6

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weight of Male Fischer Rats

Experiment Days	Control	2500 ppm	5000 ppm	10000 ppm[c]
0	132±9[a]	146±6*	133±6	133±6
3	151±6	152±7	145±8	N.D.
6[b]	153±8	166±8*	140±10*	N.D.
8	172±6	174±9	160±10*	N.D.
10	179±6	185±9	167±9*	N.D.
13	188±7	185±10	177±8*	202±10*
17	197±8	195±10	186±9*	189±10
20	209±9	202±10	193±10*	196±11*
24	218±11	208±10	195±12*	Sac.
28	228±13	219±12	210±13*	Sac.

[a]Mean ± Standard Deviation, grams

[b]Some rats were without food over the weekend.

[c]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

*Significantly different from control mean using Dunnett's test, $p < 0.05$.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

TABLE 7

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weight of Female Fischer Rats

Experiment Days	Control	2500 ppm	5000 ppm	8000 ppm[c]
0	106±6[a]	116±6*	106±6	105±4
3	114±7	119±6	112±6	N.D.
6[b]	119±7	126±6*	113±7	N.D.
8	124±8	128±6	120±7	N.D.
10	128±8	134±6	123±7	N.D.
13	126±8	130±6	128±6	130±6
17	131±9	133±6	127±6	121±6*
20	135±8	138±6	131±7	125±6*
24	139±10	140±6	134±6	Sac.
28	144±10	146±6	139±7	Sac.

[a]Mean ± Standard Deviation, grams

[b]Some rats were without food over the weekend

[c]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

*Significantly different from control mean using Dunnett's test, $p < 0.05$.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

TABLE 8

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weights of Male Sprague-Dawley Rats

Experiment Days	Control	2500 ppm	5000 ppm	8000 ppm[c]
0	220±9[a]	253±7*	236±10	221.6
3	253±10	255±8	255±10	N.D.
6[b]	250±12	247±13	235±9*	N.D.
8	278±10	269±11	261±16*	N.D.
10	292±15	285±9	270±22*	N.D.
13	311±15	296±8*	296±14*	284±12*
17	333±18	325±8	325±12	285±22*
20	351±19	344±8	343±14	251±30*
24	369±19	358±13	345±17*	Sac.
28	390±19	376±12	376±16	Sac.

[a]Mean ± Standard Deviation, grams

[b]Some rats were without food over the weekend

[c]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

*Significantly different from control mean using Dunnett's test, $p < 0.05$.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

TABLE 9

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Body Weights of Female Sprague-Dawley Rats

Experiment Days	Control	2500 ppm	6000 ppm	8000 ppm[c]
0	193±5[a]	205±4*	191±6	192±4
3	206±5	200±7	197±11	N.D.
6[b]	207±11	200±9*	192±8*	N.D.
8	210±9	209±12	205±12*	N.D.
10	225±9	222±9	215±15	N.D.
13	230±8	224±10	225±16	222±6
17	238±8	238±10	238±17	204±16*
20	244±8	246±9	242±18	202±23*
24	254±10	254±8	248±19	Sac.
28	263±8	264±11	259±16	Sac.

[a]Mean ± Standard Deviation, grams

[b]Some rats were without food over the weekend

[c]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study.

N.D. = no data. Animals were not weighed because they weren't exposed to methylene chloride during this time period.

Sac. = sacrificed.

*Significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 16

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Male CD-1 Mice

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	8000[a]
[illegible] (mg/100 ml)	10/10	31 ±4	N.D.	N.D.	29 ±19
BUN (mg/100 ml)	10/10	25 ±3	23 ±4	17* ±1	N.D.
SGPT (mU/ml)	10/10	72 ±3	N.D.	N.D.	76 ±9
SGPT (mU/ml)	10/10	76 ±7	73 ±6	76 ±8	N.D.
Body Weight (g)	10/10	37 ±4	36 ±2	38 ±2	N.D.
Liver (g)	10/10	1.99 ±0.19	1.92 ±0.23	2.14 ±0.19	N.D.
(g/100 g)	10/10	5.38 ±0.26	5.38 ±0.65	5.68 ±0.47	N.D.
Kidney (g)	10/10	0.62 ±0.07	0.56 ±0.05	0.56 ±0.03	N.D.
(g/100 g)	10/10	1.68 ±0.16	1.57 ±0.10	1.49* ±0.09	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 11

METHYLENE CHLORIDE: A FOUR WEEK-INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Female CD-1 Mice

Parameter	No. Controls/ No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	20000[a]
BUN (mg/100 ml)	7/10	30 ±2	N.D.	N.D.	25 ±6
SDH (mg/100 ml)	10/10	21 ±2	21 ±1	20 ±5	N.D.
SGPT (mU/ml)	7/10	66 ±12	N.D.	N.D.	85 ±13
SGPT (mU/ml)	10/10	61 ±3	67 ±2	71* ±4	N.D.
Body Weight (g)	10/10	30 ±2	31 ±2	33* ±2	N.D.
Liver (g)	10/10	1.44 ±0.15	1.56 ±0.10	1.81* ±0.16	N.D.
(g/100 g)	10/10	4.74 ±0.55	5.00 ±0.20	5.50* ±0.42	N.D.
Kidney (g)	10/10	0.39 ±0.03	0.38 ±0.04	0.41 ±0.03	N.D.
(g/100 g)	10/10	1.36 ±0.10	1.22 ±0.11	1.23 ±0.09	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other group inhaling methylene chloride.

N.D. = not determined.

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 12

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Male Syrian Hamsters

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	8000[a]
BUN (mg/100 ml)	4/10	25 ±5	N.D.	N.D.	22 ±9
SGPT (mU/ml)	4/10	122 ±38	N.D.	N.D.	82* ±8
SGPT (mU/ml)	10/10,9	97 ±15	96 ±9	90 ±16	N.D.
A.P. (mU/ml)	10/10	221 ±18	217 ±20	227 ±18	N.D.
Fasted Body Weight (g)	10/10,9	89 ±10	91 ±7	87 ±8	N.D.
Liver (g)	10/10,9	2.93 ±0.30	3.10 ±0.30	2.92 ±0.26	N.D.
(g/100 g)	10/10,9	3.28 ±0.20	3.39 ±0.19	3.38 ±0.05	N.D.
Kidney (g)	10/10,9	0.82 ±0.05	0.81 ±0.05	0.79 ±0.09	N.D.
(g/100 g)	10/10,9	0.92 ±0.05	0.89 ±0.04	0.92 ±0.04	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined.

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 13

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Female Syrian Hamsters

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	8000[a]
BUN (mg/100 ml)	5/10	23 ±2	N.D.	N.D.	22 ±2
SGPT (mU/ml)	5/10	95 ±18	N.D.	N.D.	104 ±21
SGPT (mU/ml)	10/10	98 ±12	119 ±62	91 ±29	N.D.
A.P. (mU/ml)	10/10	276 ±35	266 ±27	250 ±23	N.D.
Fasted Body Weight (g)	10/10	82 ±12	91 ±10	88 ±7	N.D.
Liver (g)	10/10	2.88 ±0.45	3.08 ±0.36	3.12 ±0.32	N.D.
(g/100 g)	10/10	3.29 ±0.21	3.32 ±0.19	3.53* ±0.13	N.D.
Kidney (g)	10/10	0.81 ±0.09	0.84 ±0.05	0.84 ±0.07	N.D.
(g/100 g)	10/10	0.93 ±0.03	0.93 ±0.08	0.95 ±0.04	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 14

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Male CDF Rats

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	8000[a]
BUN (mg/100 ml)	5/10	27 ±3	N.D.	N.D.	17* ±2
BUN (mg/100 ml)	10/10	25 ±2	24 ±3	22 ±2	N.D.
SGPT (mU/ml)	5/10	66 ±4	N.D.	N.D.	72 ±20
SGPT (mU/ml)	10/10	79 ±12	78 ±14	74 ±10	N.D.
Total Bilirubin (mg/100 ml)	5/10	0.2 ±0.0	N.D.	N.D.	0.1 ±0.0
A.P. (mU/ml)	10/10	284 ±24	283 ±15	278 ±23	N.D.
Fasted Body Weight (g)	10/10	208 ±11	197 ±10	193* ±12	N.D.
Liver (g)	10/10	6.21 ±0.44	5.59* ±0.32	5.79 ±0.51	N.D.
(g/100 g)	10/10	2.98 ±0.25	2.84 ±0.06	3.00 ±0.11	N.D.
Kidney (g)	10/10	1.62 ±0.12	1.48* ±0.09	1.47* ±0.12	N.D.
(g/100 g)	10/10	0.77 ±0.03	0.75 ±0.03	0.76 ±0.02	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 15

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Female CDF Rats

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	2500[a]
BUN (mg/100 ml)	5/10	29 ±1	N.D.	N.D.	19* ±5
BUN (mg/100 ml)	10/10	26 ±5	25 ±2	24 ±3	N.D.
SGPT (mU/ml)	5/10	74 ±6	N.D.	N.D.	84 ±30
SGPT (mU/ml)	10/10	82 ±18	65* ±12	81 ±16	N.D.
Total Bilirubin (mg/100 ml)	5/10	0.2 ±0.0	N.D.	N.D.	0.2 ±0.0
A.P. (mU/ml)	10/10	279 ±31	243* ±21	242* ±25	N.D.
Fasted Body Weight (g)	10/10	132 ±10	132 ±6	128 ±7	N.D.
Liver (g)	10/10	3.57 ±0.26	3.71 ±0.23	3.69 ±0.26	N.D.
(g/100 g)	10/10	2.71 ±0.15	2.82 ±0.11	2.88* ±0.15	N.D.
Kidney (g)	10/10	1.01 ±0.06	1.01 ±0.07	0.97 ±0.06	N.D.
(g/100 g)	10/10	0.77 ±0.03	0.77 ±0.04	0.76 ±0.02	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined

*Statistically significantly different from control mean using Dunnett's test, $p \leq 0.05$.

TABLE 16

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Male Sprague-Dawley Rats

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	5000	8000[a]
BUN (mg/100 ml)	3/10	25 ±2	N.D.	N.D.	34 ±11
BUN (mg/100 ml)	10/10	19 ±2	21 ±3	20 ±3	N.D.
SGPT (mU/ml)	3/10	63 ±1	N.D.	N.D.	56 ±15
SGPT (mU/ml)	10/10	53 ±4	57 ±9	66* ±11	N.D.
Total Bilirubin (mg/100 ml)	3/10	0.2 ±0.0	N.D.	N.D.	0.1 ±0.1
A.P. (mU/ml)	10/10	156 ±15	149 ±27	152 ±23	N.D.
Fasted Body Weight (g)	10/10	362 ±17	344 ±21	351 ±14	N.D.
Liver (g)	10/10	11.27 ±0.76	10.54 ±1.01	11.44 ±0.62	N.D.
(g/100 g)	10/10	3.11 ±0.15	3.06 ±0.16	3.26 ±0.13	N.D.
Kidney (g)	10/10	2.72 ±0.20	2.66 ±0.28	2.60 ±0.16	N.D.
(g/100 g)	10/10	0.75 ±0.05	0.77 ±0.06	0.74 ±0.03	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 17

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Clinical Chemistry, Fasted Body Weight, Organ and Organ Body Weight Ratios of Female Sprague-Dawley Rats

Parameter	No. Controls / No. Exposure Group	Exposure Levels (ppm) 0	2500	3900	6000[a]
ALT (mg/100 ml)	4/10	27 ±2	N.D.	N.D.	26 ±8
BUN (mg/100 ml)	10/10	23 ±5	25 ±3	21 ±3	N.D.
SGPT (mU/ml)	4/10	67 ±7	N.D.	N.D.	55* ±8
SGPT (mU/ml)	10/10	60 ±8	69 ±9	79* ±14	N.D.
Total Bilirubin (mg/100 ml)	4/10	0.2 ±0.0	N.D.	N.D.	0.1 ±0.0
A (mg/ml)	10/10	106 ±12	117 ±16	141 ±20	N.D.
Fasted Body Weight (g)	10/10	244 ±10	244 ±10	244 ±12	N.D.
Liver (g)	10/10	6.68 ±0.40	6.72 ±0.34	7.55* ±0.55	N.D.
(g/100 g)	10/10	2.74 ±0.16	2.76 ±0.15	3.09* ±0.19	N.D.
Kidney (g)	10/10	1.72 ±0.11	1.68 ±0.13	1.73 ±0.16	N.D.
(g/100 g)	10/10	0.71 ±0.04	0.69 ±0.06	0.71 ±0.04	N.D.

[a]These animals inhaled methylene chloride for 7 exposures on days 1, 14, 15, 16, 17, 20 and 21 of the study. These animals were necropsied prior to the other groups inhaling methylene chloride.

N.D. = not determined

*Statistically significantly different from control mean using Dunnett's test, $p < 0.05$.

TABLE 18

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Gross Observations on Mice and Hamsters

(PROBE STUDY)

Species	Mice				Hamsters			
Strain	CD-1				Syrian			
Sex	M	M	F	F	M	M	F	F
Dose Level (ppm)	0	0000	0	0000	0	0000	0	0000
No visible lesions	10/10	9/9	7/7	9/10	4/4	5/5	5/5	3/5
Equivocal focal areas of congestion of lungs	-	4/9	-	1/10	-	-	-	-
Slight to equivocal paleness of liver	-	-	-	5/10	-	-	-	-
Corneal cloudiness	-	-	-	-	-	-	-	2/5

Data listed as number of animals with observation/number animals examined
- Indicates observation not present in group.

TABLE 19

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Gross Observations on Sprague-Dawley and Fischer Rats

(PROBE STUDY)

Species	Rats				Rats			
Strain	Sprague-Dawley				CDF (Fisher 344)			
Sex	M	M	F	F	M	M	F	F
Dose Level(s) (ppm)	0	1000	0	8000	0	8000	0	8000
Number of Animals Examined	3	10	4	10	6	10	5	10
External								
Unkempt and hemorrhagic appearance of hair coat	-	8/10	-	1/10	-	-	-	-
Traumatic lesions to tail (cannibalism)	-	9/10	-	8/10	-	4/10	-	9/10
Traumatic lesions - facial region	-	3/10	-	3/10	-	-	-	-
Blood around facial region	-	2/10	-	-	-	-	-	-
Secretions lateral to external nares	-	2/10	-	-	-	-	-	-
Carcass decreased in overall size	-	4/10	-	2/10	-	1/10	-	-
Corneal cloudiness - (medial canthus)	1/3	-	-	-	1/6	-	1/5	1/10
Internal								
No visible lesions	2/3	2/10	2/4	-	2/6	2/10	1/5	1/10
Few circumscribed red foci in lungs, 1-2 mm in diameter	-	3/10	-	-	1/6	-	1/5	-
Several circumscribed red foci in lungs, 1-2 mm in diameter	-	-	-	1/10	-	-	1/5	-

Data listed as number rats affected/number rats examined.

- Indicates that the observation was not seen at this dose level.

TABLE 19 (cont.)

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Gross Observations on Sprague-Dawley and Fischer Rats

(PROBE STUDY)

Species	Rats Sprague-Dawley				Rats CDF (Fisher 344)			
Strain								
Sex	M	M	F	F	M	M	F	F
Dose Levels (ppm)	0	10000	0	10000	0	10000	0	10000
Number of Animals Examined	10	10	4	10	6	10	5	10
Observations:								
Internal (cont'd)								
Few circumscribed darkened foci in lungs, 1-3 mm in diameter	-	2/10	-	1/10	1/6	-	2/5	1/10
Several circumscribed darkened foci in lungs, 1-3 mm in diameter	-	-	1/4	3/10	1/6	-	1/5	1/10
Organized plug within lumen of urinary bladder	1/3	-	-	-	-	-	-	-
Body tissues appear pale (anemia secondary to hemorrhage from tail)	-	2/10	-	-	-	-	-	-
Paleness of liver (in anemic rat)	-	2/10	-	-	-	-	-	-
Decreased amounts of adipose tissue in abdominal cavity -								
Slight	-	-	-	3/10	-	4/10	-	1/10
Moderate	-	-	-	1/10	-	-	-	-
Pronounced	-	1/10	-	3/10	-	-	-	-
Lumen of stomach distended with gas and ingesta having a fluid appearance and containing hemolysed blood	-	2/10	-	2/10	-	-	-	-

Data listed as number rats affected/number rats examined.

- Indicates that the observation was not seen at this dose level.

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TABLE 19 (cont.)

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Gross Observations on Sprague-Dawley and Fischer Rats

(PROBE STUDY)

Species	Rats				Rats			
Strain	Sprague-Dawley				CDF (Fischer 344)			
Sex	M	M	F	F	M	M	F	F
Dose Levels (ppm)	0	8000	0	8000	0	8000	0	8000
Number of Animals Examined	5	10	4	10	6	10	5	10
Observations:								
Internal (cont'd)								
Lumen of stomach containing ingesta with an increased degree of fluidity and containing hemolysed blood	-	4/10	-	-	-	-	-	-
Lumen of stomach containing ingesta with an increased degree of fluidity	-	-	-	3/10	-	-	-	-
Focal ulceration and erosions of gastric mucosa	-	9/10	-	3/10	-	1/10	-	-
Contents of distal gastrointestinal tract darkened with hemolysed blood	-	2/10	-	-	-	-	-	-
One testis missing - (congenital anomaly)	-	1/10	-	-	-	-	-	-
Uterus distended with fluid due to estrus cycle	-	-	2/4	-	-	-	-	-

Data listed as number rats affected/number rats examined.

- Indicates that the observation was not seen at this dose level.

TABLE 20

METHYLENE CHLORIDE: A FOUR WEEK INHALATION TOXICITY STUDY IN RATS, HAMSTERS AND MICE

Gross Observations on Rats, Hamsters and Mice

(FIRST SHEET)

Species	Strain	Sex	No. in Group	Vapor Conc. (ppm)	No Visible Lesions Noted	Gross Observations: Lungs – Circumscribed Buff Foci 1-3 mm diam. Few	Several	Multiple	Organized Plug within Lumen of Urinary Bladder	Slight Corneal Opacity	Liver Diffusely Pale	Loss of Hair on Back	Right Kidney Enlarged	Trauma to Base of Tail
Hamster	Syrian	M	10	0	10/10	–	–	–	–	–	–	–	–	–
			10	2500	10/10	–	–	–	–	–	–	–	–	–
			9[a]	5000	8/9[a]	1/9[a]	–	–	–	–	–	–	–	–
Hamster	Syrian	F	10	0	9/10	1/10	–	–	–	–	–	–	–	–
			10	2500	10/10	–	–	–	–	–	–	–	–	–
			10	5000	10/10	–	–	–	–	–	–	–	–	–
Mice	CD-1	M	10	0	10/10	–	–	–	–	–	–	–	–	–
			10	2500	8/10	–	–	–	–	–	–	–	–	–
			10	5000	10/10	–	–	–	–	–	–	–	–	–
Mice	CD-1	F	10	0	10/10	–	–	–	–	–	–	–	–	–
			10	2500	10/10	–	–	–	–	–	–	–	–	2/10
			10	5000	10/10	–	–	–	–	–	–	–	–	–
Rat	Fisher	M	10	0	1/10	7/10	1/10	1/10	2/10	–	–	–	–	–
			10	2500	4/10	6/10	–	–	–	1/10	–	–	–	–
			10	5000	4/10	5/10	1/10	–	–	1/10	–	–	–	–
Rat	Fisher	F	10	0	5/10	3/10	–	–	–	–	2/10	–	–	–
			10	2500	1/10	6/10	2/10	–	–	–	2/10	–	–	–
			10	5000	6/10	4/10	–	–	–	–	–	–	–	–
Rat	Sprague-Dawley	M	10	0	10/10	–	–	–	–	–	–	–	–	–
			10	2500	1/10	2/10	–	–	4/10	5/10	–	–	1/10	–
			10	5000	3/10	2/10	1/10	–	3/10	1/10	–	2/10	–	–
Rat	Sprague-Dawley	F	10	0	2/10	5/10	3/10	–	–	–	–	–	–	–
			10	2500	4/10	4/10	2/10	–	–	–	–	–	–	–
			10	5000	4/10	1/10	5/10	–	–	–	–	–	–	–

Data listed as number of animals with observations/number animals examined.

[a] Escaped from cage prior to necropsy

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EFFECT OF METHYLENE CHLORIDE ON THE OXYHEMOGLOBIN DISSOCIATION CURVE OF RAT AND HUMAN BLOOD

LK 1/31/83

Chemical Name (300 per name) 25	CAS No. (10) 26
METHYLENE CHLORIDE	75-09-2

1A

8752113094

D-416

EFFECT OF METHYLENE CHLORIDE ON THE OXYHEMOGLOBIN DISSOCIATION CURVE OF RAT AND HUMAN BLOOD

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July 15, 1977

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Methylene chloride is biotransformed *in vivo* to carbon monoxide (CO) and increased carboxyhemoglobin (COHb) levels occur in animals and man following exposure. Binding of CO to hemoglobin causes a shift to the left in the position of the oxyhemoglobin dissociation curve (ODC) theoretically resulting in a diminished ability to release oxygen in tissue. This study was designed to determine the effect of methylene chloride alone and in combination with CO on altering the ODC. Methylene chloride and CO, alone and in combination were incubated with rat and human blood *in vitro* and the position of the ODC was measured by the P_{50} value. The results indicate clearly that methylene chloride alone has no effect on changing the P_{50} value of the ODC. It is concluded that any shift to the left of the ODC in individuals exposed to methylene chloride is due solely to CO formed from the biotransformation of methylene chloride. Thus, speculation that methylene chloride increases the affinity of Hb for CO is not supported.

INTRODUCTION

Methylene chloride (dichloromethane, CH_2Cl_2) is widely used as a solvent, paint stripper, degreasing fluid and low pressure aerosol propellant. Stewart, et al., (1972) reported that blood carboxyhemoglobin (COHb) levels were increased in humans inhaling methylene chloride. Subsequent studies in experimental animals showed that methylene chloride is biotransformed in the body to carbon monoxide (CO) resulting in increased COHb (Kubic, et al., 1974; Roth, et al., 1975; DiVincenzo and Hamilton, 1975; Carlsson and Hultengren, 1975).

The formation of COHb causes a shift to the left in the portion of the oxyhemoglobin dissociation curve (ODC) resulting theoretically in a diminished ability to release oxygen at the tissue level (Brewer, 1974). From studies conducted on the use of methylene chloride as a potential antisickling agent in sickle cell anemia, it was speculated that methylene chloride may interact with Hb and increase the affinity of Hb for CO (Bettis, 1975). This prompted the current investigation designed to determine if methylene chloride causes a shift to the left in the oxyhemoglobin dissociation curve. The ODC is characterized by the partial pressure of oxygen which causes 50% saturation of Hb, P_{50}.

Thus, the P_{50} was determined in rat and human RBC following incubation with methylene chloride alone or in combination with CO.

METHOD

Material. Methylene chloride (Technical grade, [illegible] Company) and carbon monoxide (Matheson Gas Products) used throughout the study were respectively 99% and 99.9% chemically pure.

Animals and blood sampling. Male Sprague-Dawley rats (Spartan Research Laboratory) weighing 200-250 g were used. All animals were housed in rooms in which constant humidity, temperature, and a 12-hour light-dark cycle (7 AM - 7 PM) were maintained. Food (Purina Rat Chow) and water were provided ad libitum except during inhalation exposure for those animals exposed to methylene chloride. Blood was obtained from unanesthetized rats by heart puncture.

Human blood was obtained from 5 healthy nonsmokers, 3 female and 2 male. Blood was drawn by venipuncture. The anticoagulant used for both human and rat blood was sodium-EDTA. Blood was obtained fresh and stored on ice until used which was always within 2 hours.

Analytical Determinations. The P_{50} was determined with the use of an IL-237 blood gas Tonometer, IL-182 CO-oximeter (Instrumentation Laboratories) and a BMS3, Mark 2 blood gas analyzer (Radiometer, Copenhagen). The CO-oximeter was

precalibrated with either rat or human blood. Two points on the ODC were measured, one on either side of the P_{50} and from this the P_{50} value was calculated. Methemoglobin levels were determined by the method of Evelyn and Malloy (1938) and carboxyhemoglobin levels were determined spectrophotometrically with the IL-182 CO-oximeter. Methylene chloride concentration in whole blood was determined with a gas chromatograph mass spectrometer (Finnigan, Model 3000 D).

In Vitro Study. Blood (rat or human) was equilibrated with the test atmosphere in a blood gas tonometer (37°C). The test atmosphere containing room air (control), methylene chloride alone (600 ppm), CO alone (2500 ppm) or methylene chloride (600 ppm) plus CO (2500 ppm) contained in a 100 l Baran bag (Anapac) was pumped into the tonometer curvette at a flow rate of 300 ml/minute with a peristaltic pump (Masterflex, Cole-Parmer). Incubation times of approximately 20 minutes were necessary to achieve the desired COHb (10-20%) and methylene chloride (25-35 µg/ml) blood levels. Following incubation the P_{50} and COHb values were determined as described above.

In Vivo Study. A group of 5 rats were exposed to an atmosphere of 500 ppm methylene chloride for 3 hours. The rats were exposed in a 14.5 cubic meter chamber under dynamic air

flow conditions. The atmosphere was generated by controlled vaporization of methylene chloride into the chamber airflow (150 l/minute). The concentration of methylene chloride in the chamber was analyzed at hour intervals by recirculating a portion of the chamber atmosphere through a gas cell of an infrared spectrophotometer (Wilks) calibrated at 7.35 μm. The mean analytical concentration determined at hour intervals for 7 hours was 48010 (80) ppm methylene chloride. Blood for determination of P_{50} and COHb was drawn by cardiac puncture at 3 hours of exposure. These blood samples were incubated in the tonometer with a gas mixture containing 800 ppm methylene chloride. This was necessary to maintain the level of methylene chloride achieved during the *in vivo* exposure (approximately 30 μg/ml).

Statistical Analysis. Differences between treatments were analyzed by Dunnett's t-test at a significance level of $p < 0.05$.

RESULTS

The effect of methylene chloride and CO on the P_{50} and COHb levels in the rat are shown in Table 1.

Exposure to methylene chloride *in vitro* or *in vivo* did not cause a significant change in the P_{50} value when compared to controls. Exposure to CO or CO plus methylene chloride caused a 9-12% COHb level and reduced the P_{50} value about 5 mmHg when compared to control. However, P_{50} values or COHb levels were not different between CO and CO plus methylene chloride treatments. *In vivo* exposure to 500 ppm methylene chloride for 3 hours caused a 6% COHb level but it did not cause a significant change in the P_{50} value when compared to controls.

The effect of methylene chloride and CO on the P_{50} and COHb levels in human blood is shown in Table 2. Similar to the results obtained in the rat, the data from human blood showed that exposure to methylene chloride alone did not significantly change the P_{50} value as compared to controls. The combined exposure to methylene chloride plus CO showed an equivalent degree of COHb compared to the CO treatment alone. However, the P_{50} value was increased by 4 mmHg when compared to the treatment with CO alone. No change in methemoglobin concentrations were observed under any test conditions.

The results of these studies clearly indicate that methylene chloride alone has no effect on the oxyhemoglobin dissociation curve (ODC) as indicated by the P_{50}. The decreased P_{50} value following methylene chloride exposure appears to be caused solely by the biotransformation of methylene chloride to CO and subsequent formation of COHb. Collison, et al., (1977) has recently reported that neither the relative affinity of hemoglobin for CO and oxygen nor the absolute affinity for CO is altered by the presence of methylene chloride. Both our observation and that of Collison, et al., (1977) are complimentary and both fail to support the speculation by Kettle (1975) that methylene chloride increases the affinity of hemoglobin for CO. Furthermore, the present study showed that methylene chloride in combination with CO in human blood caused the ODC to be shifted to the right (increased P_{50}) compared to CO exposure alone. This suggests that methylene chloride may partially ameliorate the effect of the CO induced left shift of the ODC.

The fate of methylene chloride in animals and man indicate that it is rapidly metabolized to CO and CO_2 or eliminated by the lungs unchanged. Kubic, et al., (1974, 1975) have shown that the biotransformation of methylene chloride to CO is mediated by cytochrome P-450 dependent microsomal enzyme in the liver. Furthermore, evidence for the saturation of the

metabolism of methylene chloride to CO has been observed in animals (McKenna et al., 1977) and man (Stewart, et al., 1978; Fodor, et al., 1976). Thus it appears that at atmospheric concentrations exceeding 500 ppm the metabolism of methylene chloride and subsequent formation of COHb has attained its maximum velocity. This implies that at exposures exceeding 500 ppm the amount of COHb produced will be a function of the duration of exposure rather than the exposure concentration.

In conclusion, methylene chloride alone has no effect on changing the P_{50} value of the oxyhemoglobin dissociation curve. Any shift to the left of the ODC in individuals exposed to methylene chloride is due solely to CO formed from the biotransformation of methylene chloride. The readily reversible nature of the conversion of carboxy-hemoglobin to oxyhemoglobin suggests that no cumulative toxicity from the formation of COHb would be expected.

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000013

TABLE 1

EFFECT OF METHYLENE CHLORIDE, CARBON MONOXIDE AND METHYLENE CHLORIDE PLUS CARBON MONOXIDE ON THE P_{50} VALUE OBTAINED FROM THE OXYHEMOGLOBIN DISSOCIATION CURVE OF RAT BLOOD[a]

	in vitro Air Controls (9)[b]	in vitro CH_2Cl_2 800 ppm (5)	in vitro CO 2500 ppm (4)	in vitro $CO + CH_2Cl_2$ [2500 ppm + 800 ppm] (5)	in vivo CH_2Cl_2 500 ppm 3 hrs (5)
P_{50} ± SE (mmHg)	35.3±0.7	36.5±1.9[c]	29.6±1.2[c]	30.7±0.4[c,d]	34.5±1.3[e]
% COHb ± SE	0.1±0.1	0.0	10.3±0.6	11.4±0.9	6.4±0.7

[a] Oxyhemoglobic dissociation curbe (ODC) characterized by the P_{50}.

[b] Number of animals.

[c] Significantly different from air control by Dunnett's t, $p < 0.05$.

[d] Not significantly different from CO-treatment by Dunnett's t, $p < 0.05$.

[e] Not significantly different from air control by Dunnett's t, $p < 0.05$.

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[illegible] RESEARCH | DOW CHEMICAL [illegible]

Texas Division Toxicology

[illegible] | [illegible]

[illegible] | December 30, [illegible]

DOT TEST FOR CORROSIVENESS TO SKIN ON PROPYLENE OXIDE

M. N. Pinkerton, R. [illegible] Schwebel — REPORTED BY

R. V. Johnston, D.V.M. — CHECKED BY

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ADDITIONAL INFORMATION PROCEDURES THE EFFECTS OF REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND FORMULATIONS ARE DEVELOPED [illegible]

A sample of the test material was submitted to the Comparative Toxicology Research Laboratory for evaluation of corrosiveness to skin in accordance with the Department of Transportation Hazardous Materials Regulation of the Code of Federal Regulations, Title 49, Section 173.240.

A member of the Industrial Health Team (Toxicologist, Industrial Hygienist, and/or Industrial Physician) should be consulted for recommendation of additional safety evaluations or handling procedures which may be needed to support manufacture and/or use of this test material.

A corrosive material is one that causes irreversible change or destruction to the intact skin of an albino rabbit after an exposure period of four hours. The test procedure used is described in Paragraph 1500.41 of CFR, Part 1500.

Propylene oxide is not corrosive by this test.

The TLV for propylene oxide is 100 ppm (240 mg/m^3).

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TABLE 2

EFFECT OF METHYLENE CHLORIDE, CARBON MONOXIDE AND METHYLENE CHLORIDE PLUS CARBON MONOXIDE ON THE P_{50} VALUE OBTAINED FROM THE OXYHEMOGLOBIN DISSOCIATION CURVE OF HUMAN BLOOD (IN VITRO)[a]

	Air Controls(5)[b]	CH_2Cl_2 800 ppm(4)	CO 2500 ppm 23 min.(4)	CO + CH_2Cl_2 [2500 ppm + [illegible] ppm(4)]
P_{50} ± SE (mmHg)	26.1±0.8	27.9±0.6[c]	18.5±0.9[d]	22.6±1.2[d,e]
%COHb ± SE	1.1±0.1	0.85±0.15	20.3±0.8	19.5±0.9

[a] Oxyhemoglobin dissociation curve (ODC) characterized by the P_{50}.

[b] Number of animals.

[c] Not significantly different from air control by Dunnett's t, $p < 0.05$.

[d] Significantly different from air control by Dunnett's t, $p < 0.05$.

[e] Significantly different from CO treatment by Dunnett's t, $p < 0.05$.

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Doc Title: CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-09-2

6K
5.314

1A

878211311-c

D-420

R & D REPORT

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OCCUPATIONAL HEALTH AND MEDICAL RESEARCH

CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

PAGES OF FULL REPORT

R. V. Johnston, J. W. Rampy,* B. J. Dabney, T. Barnes-Lloyd

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.)

Four groups of 10 Sprague-Dawley albino rats (Spartan sub-strain, SPF-derived) were exposed to 3500, 1500, 500, and 0 (control) concentrations of methylene chloride in air for 6 hrs/day, 5 days/week for 6 months. Bone marrow cells from five rats of each sex killed at the end of each of four 6-month exposure periods were collected and cultured for cytogenetic evaluation. Blind analysis of 200 cells per animal was performed by four accredited medical technicians of Occupational Health and Medical Research, Freeport, Texas. The cells were evaluated for chromatid and chromosome breaks; dicentrics, rings, and exchanges; miscellaneous aberrations; severe damage; and the number and percentage of abnormal cells, an abnormal cell being defined as a cell with at least one aberration or abnormality. It was found that values for the various aberration categories at the 3500, 1500, and 500 ppm dose levels were not significantly different from the control group, and it was concluded that longterm, intermittent, relatively-high concentrations of methylene chloride in air are unlikely to induce increased cytogenetic aberration rates in bone marrow cells of rats of this sub-strain.

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CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

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CYTOGENETIC EVALUATION OF BONE MARROW CELLS FROM RATS EXPOSED TO METHYLENE CHLORIDE FOR SIX MONTHS

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INTRODUCTION

We report here a study of cytogenetic aberrations in rats exposed to methylene chloride (CH_2Cl_2). This investigation was undertaken as part of a 24-month inhalation study of chronic toxicity of methylene chloride in rats and hamsters that is being conducted by the Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, Michigan, and which is scheduled for completion in April, 1978.

Cytogenetic evaluation is a recognized and useful tool for the estimation of genetic risk (Kucerova, 1976). The value of *in vivo* cytogenetic evaluation lies in the fact that effects of various mutagenic agents upon the genetic material--the chromosomes--of the living animal can be directly observed. Since specifically mammalian metabolic processes are taken into account, cytogenetic studies in mammals exposed to high levels of a suspect agent can be particularly useful in arriving at judgments about the dimensions of potential risk to members of the human species exposed to lower concentrations of the substance.

MATERIALS AND METHODS

Four groups of ten Sprague-Dawley albino rats (Spartan substrain, SPF-derived) were exposed in inhalation chambers of the

*Toxicology Research Laboratory, Health and Environmental Research, Midland, Michigan

Methylene Chloride Cytogenetics
Materials and Methods

Midland Toxicology Research Laboratory to 1500, 1250, 500, and 0 (control) concentrations of methylene chloride in air for 6 hrs/day, 5 days/week for six months. The animals were group-caged and kept in the chamber with a supply of clean air during the non-exposure intervals. Food was removed during the exposure periods, but was otherwise freely available. Water, via an automatic watering system, remained available at all times. The animals were evaluated daily for signs of toxicity and changes in appearance and behavior. The results of these observations and other findings will be reported separately.

The five rats of each sex to be killed at the end of each of the four six-month exposure periods and used for cytogenetic evaluations were identified before the exposures began.

Following sacrifice, bone marrow cells were collected and cultured, and coded slides were prepared as described in Table 1. Blind analysis of the slides was performed in the Comparative Toxicology Research Laboratory of Occupational Health and Medical Research, Freeport, Texas, by four accredited medical technicians. The cells were evaluated and scored by the criteria of the World Health Organization (Buckton and Evans, 1973). Two hundred cells per animal were scored. The cells were evaluated for chromatid and chromosome breaks, discentrics, rings, and exchanges; miscellaneous aberrations; severe damage, and the number and percentage of abnormal cells. An abnormal cell is defined as a cell with at least one aberration or abnormality. Findings for each cell, along with

the vernier coordinates, were recorded on a score sheet similar to Figure 3 in Kilian et al. (1977). A photograph was made of every abnormal cell scored.

RESULTS

Results of the cytogenetic evaluation are shown in the Tables 2 and 3. Very few aberrations were found in any of the control or test animals. In the total of 8000 cells examined, only 37 (0.46%) abnormal chromosomes were found. Most of the aberrations were chromatid breaks (27) and chromosome breaks (7). One exchange figure was observed at the 1500 ppm dose, and 2 rings were noted at the 3500 ppm dose. Analysis of variance showed that none of the aberration rates in any of the treated groups was significantly different at P≤0.05 from the control group. Also, when all the so-called "stable" aberrations (dicentrics, rings and exchanges) are pooled, the differences are not significant.

SUMMARY

Four groups of 10 Sprague-Dawley albino rats (Spartan sub-strain, SPF-derived) were exposed to 7500, 1500, 500 and 0 (control) ppm of methylene chloride in air for 6 hrs/day, 5 days/week for 6 months. Bone marrow cells from five rats of each sex killed at the end of each of four 6-month exposure periods were collected and cultured for cytogenetic evaluation. Blind analysis of 200 cells per animal was performed by four accredited medical technicians of Occupational Health and Medical Research, Freeport, Texas. The cells were evaluated for chromatid and chromosome breaks; dicentrics, rings, and exchanges; miscellaneous

aberrations; severe damage; and the number and percentage of abnormal cells, an abnormal cell being defined as a cell with at least one aberration or abnormality. It was found that values for the various aberration categories at the 3500, 1500, and 500 ppm dose levels were not significantly different from the control group, and it was concluded that long-term, intermittent, relatively-high concentrations of methylene chloride in air are unlikely to induce increased cytogenetic aberration rates in bone marrow cells of rats of this sub-strain.

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TABLE I

PROCEDURES FOR CELL COLLECTION AND SLIDE PREPARATION FOR CHROMOSOME ANALYSIS

A. Cell Collection and Preparation

1. The rats were injected intraperitoneally with colchicine (0.4 mg/kg) four hours before being decapitated. The head of the femur was removed and the bone marrow was aspirated using a 10 cc syringe and a 20 gauge needle.

2. The bone marrow cells were mixed in 10 ml Hank's balanced salt solution using a disposable pipette.

3. The cells were centrifuged at 2000 RPM for 10 minutes.

4. The Hank's balanced salt solution was removed and 10 ml of 0.07 KCl was added to the tubes. They were then incubated for 20 minutes in a 37° water bath. (Critical)

5. The cells were then centrifuged for 10 minutes at 2000 RPM.

6. The supernatant was removed and a 3:1 methanol/acetic acid fixative was used to resuspend the cells.

7. The cells were then stored at 4°C for later preparation of slides.

B. Slide Preparation

1. Centrifuge as in A-5 above, decant the fixative and add fresh 3:1 fixative.

2. Remove the slides and place approximately 5 drops of a cell suspension on a slide; pass the slide through a flame. The slides were then dried on a warming plate at 60°.

3. The slides were rinsed gently in distilled H$_2$O and were placed in Giemsa Stain for 10 minutes. Dehydration was through 2 acetone washes, 2 acetone-xylol changes. The slides were coverslipped from xylol using Harleco Synthetic Resin. (HRH) as a mounting medium and were dried in an oven overnight.

TABLE 2. CYTOGENETIC ABERRATIONS OBSERVED IN BONE MARROW CELLS FROM RATS EXPOSED TO 3500, 1500, 500 AND 0 PARTS PER MILLION CONCENTRATIONS IN AIR OF METHYLENE CHLORIDE FOR 6 HR/DAY, 5 DAY/WEEK FOR 6 MONTHS

Animal Number	Dose PPM	Chromatid Breaks w/ Frag=	Chromatid Breaks w/o Frag=	Chromosome Break w/ Frag=	Chromosome Break w/o Frag=	Diacentrics	Rings	Tetraploid	No. of Abnormal Cells
3903	0	1	0	0	0	0	0	0	1
3903	0	0	0	0	0	0	0	0	0
3904	0	0	0	0	0	0	0	0	0
3905	0	3	0	1	0	0	0	0	4
3906	0	2	0	1	0	0	0	0	3
4076	0	1	0	1	0	0	0	0	2
4077	0	0	0	0	0	0	0	0	0
4078	0	1	0	0	0	0	0	0	1
4079	0	0	0	0	0	0	0	0	0
4080	0	1	0	0	0	0	0	0	1
	X =	0.9	0	0.3	0	0	0	0	1.1
	SD =	0.99	0	0.42	0	0	0	0	1.20
3947	500	0	0	0	0	0	0	0	0
3948	500	0	0	1	0	0	0	0	1
3949	500	1	0	1	0	0	0	0	2
3950	500	0	0	0	0	0	0	0	0
3951	500	1	0	0	0	0	0	0	1
4721	500	1	0	0	0	0	0	0	1
4722	500	0	0	0	0	0	0	0	0
4723	500	0	0	0	0	0	0	0	0
4724	500	0	0	0	0	0	0	0	0
4725	500	1	0	0	0	0	0	0	1
	X =	0.5	0.0	0.2	0	0	0	0	0.6
	SD =	0.707	0.0	0.42	0	0	0	0	0.699
3818	1500	0	0	0	0	0	0	0	0
3819	1500	0	0	0	0	0	0	0	0
3820	1500	0	0	0	0	0	0	0	0
3821	1500	0	1	0	0	0	0	0	1
3822	1500	0	0	0	0	0	0	0	0
4592	1500	1	0	0	0	0	0	0	1
4593	1500	0	0	0	0	0	0	0	0
4594	1500	0	0	0	0	0	0	0	0
4595	1500	1	0	3	0	0	0	0	4
4596	1500	3	0	1	0	0	0	0	4
	X =	0.5	0.1	0.1	0	0	0	0.1	0.8
	SD =	0.97	0.316	0.316	0	0	0	0.316	1.229
3689	3500	1	0	0	0	0	1	0	2
3690	3500	1	0	0	0	0	1	0	2
3691	3500	1	0	0	0	0	1	0	2
3692	3500	0	0	0	0	0	0	0	0
3693	3500	1	0	1	0	0	0	0	2
4463	3500	1	0	1	0	0	0	0	2
4464	3500	0	0	0	0	0	0	0	0
4465	3500	1	0	0	0	0	0	0	1
4466	3500	0	0	0	0	0	0	0	0
4467	3500	1	0	0	0	0	0	0	1
	X =	0.7	0	0.2	0	0	0.2	0	1.1
	SD =	0.48	0	0.421	0	0	0.421	0	0.07
	F =	0.91	1.0	0.157			2.25	1.0	0.548
	P =	0.65	0.405	0.921			0.098	0.40	0.660

TABLE 3. SUMMARY OF CYTOGENETIC ABERRATIONS DETECTED IN BONE MARROW CELLS FROM RATS EXPOSED TO 3500, 1500, 500, and 0 PARTS PER MILLION CONCENTRATIONS IN AIR OF METHYLENE CHLORIDE FOR 6 HR/DAY, 5 DAY/WEEK FOR SIX MONTHS

				TYPE OF ABERRATION					
	No. of Rats	No. Cells Scored	Dose	Chromatid Breaks	Chromosome Breaks	Dicentrics, Rings, Exchanges, Misc.	Severely Damaged	No. Abnormal Cells	Percent Abnormal Cells
	10	2000	0	9	2	0	0	11	0.55
	10	2000	500	5	2	0	0	6	0.30
	10	2000	1500	6	1	1[a]	0	8	0.40
	10	2000	3500	7	2	2[b]	0	11	0.55
TOTAL	40	8000		27	7	3	0	36	avg. 0.45

[a]Exchange

[b]Rings

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METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

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METHYLENE CHLORIDE	75-Ø9-2

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METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

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ABSTRACT

Male and female Sprague-Dawley rats were exposed to 0, 1600, 3500, or 8000 ppm methylene chloride (CH_2Cl_2) for 6 hr/day, and were killed for pathological examination immediately after 1 or 3 exposures. The acute toxicity of inhaled CH_2Cl_2 was assessed based on body weights, liver weights, clinical observations, gross pathology, and histopathology.

Exposure-related effects were observed in rats exposed to 8000 ppm. These effects included body weight loss relative to control (in rats exposed for 3 days) and a 10% decrease in relative liver weights (in rats exposed for 1 day only). Edema and acute inflammation of the skin and subcutaneous tissues around the external nares in the region of the vibrissae (sensory hairs) were observed, and the edema appeared to be more pronounced after just one exposure. Staggering and changes in activity occurred during exposure, but were not detected 1/2 hour post-exposure. Lymphoid necrosis of the thymus and some lymph nodes was evident in some rats exposed for 1 day and in some exposed for 3 days. A generalized depleted appearance of the parotid salivary gland was present, which consisted of a decreased acinar size. This was probably a nonspecific reversible alteration. Acute inflammation or hemorrhage in the cervical lymph nodes was observed. This was thought to be secondary to the inflammatory reaction around the external nares. No exposure-related alterations were apparent in any rats exposed to 1600 or 3500 ppm for either 1 or 3 days.

INTRODUCTION

Methylene chloride has applications as a paint remover, propellant, and an industrial solvent. It has the following reported properties:

Molecular formula:	CH_2Cl_2
Synonyms:	dichloromethane, $MeCl_2$
Boiling Point:	40°C at 760 mmHg
Molecular Weight:	84.94 daltons
Vapor Pressure:	410 mmHg at 25°C
Explosive Limits:	12.8 to 34.6% (in air)

Because methylene chloride ($MeCl_2$) is very volatile, the principle route of human exposure is by inhalation. The current ACGIH-TLV and the Dow Chemical Company IHG is 100 ppm.

Exposure to sufficient concentrations of $MeCl_2$ can cause a number of effects. A 6 hour exposure to 17,000 ppm is reportedly lethal to rats, and 15,000 ppm produces deep narcosis within 21 minutes (Schumacher, 1923). Ten rats remained "alive and well" for one month after a single 7 hour 10,000 ppm exposure (Heppel, et al., 1944).

Burek, et al. (1981) conducted a methylene chloride chronic toxicity and oncogenicity study in Sprague-Dawley rats and in hamsters. Animals were exposed to 0, 500, 1500 or 3500 ppm methylene chloride for 6 hr/day, 5 days/wk for 2 years. The liver appeared to be the primary target organ in the rat with slight exposure-related effects in both male and female rats exposed to 500, 1500 or 3500 ppm. Histopathologically, the liver effects in rats appeared to progress slightly between 12 and 18 months of exposure but did not progress further between 18 and 24 months. While the number of female rats with a benign tumor was not increased, the total number of benign mammary tumors was increased in female rats exposed to 500, 1500 or 3500 ppm of methylene chloride. The total number of benign mammary tumors increased in an exposure-related manner with 165 in the controls and 218, 245 and 287 in females exposed to 500, 1500 or 3500 ppm respectively. This effect was evident in male rats, but to a lesser extent than in females and with apparent effects only in the 1500 and 3500 ppm exposure groups. Male rats exposed to 1500 or 3500 ppm appeared to have an increased number of sarcomas (malignant mesenchymal tumors) in the ventral neck region that were in or around the salivary glands. This increase was statistically significant in the 3500 ppm

group, with a trend (numerical increase, but not statistically significant) for an increase in the 1500 ppm group. No treatment-related histopathological effects were observed in hamsters in any of the 3 exposure groups.

Acute toxicity studies were undertaken to elucidate possible mechanisms and to evaluate the toxicological significance of the effects observed in the 2-year study. Although 6 months of repeated $NaCl_2$ exposure at concentrations up to 3500 ppm caused no histopathologically detectable changes in any tissues except the presence of hemosiderin in the liver of some male rats (Burek et al., 1981), it was not known whether initial exposures caused tissue reaction or tissue injury. Subsequent accommodation could mask this possible effect.

The increases in salivary gland tumors were apparently sex specific. Therefore, it is possible that the effects of $NaCl_2$ may be secondary to an effect on sex hormones. Gluckman and Cherry (1971) have reported that direct injection of 1/2 or 1% DMBA (dimethylbenzathracene) into the salivary gland caused twice as many neoplasms of the salivary gland in male rats than in females. Administration of testosterone to females is reported to double the incidence of these neoplasms, and administration of estrogens to males halves the incidence at these dose levels. This evidence indicates that sex hormones can affect DMBA-induced salivary gland neoplasms.

Hormones are known to play a role in development of mammary neoplasia in laboratory animals and in humans, although the nature of this involvement is not certain (Nandi, 1978). Studies have not established a relation between $NaCl_2$ exposure and some sex hormones (Burek, et al., 1981), but histological evaluation of endocrine hormone sensitive tissues immediately after acute exposure to high $NaCl_2$ concentrations may provide more information. Ethanol has been shown to have several effects on sex hormones. Chronic ethanol administration can cause hypogonadism and feminization in males, possibly by three mechanisms: at the hypothalamic/pituitary and gonadal levels, and alteration of steroid hormone metabolism by the liver (Gordon et al., 1979). Although there is currently no direct evidence that $NaCl_2$ alters hormone concentrations, this possible relation was thought to deserve further investigation.

An acute toxicity study was conducted to investigate possible initial effects of $NaCl_2$ exposure. The route of exposure (inhalation) and strain of rats (Sprague-Dawley) were chosen because they were

used in the chronic toxicity study of Burek, et al. (1981). Sexually mature rats were used in the acute inhalation toxicity study because sex-dependent effects were observed in the chronic toxicity study. This study was designed to assess possible immediate but transient effects of $MeCl_2$ exposure by histopathological evaluation of target organs and hormone sensitive tissues from rats killed within approximately 2 hours of (1) a single 6 hour exposure, and (2) three 6 hour exposures on consecutive days. The two necropsies were chosen to maximize the likelihood of detecting an effect.

METHODS

Male and female rats were exposed to 0, 1500, 3500, or 8000 ppm $MeCl_2$ for 6 hours ("1 day" exposure group) or 6 hr/day for 3 consecutive days. Animals underwent necropsy as soon as possible after their last scheduled exposure; this was from ~1/2 hr post-exposure to ~2-1/2 hr post-exposure.

Test Material.

Methylene chloride (lot #T-100) was obtained from Dow Chemical U.S.A., Plaquemine, LA. Technical grade $MeCl_2$ was used. The stabilizer and principle impurities identified (those above 10 ppm) in the test material were cyclohexane (a stabilizer, 383 ppm), trans-1,2-dichloroethylene (100 ppm), vinylidene chloride (42 ppm), and water (22 ppm).

Animals.

Male and female Spartan Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) were received at age ~11 weeks and were exposed starting at age ~13 weeks. Five rats/sex/exposure concentration/necropsy group (i.e. 1 or 3 days of exposure) were assigned to their exposure groups by a computer-generated randomization program, and were individually identified with metal ear tags. Animals were housed in cages with wire mesh floors (1 or 2 rats/cage), and were provided Purina Certified Rodent Chow (Ralston Purina Co., St. Louis, MO) and water ad libitum except during exposure. Animals were housed in stainless steel wire mesh exposure cages (5 rats/cage) during exposures.

Vapor Generation and Analysis

Methylene chloride vapors were analyzed with a Varian 3400 GC with flame ionization detector. Helium was used as the carrier gas, and a 1/8" x 6' stainless steel column packed with Poropak QS9 was used. Standard bags were made by adding $MeCl_2$ liquid to a U-tube; then 100

l of dry, filtered air was metered through the tube into a SARAN® bag. The test material was heated to achieve complete vaporization. A series of standard bags were analyzed to produce a standard curve (peak height response vs. $MeCl_2$ concentration). Chamber $MeCl_2$ concentrations were determined by interpolating on this curve. The analytical equipment was checked before each exposure by analyzing a standard bag.

Methylene chloride vapors were generated with a J-tube apparatus (Miller, et al., 1980) and 157 ℓ stainless steel and glass exposure chambers were run with an airflow of 25 lpm.

Chambers were sampled by drawing test atmosphere through Teflon® tubing with an FMI pump (Fluid Metering, Inc., Oyster Bay, NY) to an 8-port valve with 1 ml sample loops. A stream selector and automatic valve actuator were used to select the chambers for analyses. A microprocessor recorded the "peak height" response from the GC and calculated the chamber $MeCl_2$ concentration.

CRITERIA OF RESPONSE

Animals were observed before exposure and at the end of exposure. Body weights were determined before exposure and at necropsy.

At necropsy, each rat was anesthetized with methoxyflurane, then the trachea was clamped, and the rat killed by decapitation. Each rat was examined externally and internally by a veterinary pathologist and all observations were recorded. Immediately following decapitation, the eyes were examined *in situ* by gently pressing a glass slide against the cornea and examining the eye under fluorescent illumination. Any ocular abnormalities noted were recorded as part of the gross pathologic observations. Body and liver weights were determined and recorded on all rats. Representative portions of the organs and tissues listed in Table 1 were preserved in phosphate-buffered 10% formalin from each rat.

Hematoxylin and eosin-stained sections were prepared from a complete set of tissues from the rats exposed to 0 or 8000 ppm. Sections of the liver, thymus, salivary glands (at least one section through the parotid, sublingual, and submaxillary glands), skin (from the inguinal region and from the region of the external nares containing vibrissae) and cervical

lymph nodes were prepared from rats exposed to 1500 or 3500 ppm. All sections were examined by light microscopy. The actual number of sections examined are summarized in Table 5 for the 1 and 3 day necropsies. In some cases, a tissue specimen was not obtained at necropsy, or was missed during histological preparation. Also, parathyroids were examined only to the extent that they were included in the routine sections of the thyroid gland.

STATISTICAL PROCEDURES

Analysis of body weight, organ weights, and relative organ weights were done with a computer program that performed Bartlett's test for homogeneity of variance, then an analysis of variance (parametric or non-parametric as indicated by Bartlett's). If ANOVA was statistically significant at $p < 0.1$, Dunnett's (parametric) or Wilcoxon's (non-parametric) tests were used to determine whether there were statistically significant differences between exposed and control groups. The final level of statistical significance (for establishing a difference between control and exposed groups) chosen was $p < 0.05$. Statistical outliers ($p < 0.02$) were identified, but were not normally excluded from the analysis.

RESULTS

Chamber Concentrations.

The mean measured $MeCl_2$ concentrations were within 0% of the targeted concentrations (0, 1500, 3500, and 8000 ppm), and the nominal concentrations were within 5% of the measured concentrations; this data is presented in Table 2. Temperature and relative humidity of the chambers ranged from 20 to 25°C and 34 to 50%, respectively.

General Observations.

Animals exposed to 8000 ppm $MeCl_2$ exhibited a number of effects during exposure, including increased activity, blood on coats (probably from self-mutilation or fighting), swelling near the nose, tremors, staggering, gasping. On one occasion, a female rat pushed her nose through the wire mesh cage. These 8000 ppm exposed animals were obviously distressed during exposures. Many of the effects that were observed (notably the increased activity, staggering, etc.) were markedly

diminished within 1/2 hour after exposure ended. The swelling in the nasal region decreased overnight. No effects were observed during exposure of the 1500 or 3500 ppm groups.

Body and Organ Weights.

There were statistically significant (p < 0.01) decreases in liver to body weight ratios in 8000 ppm exposed (1 day exposure group only) male and female rats (Table 3a). Statistically significant body weight loss was detected in male and female rats exposed to 8000 ppm $MeCl_2$ for 6 hr/day for 3 days (Table 3b and 4). No effects on body weight, liver weight, or liver to body weight ratio was detected in animals exposed to 1500 or 3500 ppm $MeCl_2$.

PATHOLOGY

Methylene chloride exposure for 6 hr/day for 1 or 3 days resulted in exposure-related pathologic alterations in male and female rats exposed to 8000 ppm, but not to 1500 or 3500 ppm. Tables 5 and 6 show the summarized data for the gross and histopathologic observations from these rats.

Exposure to 8000 ppm resulted in grossly observed swelling of the skin and subcutaneous tissues around the external nares in the region of the vibrissae in all males and females that were examined after 1 or 3 days of exposure. This edema appeared to be less extensive in rats sacrificed after 3 days of exposure. The swelling around the external nares was confirmed by histological examination to be due to edema and acute inflammation. The inflammatory reaction consisted of mixed cellular infiltration of lymphocytes, plasma cells, mast cells and neutrophils. The inflammatory reaction extended into the base of one or more vibrissae (but not the other types of hairs) in some, but not all, of the male and female rats. Focal ulceration was observed near the external nares in 3/5 of the males exposed to 8000 ppm. Enlarged or darkened cervical lymph nodes were observed in 3/5 of the males exposed to 8000 ppm for 6 hours. Acute inflammation and/or hemorrhage were observed histologically in the cervical lymph nodes of several rats of each sex that were exposed to 8000 ppm for 1 or 3 days. Since these lymph nodes drain the head region, it is likely that the

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cervical lymph node reaction was secondary to the inflammatory reaction around the external nares.

Two of 5 males exposed to 8000 ppm for 6 hours had grossly evident edema around the salivary glands. Histologically the edema was confirmed in one of these male rats along with slight focal degeneration in the submaxillary salivary gland.

In rats exposed to 8000 ppm for 3 days, the parotid salivary glands showed a decreased size of the acini. This lesion was multifocal or diffuse in 4/5 males and 5/5 females. This alteration appeared to be the result of a decreased amount of secretory material in the individual cells and glands rather than a degenerative lesion. The submaxillary and sublingual glands were also examined and they were normal in appearance.

Facial matting due to porphyrin was observed in 2/5 of the males and 2/5 of the females exposed to 8000 ppm for 3 days.

Some exposure-related lesions were histologically recognized, although they were not apparent on gross observation. For example, slight lymphoid necrosis was present in the cortex of the thymus in 3/5 males and 5/5 females exposed to 8000 ppm for 1 day and all of the rats exposed to 8000 ppm for 3 days. Also, slight lymphoid necrosis was present in one or more lymph nodes from several rats (males and females) exposed to 8000 ppm for 1 or 3 days.

Air was observed in the lumen of the stomach in male rats exposed to 8000 ppm for 1 day (3 of 5 rats) and for 3 days (1 of 5 rats).

A few additional gross and histopathologic observations were made, but most were typical of spontaneously occurring lesions that are often seen in rats of this age and strain.

DISCUSSION

Rats exposed to 8000 ppm $MeCl_2$ were markedly affected during exposure. The staggering and changes in activity could reflect the CNS depressant activity that was reported by Schumaker (1923). The increased activity could reflect stage II anesthesia (excitement). Irritation of the sensory hairs near the external nares (as suggested by the edema and inflammation in this area) might also affect activity. The rats' activity appeared normal within ~1/2 hour after a 6 hour exposure.

The severe distress of the 8000 ppm exposed rats is suggested by the observation of air in the stomach. This is often connected with agonal

or near agonal gasping, although no deaths occurred in this study. Exposure of rats to 17,000 ppm for 6 hours is reportedly lethal (Schumacher, 1923).

The effects observed in the cervical lymph nodes and salivary glands may be secondary. The cervical lymph nodes drain the area near the external nares that were edematous after a 6 hour 8000 ppm exposure. Edema was also observed externally (during gross pathological examination) in the area near the salivary glands in 2/5 of the males exposed to 8000 ppm for 6 hrs. This is likely to be an extension of the edema observed near the vibrissae.

One explanation for the localized edema is that $MgCl_2$ could be absorbed in the nasal turbinates, and high $MgCl_2$ concentrations in the area of the nares could cause edema due to direct or indirect effects on the vasculature. The base of the sensory hairs are more vascularized than other hair follicles, and this might explain the reaction at the base of the vibrissae. One major problem with the explanation of absorption in the turbinates causing the edema is that the turbinates are surrounded by a bone structure that would probably act as a diffusional barrier. Of course systemic absorption via the turbinates is possible. It is also possible that $MgCl_2$ acts externally on the vibrissae.

In most of the rats exposed to 8000 ppm $MgCl_2$ for 6 hr/day for 3 days, acini of the parotid salivary glands were decreased in size. This is thought to reflect a decreased amount of secretory material in the acini rather than a degenerative lesion.

Several observations were made on the salivary glands of rats exposed to 1500 and 3500 ppm (for 1 but not 3 days) that are judged to be not treatment related. These observations included a very slight focal aggregation of mononuclear (predominantly lymphoid cells) in the interstitium in 1 male rat exposed to 1500 ppm, very slight or slight subacute focal interstitial inflammation in 1 of 5 females exposed to 1500 or 3500 ppm, and slight subacute to chronic focal interstitial inflammation in 1 male exposed to 1500 ppm. These observations are thought to be spontaneous and not treatment-related because (1) these cellular reactions would take more than 6 hours to develop (they were only observed in the single 6 hour exposed animals, (2) these lesions can be observed in non-exposed animals, and (3) there is no dose-dependency to these effects.

The lack of toxicity in the liver and kidney at exposure concentrations that are 1/2 of a lethal concentration (based on Schwanker, 1983) is noteworthy. The slight (~10%) decrease in liver to body weight ratio was observed after 1, but not 3 exposures; and there were no treatment-related histopathological effects detected in either of these tissues.

Judging by the methods utilized in this study, acute inhalation exposures at concentrations up to 8000 ppm do not result in immediate effects on sex hormone sensitive tissues. No treatment-related changes in the pituitary gland, adrenal gland, thyroid gland, parathyroid gland, mammary gland, uterus, or testicle were detectable by gross pathology or histopathology.

Studies by Schumann, et al. (1981) indicate that ^{14}C is not preferentially concentrated in any of the salivary glands (parotid, submaxillary and sublingual) of rats exposed to 3500 ppm ^{14}C-$MeCl_2$ for 6 hours, nor is there more apparent ^{14}C macromolecular binding in the salviary glands relative to liver or kidney. Repeated exposures (3500 ppm, 6 hr/day for 3 days) did not significantly alter DNA synthesis in any of the three salivary glands, in liver, or in kidney. Although these measurements were done in animals exposed to 3500 ppm, not 8000 ppm, these results do not provide an obvious explanation for the histopathological effects that were observed after acute exposures to 8000 ppm.

The mechanism and biological significance of the edema and inflammation in the 8000 ppm exposed rats is not clear, and this effect is not common. However, based on currently available information, there is no apparent connection with the occurence of mesenchymal tumors in the ventral neck region of cronically exposed male rats. The following points should be considered: (1) the primary area of edema and inflammation was near the external nares, not the ventral neck region, (2) edema and inflammation was observed in males and females, increased mesenchymal tumors were observed in males only, (3) edema and inflammation occurred at 8000 ppm, but not at 3500 ppm, (4) the acute effect observed in the parotid salivary gland (decreased acinar size) was thought to be a reversible nonspecific alteration, and (5) DNA synthesis in salivary glands does not increase in response to 3500 ppm $MeCl_2$, 6 hr/day for 3 days, suggesting that there is not a hyperplastic response to tissue injury.

SUMMARY AND CONCLUSIONS

Male and female Sprague-Dawley rats were exposed to 0, 1500, 3500, or 8000 ppm methylene chloride ($MeCl_2$) for 6 hr/day, and were killed for pathological examination immediately after 1 or 3 exposures. The acute toxicity of inhaled $MeCl_2$ was assessed based on body weights, liver weights, clinical observations, gross pathology, and histopathology.

Exposure to 0, 1500, 3500, or 8000 ppm $MeCl_2$ for 6 hr/day for 1 or 3 days resulted in exposure-related effects only in rats exposed to 8000 ppm. These effects included increased body weight loss relative to control, body weight change (in rats exposed for 3 days) and a 10% decrease in relative liver weights (in rats exposed for 1 day only). Edema and acute inflammation of the skin and subcutaneous tissues around the external nares in the region of the vibrissae (sensory hairs) were observed, and the edema appeared to be more pronounced after just one exposure. Transient apparent CNS effects (staggering, changes in activity) occurred during exposure, but were not detected 1/2 hour post-exposure. Lymphoid necrosis of the thymus and some lymph nodes was evident in some rats exposed for 1 day and in some exposed for 3 days. A generalized depleted appearance of the parotid salivary gland was present. This was probably a nonspecific reversible alteration. Acute inflammation or hemorrhage in the cervical lymph nodes was observed, this was thought to be secondary to the inflammatory reaction around the external nares. No exposure-related alterations were apparent in any rats exposed to 1500 or 3500 ppm for 1 or 3 days.

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000016

TITLE OF STUDY: METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS
MET-K-1712-(22)

In compliance with Good Laboratory Practice Regulations, the study phases were inspected by the Quality Assurance Unit and the results of these inspections reported to Management and the Study Director on 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 reports are located at the submitting laboratory.

Study Started:	19 Jan 1981	Report Issued Date:	3 Sept 1981
Date of Inspection:	12 Jan 1981	Date of Report:	12 Jan 1981
	8 July 1981		8 July 1981

W. E. Hoover 3 Sept 81
Date

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TABLE 1

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Routine Tissues Collected at Gross Necropsy

Adrenal Glands
Aorta
Bone
Bone Marrow
Brain
Cecum
Cervical Lymph Nodes
Coagulating Gland
Epididymis
Esophagus
Eye
Heart
Kidney
Large Intestine
Larynx
Liver
Lungs
Mammary Gland
Mediastinal Lymph Nodes
Mediastinal Tissue
Mesenteric Lymph Nodes
Nasal Turbinates
Ovary
Oviduct
Pancreas
Parathyroid Gland
Peripheral Nerve
Pituitary
Prostate
Salivary Glands (parotid, sublingual, submandibular)
Seminal Vesicle
Skeletal Muscle
Skin
Small Intestine
Spleen
Spinal Cord
Stomach
Testicle
Thymus
Thyroid Gland
Tongue
Trachea
Urethra
Ureter
Urinary Bladder
Uterus
Vagina

TABLE 2

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Summary of Chamber $MeCl_2$ Concentrations

Sex	Number of Exposures	Targeted ($MeCl_2$) (ppm) 0	1600	3600	8000
Male	1	0	1610±70	3670±130	7960±120
Male	3	0	1620±70	3620±120	8000±200
Female	1	0	1480±60	3580±90	8040±210
Female	3	0	1630±70	3670±120	7950±220

Values are the mean $MeCl_2$ concentration ± S.D.
Nominal concentrations for all exposures were 0, 1600, 3610, and 8100 ppm.

TABLE 3a

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Body Weights, Liver Weights, and Relative Liver Weights of Rats Exposed to $MeCl_2$ for 6 hr/day for 1 Day

Exposure Conc. (ppm)	Sex	Body Weight (g)	Liver Weight (g)	Liver Wt/Body Wt (g/100g)
0	M	385±15	14.9±0.6	3.9±0.1
	F	268± 6	9.8±0.3	3.7±0.1
1500	M	387±25	10.1±1.9	3.9±0.2
	F	257±17	9.2±1.2	3.6±0.3
3500	M	384±18	14.1±0.9	3.7±0.2
	F	264±10	9.4±0.4	3.6±0.2
8000	M	387±19	13.7±1.3	3.5±0.2*
	F	263± 9	8.7±0.5	3.3±0.1*

Values are $\bar{X} \pm$ S.D., n = 5.

*Statistically significant difference from control ($p < 0.05$).

There was no statistically significant body weight loss in any exposure group relative to controls.

000020

TABLE 3b

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Body Weights, Liver Weights, and Relative Liver Weights of Rats Exposed to CH_2Cl_2 for 6 hr/day for 3 Days

Exposure Conc. (ppm)	Sex	Body Weight (g)	Liver Weight (g)	Liver Wt/Body Wt (g/100g)
0	M	400±19	14.3±0.9	3.6±0.2
	F	258±14	9.4±0.8	3.7±0.3
1500	M	389±26	14.9±1.6	3.8±0.2
	F	264±10	9.0±0.4	3.4±0.1
3500	M	394±21	14.4±1.0	3.7±0.1
	F	267±13	9.1±0.7	3.4±0.1
8000	M	357±27*[a]	12.9±1.6	3.6±0.3
	F	250±28[a]	8.5±0.9	3.4±0.1

Values are $\bar{X}$ ± S.D., n = 5.

*Statistically significant difference from control ($p<0.05$).

[a]Statistically significant body weight loss relative to control body weight change ($p<0.05$).

TABLE 4

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Body Weight Loss in Rats Exposed to $MeCl_2$ for 1 or 3 Days

Exposure Conc. (ppm)	Male 1 Day	Female 1 Day	Male 3 Days	Female 3 Days
0	8.20	5.66	4.94	6.56
	3.65	2.20	4.00	4.51
1600	4.96	8.06	2.96	8.16
	3.51	2.46	7.85	4.06
3500	1.30	9.72	3.74	7.90
	5.69	4.66	9.27	5.96
8000	3.00	9.64	44.32*	31.56*
	4.60	3.45	7.48	9.94

Values are the mean weight loss of rats (in grams, ± S.D.) weighed 1 day before $MeCl_2$ exposure, and at necropsy following either one 6 hour or three 6 hour exposures on consecutive days.

*Significant difference from control value ($p < 0.05$, Dunnett's test)

TABLE 6a

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 1 Day

LUNGS								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
HEART								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
KIDNEY								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
BRAIN								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
PITUITARY								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
ADRENAL GLAND								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
PERIPHERAL NERVE								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
PANCREAS								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
LIVER								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1
ABDOMEN								
NO LESIONS RECOVERED. TRACT, BILATERAL.	1	1	1	1	1	1	1	1
FEMUR								
NO LESIONS RECOVERED. SPLEEN, PELVIS, MUSCLE	1	1	1	1	1	1	1	1
STOMACH								
NO LESIONS RECOVERED. GAS, LUMEN	1	1	1	1	1	1	1	1
SMALL INTESTINE								
NO LESIONS RECOVERED.	1	1	1	1	1	1	1	1

000023

TABLE 5a

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 1 Day

[illegible]		Males				Females			
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]									
NO LESIONS RECOGNIZED. GAS, LUMEN		5 5	5 5	5 5	4 1	5 5	5 0	5 0	5 5
TESTICLE									
NO LESIONS RECOGNIZED. ABSENT, LEFT		4 1	5 0	5 0	5 0	- -	- -	- -	- -
EPIDIDYMIS									
NO LESIONS RECOGNIZED. DECREASED SIZE, LEFT	- SEVERE	4 1	5 0	5 0	5 0	- -	- -	- -	- -
SEMINAL VESICLE									
NO LESIONS RECOGNIZED.		5	5	5	5	-	-	-	-
COAGULATING GLAND									
NO LESIONS RECOGNIZED.		5	5	5	5	-	-	-	-
PROSTATE									
NO LESIONS RECOGNIZED.		5	5	5	5	-	-	-	-
MAMMARY GLANDS									
NO LESIONS RECOGNIZED. PROTEINACEOUS FLUID, LUMEN		[illegible]	[illegible]	[illegible]	[illegible]	5 0	5 0	5 0	5 0
[illegible]									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
[illegible]									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
OVARY									
NO LESIONS RECOGNIZED.		-	-	-	-	5	5	5	5
UTERUS									
NO LESIONS RECOGNIZED.		-	-	-	-	5	5	5	5
VAGINA									
NO LESIONS RECOGNIZED. NORMAL CYCLIC CHANGES		- -	- -	- -	- -	[illegible]	[illegible]	[illegible]	[illegible]
CERVIX									
NO LESIONS RECOGNIZED.		-	-	-	-	5	5	5	5

- Indicates not applicable

TABLE 5a

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 1 Day

[illegible]		MALES				FEMALES			
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TRACHEA									
NO LESIONS OBSERVED.		-	-	-	-	9	9	9	9
LUNGS									
NO LESIONS OBSERVED.		8	8	8	1	8	8	8	8
FOCUS - DARK, MULTIFOCAL	- VERY SLIGHT	1	0	0	0	0	0	1	0
FOCUS - DARK, MULTIFOCAL	- SLIGHT	0	1	1	4	1	1	0	1
FOCUS - DARK, MULTIFOCAL	- MODERATE	0	1	0	0	1	0	0	0
SKELETAL MUSCLE									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
SALIVARY GLAND									
NO LESIONS RECOGNIZED.		8	8	8	1	8	8	8	8
ESCHA, BILATERAL, SEPTUM	- SLIGHT	0	0	0	1	0	0	0	1
THYMUS									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
AORTA									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
PERICARDIUM									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
THYROID GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
PARATHYROID GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
TRACHEA									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
HARDERS GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
EYE									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
TONGUE									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9

- Indicates not applicable

000025

TABLE 5a

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 1 Day

Rat		Male				Female			
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ORAL CAVITY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
NASAL TURBINATES									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
LACRIMAL GLAND									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
LARYNX									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
ADRENAL GLAND									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
ABDOMINAL CAVITY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
FAT - STRANGULATED PORTION, OMENTUM :		0	0	0	0	0	0	0	1
EXTERNAL AND SKIN									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	0
EDEMA, EXTERNAL NARES :		0	0	0	0	0	0	0	5
EDEMA, EXTERNAL NARES :	- SLIGHT	0	0	0	1	0	0	0	0
EDEMA, EXTERNAL NARES :	- MODERATE	0	0	0	1	0	0	0	0
TRAUMA, FOREFOOT , RIGHT, FOCAL:		0	0	0	0	0	0	0	1
TRAUMA, FOREFOOT , LEFT:		0	0	0	0	0	0	0	1
TRAUMA, INGUINAL , RIGHT, FOCAL:	- SLIGHT	0	0	0	1	0	0	0	0
TRAUMA, SCROTUM , LEFT, FOCAL:	- SLIGHT	0	0	0	1	0	0	0	0
SKIN									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
LYMPH NODES									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
INCREASED SIZE, CERVICAL , BILATERAL:	- SLIGHT	0	0	0	1	0	0	0	0
DARK, CERVICAL , BILATERAL:		0	0	0	1	0	0	0	0
DARK, CERVICAL , BILATERAL, DIFFUSE:		0	0	0	1	0	0	0	0
PERITUM									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
THORACIC CAVITY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
VASCULATURE									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5

TABLE 5b

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 3 Days

[illegible]		MALES				FEMALES			
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
LIVER									
NO LESIONS RECOGNIZED.		5	5	5	5	4	5	5	5
NEPHIA, LEFT MIDDLE LOBE !		0	0	0	0	1	0	0	0
HEART									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
SPLEEN									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
INCREASED SIZE:	- SLIGHT	1	0	0	1	0	0	0	0
BRAIN									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
PITUITARY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
SPINAL CORD									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
PERIPHERAL NERVE									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
PANCREAS									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
DUNE									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
ARRENAL									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
KIDNEY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
STOMACH									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
GAS, LUMEN !		0	0	0	1	0	0	0	0
SMALL INTESTINE									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5

TABLE 6b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 3 Days

[illegible]	MALES				FEMALES			
SKIN								
NO LESIONS RECOGNIZED.	5	5	5	5	5	5	5	5
PERITONEUM								
NO LESIONS RECOGNIZED.	5	5	5	5	-	-	-	-
PERIGONADIA								
NO LESIONS RECOGNIZED.	5	5	5	5	-	-	-	-
SEMINAL VESICLE								
NO LESIONS RECOGNIZED.	5	5	5	5	-	-	-	-
COAGULATING GLAND								
NO LESION RECOGNIZED.	5	5	5	5	-	-	-	-
PROSTATE								
NO LESIONS RECOGNIZED.	5	5	5	5	-	-	-	-
PRIMARY GLANDS								
NO LESIONS RECOGNIZED.	5	3	4	4	5	5	5	5
PROTEINACEOUS PLUG	3	0	1	3	3	3	0	3
PROTEINACEOUS PLUG, LUMEN	0	1	0	1	1	1	0	0
UTERUS								
NO LESIONS RECOGNIZED.	5	5	5	5	5	5	5	5
VAGINA								
NO LESIONS RECOGNIZED.	5	5	5	5	5	5	5	5
OVARY								
NO LESIONS RECOGNIZED.	-	-	-	-	5	5	5	5
UTERUS								
NO LESIONS RECOGNIZED.	-	-	-	-	5	5	5	5
VAGINA								
NO LESIONS RECOGNIZED.	-	-	-	-	5	5	5	5
NORMAL CYCLIC CHANGES:	-	-	-	-	1	1	1	0
CERVIX								
NO LESIONS RECOGNIZED.	-	-	-	-	5	5	5	5

- Indicates not applicable

TABLE 5b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr/day for 3 Days

		MALES				FEMALES			
		1	1	1	1	1	1	1	1
VAGINA									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
LUNGS									
NO LESIONS RECOGNIZED.		1	1	1	1	1	1	1	0
AREA = DARK	= SLIGHT	0	0	0	0	0	1	0	0
FOCUS = DARK, MULTIFOCAL	= SLIGHT	0	0	0	0	0	0	0	1
SKELETAL MUSCLE									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
SALIVARY GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
THYMUS									
NO LESIONS RECOGNIZED.		0	0	0	0	0	0	0	0
FOCUS = DARK, MULTIFOCAL	= SLIGHT	0	0	0	0	0	1	1	0
AORTA									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
EPIPHYSIS									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
THYROID GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
PARATHYROID GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
TRACHEA									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
MANDIBULAR GLAND									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
EYE									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9
TONGUE									
NO LESIONS RECOGNIZED.		9	9	9	9	9	9	9	9

- indicates not applicable

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TABLE 5b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Gross Pathologic Observations of Rats Exposed for 6 hr day for 3 Days

KEY		MALES				FEMALES			
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
ORAL CAVITY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
FRACTURED/MISSING TEETH:		0	0	0	1	0	0	0	0
NASAL TURBINALES									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
LACRIMAL GLAND									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
LARYNX									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
SYMBAL GLAND									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
ABDOMINAL CAVITY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
EXTERNAL AND SKIN									
NO LESIONS RECOGNIZED.		5	5	5	0	5	5	5	1
ECEMA, EXTERNAL NARES :		0	0	0	0	0	0	0	0
ECEMA, EXTERNAL NARES :	- VERY SLIGHT	0	0	0	0	0	0	0	1
HEMORRHAGE, MIND FOOT , RIGHT, FOCAL:	- SLIGHT	0	0	0	1	0	0	0	0
ULCER, EXTERNAL NARES , FOCAL:	- SLIGHT	0	0	0	0	0	0	0	0
ULCER, EXTERNAL NARES , MULTIFOCAL:	- SLIGHT	0	0	0	1	0	0	0	0
ULCER, NECK , FOCAL:	- SLIGHT	0	0	1	0	0	0	0	1
FACIAL SOILING - PORPHYRIN, RIGHT:	- SLIGHT	0	0	0	1	0	0	0	0
FACIAL SOILING - PORPHYRIN, LEFT:		0	0	0	1	0	0	0	0
FACIAL SOILING - PORPHYRIN, BILATERAL:		0	0	0	0	0	0	0	0
COLON									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
LYMPH NODES									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
RECTUM									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
THORACIC CAVITY									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5
VASCULATURE									
NO LESIONS RECOGNIZED.		5	5	5	5	5	5	5	5

TABLE 6a

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 1 Day

		MALES				FEMALES			
LIVER (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		2	1	1	1	2	1	1	2
EOSINOPHILIC HEPATOCYTOSIS, MULTIFOCAL	- VERY SLIGHT	1	0	0	0	1	1	0	1
VACUOLIZATION, HEPATOCELLULAR	- SLIGHT	1	0	0	0	0	0	0	0
VACUOLIZATION, HEPATOCELLULAR, MULTIFOCAL	- VERY SLIGHT	1	0	1	1	0	1	0	0
HEART (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
KIDNEY (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
BRAIN (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
PITUITARY (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
DILATED, FOLLICLE, FOCAL	- VERY SLIGHT	1	0	0	0	0	0	0	1
SPINAL CORD (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
PERIPHERAL NERVE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
PANCREAS (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	1	1	0	0	1
INFLAMMATION, SUBACUTE, FOCAL	- VERY SLIGHT	0	0	0	1	0	0	0	0
INFARCTION, LOBULE, FOCAL	- SLIGHT	1	0	0	0	0	0	0	0
BONE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECORDED		1	0	0	1	1	0	0	1
BONE MARROW (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
ADRENAL (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	0	1	0	0	1
VACUOLIZATION, BILATERAL	- VERY SLIGHT	1	0	0	0	0	0	0	0
VACUOLIZATION, CORTEX, BILATERAL, MULTIFOCAL	- VERY SLIGHT	1	0	0	1	0	0	0	0
ADRENAL (NO. OF TISSUES EXAMINED)		1	0	0	1	1	1	0	1
NO LESIONS RECOGNIZED		2	0	0	2	1	0	0	1

TABLE 6a (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 1 Day

[illegible]		Males				Females			
[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] Continued:									
CHRONIC PROGRESSIVE GLOMERULONEPHROPATHY, UNILATERAL:									
	- VERY SLIGHT	1	0	0	1	0	0	0	0
CHRONIC PROGRESSIVE GLOMERULONEPHROPATHY, BILATERAL:									
	- VERY SLIGHT	2	0	0	0	0	0	0	0
BILATES, PELVIS , UNILATERAL:	- MODERATE	0	0	0	0	0	1	0	0
FIBROSIS, INTERSTITIUM , UNILATERAL, FOCAL:	- VERY SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - SUBACUTE, INTERSTITIUM , UNILATERAL, FOCAL:									
	- VERY SLIGHT	0	0	0	0	1	0	0	0
MINERALIZATION, CORTICOMEDULLARY JUNCTION , BILATERAL, MULTIFOCAL:	- SLIGHT	0	0	0	1	0	0	0	0
STOMACH (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
SMALL INTESTINE (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
SEGMENT (NO. OF TISSUES EXAMINED)		5	0	0	5	4	0	0	4
NO LESIONS RECOGNIZED:		5	0	0	5	4	0	0	4
LARGE INTESTINE (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
CERVICAL LYMPH NODE (NO. OF TISSUES EXAMINED)		5	5	5	5	5	5	5	5
NO LESIONS RECOGNIZED:		5	5	5	5	5	4	5	1
HEMORRHAGE:	- SLIGHT	0	0	0	0	0	0	0	1
HEMORRHAGE:	- MODERATE	0	0	0	1	0	0	0	0
INFLAMMATION - ACUTE:	- VERY SLIGHT	0	0	0	0	0	0	0	1
INFLAMMATION - ACUTE:	- SLIGHT	0	0	0	0	0	1	0	0
INFLAMMATION - ACUTE:	- MODERATE	0	0	0	0	0	0	0	0
MEDIASTINAL LYMPH NODE (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	0	5	0	0	1
HEMORRHAGE, SINUSOIDS :	- SLIGHT	0	0	0	0	0	0	0	1
NECROSIS, CORTEX :	- VERY SLIGHT	0	0	0	0	0	0	0	1
NECROSIS, CORTEX :	- SLIGHT	0	0	0	0	0	0	0	0
NECROSIS, CORTEX , MULTIFOCAL:	- VERY SLIGHT	0	0	0	0	0	0	0	0
NECROSIS, CORTEX , MULTIFOCAL:	- SLIGHT	0	0	0	0	0	0	0	1
MESENTERIC LYMPH NODE (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	4	5	0	0	4
NECROSIS, CORTEX :	- SLIGHT	0	0	0	1	0	0	0	0
NECROSIS, CORTEX , MULTIFOCAL:	- VERY SLIGHT	0	0	0	0	0	0	0	1
OTHER MISCELLANEOUS LYMPH NODES (NO. OF TISSUES EXAMINED)		0	0	0	0	0	0	0	1
NO LESIONS RECOGNIZED:		0	0	0	0	0	0	0	1

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TABLE 5A (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 or 24 hr, for 1 Day

[illegible] NO. OF TISSUES EXAMINED	1	0	1	1	0	0	0	0
NO LESIONS REPORTED	1	1	1	1	0	0	0	0
[illegible] NO. OF TISSUES EXAMINED	1	1	1	1	0	0	0	0
[illegible]	1	1	1	1	0	0	0	0
[illegible] NO. OF TISSUES EXAMINED	1	1	1	1	0	0	0	0
NO LESIONS REPORTED	1	0	1	1	0	0	0	0
[illegible] NO. OF TISSUES EXAMINED	1	1	1	1	0	0	0	0
NO LESIONS REPORTED	1	1	0	1	0	0	0	0
[illegible] NO. OF TISSUES EXAMINED	1	0	1	1	0	0	0	0
NO LESIONS REPORTED	1	1	1	1	0	0	0	0
INFLAMMATION, SUBACUTE, INTERSTITIAL, FOCAL	• SLIGHT	1	1	1	0	0	0	0
[illegible] NO. OF TISSUES EXAMINED		1	1	1	1	1	0	0
NO LESIONS REPORTED		1	1	1	1	1	1	1
[illegible] NO. OF TISSUES EXAMINED		1	0	0	0	1	1	1
NO LESIONS REPORTED		0	0	0	1	1	1	1
[illegible] NO. OF TISSUES EXAMINED		0	0	0	0	1	1	1
NO LESIONS REPORTED		0	0	0	0	1	1	1
[illegible] NO. OF TISSUES EXAMINED		0	0	0	1	1	1	1
NO LESIONS REPORTED		0	0	0	1	0	1	1
[illegible] NO. OF TISSUES EXAMINED		0	0	0	0	1	1	1
NO LESIONS REPORTED		0	0	0	0	1	1	1
[illegible] NO. OF TISSUES EXAMINED		1	1	1	1	1	1	1
INFLAMMATION, SUBACUTE, MULTIFOCAL	• VERY SLIGHT	1	1	1	1	1	1	1
INFLAMMATION, SUBACUTE, MULTIFOCAL	• SLIGHT	1	1	1	0	1	1	1
[illegible] NO. OF TISSUES EXAMINED		1	1	1	1	1	1	1
NO LESIONS REPORTED		1	1	1	1	1	0	1
[illegible] NO. OF TISSUES EXAMINED		1	1	1	1	1	1	1
NO LESIONS REPORTED		1	1	1	0	1	1	1
ASPARTATE [illegible] NO. OF [illegible]	[illegible]	1	1	1	1	1	0	0
INFLAMMATION, FOCAL	• VERY SLIGHT	1	1	1	1	1	0	0
DEGENERATION, MULTIFOCAL, [illegible]	• SLIGHT	1	1	1	1	1	0	0
[illegible] INFLAMMATION, BILATERAL, MULTIFOCAL [illegible]	• SLIGHT	1	1	1	1	1	1	1

• Indicates not applicable

TABLE 6a (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 1 Day

		MALES				FEMALES			
[illegible]									
SALIVARY GLAND (Continued)									
INFLAMMATION - SUBACUTE, INTERSTITIUM, FOCAL	- VERY SLIGHT	0	1	1	0	1	0		0
INFLAMMATION - SUBACUTE, INTERSTITIUM, MULTIFOCAL									
INFLAMMATION - SUBACUTE TO CHRONIC, INTERSTITIUM, FOCAL	- SLIGHT	0	0	0	0	0	1	0	0
	- SLIGHT	0	1	0	0	0	0	0	0
THYMUS (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
NECROSIS, CORTEX	- VERY SLIGHT	0	0	0	1	0	0	0	0
NECROSIS, CORTEX	- SLIGHT	0	0	0	0	0	0	0	0
NECROSIS, CORTEX, MULTIFOCAL	- VERY SLIGHT	0	0	0	0	0	0	0	0
PERIVISCERAL TISSUE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
AGGREGATE(S) OF MONONUCLEAR (PREDOMINANTLY LYMPHOID) CELLS, MULTIFOCAL	- SLIGHT	1	0	0	0	0	0	0	1
AORTA (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
ESOPHAGUS (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
THYROID GLAND (NO. OF TISSUES EXAMINED)		1	0	1	1	1	0	1	1
NO LESIONS RECOGNIZED		1	0	1	1	1	0	1	1
CYSTIC, WITH KERATINOUS DEBRIS, FOCAL	- VERY SLIGHT	1	0	0	0	1	0	0	0
CYST(S) WITH KERATINOUS DEBRIS, MULTIFOCAL	- SLIGHT	0	1	0	0	1	0	0	1
PARATHYROID GLAND (NO. OF TISSUES EXAMINED)		1	0	0	1	0	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
TRACHEA (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
INFLAMMATION - SUBACUTE, SUBMUCOSA, MULTIFOCAL	- SLIGHT	1	0	1	1	1	0	0	1
SKIN (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		1	0	0	1	1	1	1	0
EDEMA, SUBCUTANEOUS	- VERY SLIGHT	0	0	0	1	0	0	0	0
GRANULOMA(S) - FOREIGN BODY, SUBCUTANEOUS, FOCAL	- MODERATE	0	0	0	0	0	0	0	0
INFLAMMATION - ACUTE, HAIR FOLLICLES, FOCAL	- VERY SLIGHT	1	1	1	1	0	0	0	0
INFLAMMATION - ACUTE, HAIR FOLLICLES, FOCAL	- SLIGHT	0	1	0	1	0	0	0	0
INFLAMMATION - ACUTE, HAIR FOLLICLES, MULTIFOCAL	- SLIGHT	0	0	0	0	0	0	0	0
INFLAMMATION - ACUTE, SUBCUTANEOUS	- SLIGHT	1	1	1	1	1	0	0	1
	- VERY SLIGHT	0	0	0	0	0	0	0	0

TABLE 6a (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 1 Day

		MALES				FEMALES			
AORTA (CONT'D)									
INFLAMMATION - ACUTE, SUBCUTANEOUS	- MODERATE	0	0	0	1	0	0	0	1
HEMORRHAGE, SUBCUTANEOUS	- MODERATE	0	0	0	1	0	0	0	1
URINARY BLADDER (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	1	1	0	0	1
HYPERPLASIA, AZINE	- SLIGHT	0	0	0	1	0	0	0	0
HYPERPLASIA, INTERSTITIUM	- SLIGHT	0	0	0	1	0	0	0	0
EYE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
TONGUE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
INFLAMMATION - ACUTE, SUBMUCOSA, FOCAL	- SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - SUBACUTE TO CHRONIC, SUBMUCOSAL GLANDS, FOCAL	- VERY SLIGHT	0	1	0	0	1	0	0	0
NASAL TURBINATES (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
MESENTERIC LYMPH (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	1	1	1	1	0	0	1
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, MESENTERY, MULTIFOCAL	- SLIGHT	1	1	1	1	0	0	1	1
EXPANSULATED OR NECROTIC FAT, MESENTERY		1	0	0	0	0	0	1	1
LACRIMAL GLAND (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, FOCAL	- VERY SLIGHT	0	0	1	0	0	0	0	0
INFLAMMATION - SUBACUTE, MULTIFOCAL	- SLIGHT	0	0	0	1	1	0	1	1
LARYNX (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	1	0	1	1	1	0	1

TABLE 65

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 3 Days

[illegible]		MALES				FEMALES			
		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
LIVER (NO. OF TISSUES EXAMINED)		5	5	5	5	5	5	5	5
NO LESIONS RECOGNIZED:		3	2	4	2	2	0	2	1
ARCHITECTURE ALTERED SECONDARY TO DIAPHRAGMATIC HERNIA:		0	0	0	0	1	0	0	0
EXTRAMEDULLARY HEMATOPOIESIS, FOCAL:	- VERY SLIGHT	0	0	0	1	0	0	0	0
EXTRAMEDULLARY HEMATOPOIESIS, MULTIFOCAL:	- VERY SLIGHT	2	0	0	1	0	1	2	0
FOCUS(S) OF ALTERED CELLS, HEPATOCELLULAR, FOCAL:	- VERY SLIGHT	0	0	1	0	[illegible]	0	0	0
INFLAMMATION - SUBACUTE, MULTIFOCAL:	- SLIGHT	0	0	0	0	0	0	0	1
VACUOLIZATION, HEPATOCELLULAR:	- VERY SLIGHT	0	0	0	0	0	0	0	1
VACUOLIZATION, HEPATOCELLULAR:	- SLIGHT	0	0	0	0	1	0	0	0
VACUOLIZATION, HEPATOCELLULAR, MULTIFOCAL:	- VERY SLIGHT	0	0	0	1	1	0	2	2
VACUOLIZATION, HEPATOCELLULAR, MULTIFOCAL:	- SLIGHT	0	0	0	0	1	0	0	0
ABSORPTIONS OF RE CELLS PRESENTLY ADJACENT TO DEGENERATIVE IN NECROTIC HEPATOCYTES, FOCAL:	- VERY SLIGHT	0	0	0	0	1	0	0	1
PEARS (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		4	0	0	4	5	0	0	5
DEGENERATION WITH OR WITHOUT INFLAMMATION, MYOCARDIUM, FOCAL:	- VERY SLIGHT	0	0	0	1	0	0	0	0
DEGENERATION WITH OR WITHOUT INFLAMMATION, MYOCARDIUM, FOCAL:	- SLIGHT	1	0	0	0	0	1	0	1
SPLEEN (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
NECROSIS, FOLLICLES (WHITE PULP):	- VERY SLIGHT	0	0	0	1	0	0	0	0
NECROSIS, FOLLICLES (WHITE PULP):	- SLIGHT	0	0	0	1	0	0	0	0
BRAIN (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
PITUITARY (NO. OF TISSUES EXAMINED)		5	0	0	4	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	4	5	0	0	5
SPINAL CORD (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
PERIPHERAL NERVE (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
PANCREAS (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5
BONE (NO. OF TISSUES EXAMINED)		5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:		5	0	0	5	5	0	0	5

TABLE 6b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 3 Days

		MALES				FEMALES			
[illegible]									
[illegible]									
LUNG, LOBAR (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
ADRENAL (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	0	1	0	0	0
VACUOLIZATION, CORTEX, BILATERAL, MULTIFOCAL	- VERY SLIGHT	0	0	0	1	0	0	0	0
KIDNEY (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	0	0	0	0	0
CHRONIC PROGRESSIVE GLOMERULONEPHROPATHY, BILATERAL		1	0	0	1	1	0	0	1
DILATED, TUBULES, UNILATERAL, MULTIFOCAL	- VERY SLIGHT	1	0	0	0	0	0	0	0
FIBROSIS, INTERSTITIUM, UNILATERAL, FOCAL	- SLIGHT	0	0	0	0	0	0	0	0
	- SLIGHT	1	0	0	0	0	0	0	1
BRAIN (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
SMALL INTESTINE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
CECUM (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
LARGE INTESTINE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
MEDIASTINAL LYMPH NODE (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		1	1	1	0	1	1	1	0
HEMORRHAGE	- SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - ACUTE	- VERY SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - ACUTE	- SLIGHT	0	0	0	1	0	0	0	0
NECROSIS, CORTEX	- SLIGHT	0	0	0	0	0	0	0	1
MEDIASTINAL LYMPH NODE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
NECROSIS, CORTEX	- SLIGHT	0	3	0	0	1	0	0	0
NECROSIS, CORTEX	- VERY SLIGHT	1	0	0	0	0	0	0	0
NECROSIS, CORTEX	- SLIGHT	0	0	0	1	0	0	0	1
NECROSIS, CORTEX, MULTIFOCAL	- VERY SLIGHT	0	0	0	0	0	0	0	1
NECROSIS, CORTEX, MULTIFOCAL	- SLIGHT	0	0	0	1	0	0	0	0
MESENTERIC LYMPH NODE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	1	1	0	0	1
NECROSIS, CORTEX	- VERY SLIGHT	0	0	0	1	0	0	0	0
NECROSIS, CORTEX	- SLIGHT	0	0	0	0	0	0	0	1
NECROSIS, CORTEX	- MODERATE	1	0	0	0	0	0	0	0

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TABLE 6b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 Hr/day for 3 Days

[illegible]	MALES				FEMALES			
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
OTHER MISCELLANEOUS LOWER ORGANS (NO. OF TISSUES EXAMINED)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
NO LESIONS RECOGNIZED:	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TESTICLE (NO. OF TISSUES EXAMINED)	5	0	0	5	-	-	-	-
NO LESIONS RECOGNIZED:	5	0	0	5	-	-	-	-
EPIDIDYMIS (NO. OF TISSUES EXAMINED)	5	0	0	5	-	-	-	-
NO LESIONS RECOGNIZED:	5	0	0	5	-	-	-	-
SEMINAL VESICLE (NO. OF TISSUES EXAMINED)	5	0	0	5	-	-	-	-
NO LESIONS RECOGNIZED:	5	0	0	5	-	-	-	-
COAGULATING GLAND (NO. OF TISSUES EXAMINED)	5	0	0	5	-	-	-	-
NO LESIONS RECOGNIZED:	5	0	0	5	-	-	-	-
PROSTATE (NO. OF TISSUES EXAMINED)	5	0	0	5	-	-	-	-
NO LESIONS RECOGNIZED:	4	0	0	4	-	-	-	-
AGGREGATE(S) OF MONONUCLEAR (PREDOMINATELY LYMPHOID) CELLS, INTERSTITIAL, MULTIFOCAL: - SLIGHT	1	0	0	0	-	-	-	-
INFLAMMATION - SUBACUTE, INTERSTITIAL, MULTIFOCAL: - SLIGHT	0	0	0	1	-	-	-	-
URINARY BLADDER (NO. OF TISSUES EXAMINED)	5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:	5	0	0	5	5	0	0	5
OVARY (NO. OF TISSUES EXAMINED)	-	-	-	-	5	0	0	5
NO LESIONS RECOGNIZED:	-	-	-	-	5	0	0	5
OVIDUCT (NO. OF TISSUES EXAMINED)	-	-	-	-	5	0	0	5
NO LESIONS RECOGNIZED:	-	-	-	-	5	0	0	5
UTERUS (NO. OF TISSUES EXAMINED)	-	-	-	-	5	0	0	5
NO LESIONS RECOGNIZED:	-	-	-	-	5	0	0	5
CERVIX (NO. OF TISSUES EXAMINED)	-	-	-	-	5	0	0	5
NO LESIONS RECOGNIZED:	-	-	-	-	5	0	0	5
LUNGS (NO. OF TISSUES EXAMINED)	5	0	0	5	5	0	0	5
ALVEOLAR HISTIOCYTOSIS, MULTIFOCAL: - SLIGHT	0	0	0	0	0	0	0	[illegible]
INFLAMMATION - SUBACUTE, MULTIFOCAL: - VERY SLIGHT	0	0	[illegible]	1	0	0	0	2
INFLAMMATION - SUBACUTE, MULTIFOCAL: - SLIGHT	5	0	[illegible]	4	5	0	0	3
SKELETAL MUSCLE (NO. OF TISSUES EXAMINED)	5	0	0	5	5	0	0	5
NO LESIONS RECOGNIZED:	5	0	0	5	5	0	0	5

- Indicates not applicable

TABLE 6b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 3 Days

		MALES				FEMALES			
LACRIMAL GLAND (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		1	1	1	1	1	1	1	1
HEMORRHAGE SUBC, ACINI , MULTIFOCAL (PAROTID GLAND)	- SLIGHT	1	0	0	0	0	0	0	1
HEMORRHAGE SUBC, ACINI , DIFFUSE (PAROTID GLAND)	- SLIGHT	0	0	0	1	0	0	0	0
EDEMA, INTERSTITIUM , MULTIFOCAL	- SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - SUBACUTE, INTERSTITIUM , MULTIFOCAL	- VERY SLIGHT	0	0	0	0	0	0	1	0
INFLAMMATION - SUBACUTE, INTERSTITIUM , MULTIFOCAL	- SLIGHT	0	0	0	0	0	0	1	0
LUNGS (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		1	1	1	0	1	1	1	0
HEMORRHAGE, CENTER	- SLIGHT	0	0	0	0	0	0	0	0
HEMORRHAGE, CENTER , MULTIFOCAL	- SLIGHT	0	0	0	1	0	0	0	1
MYELENCEPHALON TISSUE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
AORTA (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
ESOPHAGUS (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
SALIVARY GLAND (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
PARATHYROID GLAND (NO. OF TISSUES EXAMINED)		2	0	0	2	1	0	0	1
NO LESIONS RECOGNIZED		2	0	0	2	1	0	0	1
TRACHEA (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
SKIN (NO. OF TISSUES EXAMINED)		1	1	1	1	1	1	1	1
NO LESIONS RECOGNIZED		0	1	1	0	1	1	1	0
EDEMA, SUBCUTANEOUS	- VERY SLIGHT	1	0	0	0	0	0	0	1
EDEMA, SUBCUTANEOUS	- SLIGHT	0	0	0	1	0	0	0	0
EDEMA, SUBCUTANEOUS	- MODERATE	0	0	0	1	0	0	0	0
GRANULOMA(S) - FOREIGN BODY, SUBCUTANEOUS , FOCAL	- VERY SLIGHT	0	1	1	1	0	0	1	1
INFLAMMATION - ACUTE, HAIR FOLLICLES , FOCAL	- SLIGHT	0	0	0	1	0	0	0	1
INFLAMMATION - ACUTE, HAIR FOLLICLES , MULTIFOCAL	- SLIGHT	0	0	0	1	0	0	0	1
INFLAMMATION - ACUTE, HAIR FOLLICLES , MULTIFOCAL	- MODERATE	0	0	0	1	0	0	0	0
INFLAMMATION - ACUTE, SUBCUTANEOUS	- SLIGHT	0	0	0	0	0	0	0	0
INFLAMMATION - ACUTE, SUBCUTANEOUS	- MODERATE	0	0	0	1	0	0	0	0
NECROSIS, SUBCUTANEOUS	- SLIGHT	0	0	0	1	0	0	0	0
NECROSIS, SUBCUTANEOUS	- MODERATE	0	0	0	1	0	0	0	0
ULCER, FOCAL	- SEVERE	0	0	0	0	0	0	0	1

TABLE 6b (cont)

METHYLENE CHLORIDE: AN ACUTE INHALATION TOXICITY STUDY IN RATS

Histopathologic Observations of Rats Exposed for 6 hr/day for 3 Days

(a)		MALES				FEMALES			
PARTIAL BLANK (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	0	1	0	0	1
HYPERPLASIA, ACINI	• VERY SLIGHT	1	0	0	0	0	0	0	0
HYPERPLASIA, ACINI	• SLIGHT	0	0	0	1	0	0	0	0
EYE (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
ESOPHAGUS (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	0	1	0	0	1
INFLAMMATION - ACUTE, SUBMUCOSA, FOCAL	• SLIGHT	0	0	0	1	0	0	0	0
INFLAMMATION - SUBACUTE TO CHRONIC, MUSCLE, FOCAL	• SLIGHT	1	0	0	0	0	0	0	0
NASAL TURBINATES (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	0	1	0	0	0
INFLAMMATION - ACUTE, RESPIRATORY EPITHELIUM, MULTIFOCAL	• SLIGHT	1	0	0	0	0	0	0	0
INFLAMMATION - ACUTE, RESPIRATORY EPITHELIUM, MULTIFOCAL	• MODERATE	0	0	0	1	0	0	0	0
INFLAMMATION - ACUTE, TOOTH, BILATERAL	• MODERATE	0	0	0	0	1	0	0	1
INFLAMMATION - SUBACUTE, SUBMUCOSA, MULTIFOCAL	• SLIGHT	1	0	0	0	0	0	0	0
HARDERIAN GLAND (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1
LACRIMAL GLAND (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		0	0	0	0	0	0	0	0
INFLAMMATION - SUBACUTE, MULTIFOCAL	• SLIGHT	1	0	0	1	1	0	0	1
LARYNX (NO. OF TISSUES EXAMINED)		1	0	0	1	1	0	0	1
NO LESIONS RECOGNIZED		1	0	0	1	1	0	0	1

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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METHYLENE CHLORIDE - TECHNICAL GRADE: ACUTE ORAL TOXICITY

LK 28

Chemical Name (300 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75-Ø9-2

1A

D-427 878211313

METHYLENE CHLORIDE - TECHNICAL GRADE: ACUTE ORAL TOXICITY

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August 4, 1981

SUMMARY

The single-dose oral LD_{50} for Methylene Chloride - Technical Grade for male rats was 2524 mg/kg (1867 - 3414 mg/kg, 95% confidence interval). Rats were administered 630, 1300, 2500 or 5000 mg/kg of the undiluted test material. The following in-life signs of toxicity were observed in test animals (dose groups affected are in parantheses): lethargy, lack of physical coordination (all), blackening of the tip of the tail along with sloughing and/or breakage at the tip of the tail (630-2500 mg/kg), palpebral closure, rapid shallow breathing (1300-5000 mg/kg) and exudate staining of the nares (630 mg/kg). The majority of rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats 2 weeks post-treatment, rats given 2500 and 1300 mg/kg had internal, treatment related lesions which consisted of gastric ulcers and fibrous adhesions between the stomach, liver, spleen and adjacent peritoneum. Similar lesions of lesser severity were noted on rats administered 630 mg/kg.

INTRODUCTION

A sample of Methylene Chloride - Technical Grade also known as Dichloromethane or Methylene dichloride (Lot #916304776) was submitted to the Toxicology Research Laboratory for assessment of acute oral toxicity. The source of this clear liquid was the Inorganic Chemicals Laboratory, Midland, Michigan.

To assist the reader in evaluating the acute toxicological properties of this formulation, copies of the original laboratory work sheets are appended to this report.

Test Animals

Acute oral toxicity tests of this material were conducted on male CD1 (Fischer 344-derived) rats (Charles River Breeding Laboratories, Portage, Michigan), weighing 162-207 g. All animals were maintained on a 12 hour light and dark cycle. They were supplied with commercial certified laboratory chow (Ralston Purina Company, St. Louis, Missouri) and water ad libitum. All animals were acclimated to the laboratory environment at least 1 week prior to testing, and were housed 2 or 3 per cage. Each animal was uniquely identified by an individual metal eartag.

ACUTE ORAL TOXICITY

Method

Four groups of rats, each consisting of 6 rats/dose level, were fasted overnight prior to dosing. The following morning, each group received 630, 1300, 2500 or 5000 mg/kg/rat of the test material by single-dose gavage. Each rat was weighed immediately prior to treatment, the day after treatment and weekly thereafter for a 2 week post-treatment observation period. The rats were observed periodically during this time for signs of toxicity. All surviving rats were submitted for a

gross necropsy examination 2 weeks post-treatment. The acute oral median lethal dose was calculated by the moving average method (Thompson and Weil, 1952). The slope was calculated as 2/log (LD_{84}/LD_{16}).

Results

Results of acute oral toxicity tests are given in Table 1. The single-dose oral LD_{50} for male rats was 2524 mg/kg (1807-3414 mg/kg, 95% confidence interval), with an approximate slope of 4.4.

Results of animal observation during the 2 week post-treatment observation period are given in Table 2. In-life signs of toxicity observed in test animals included the following: lack of physical coordination, lethargy, palpebral closure, rapid shallow breathing, a blackening of the tip of the tail often with secondary sloughing of up to 1/3 of the distal end of the tail. The majority of rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats 2 weeks post-treatment, all rats given 2500 and 1300 mg/kg had internal, treatment-related lesions which consisted of gastric ulcers and fibrous adhesions between the stomach, liver, spleen and adjacent peritoneum. Similar lesions of lesser severity (gastric ulcers 3/6, fibrous adhesions 2/6 and focal hyperkeratosis of the nonglandular gastric epithelium 6/6) were noted in rats administered 630 mg/kg. In addition, one of these rats had a decreased amount of thymic and abdominal adipose tissue.

REFERENCES

Thompson, W. R. and C. S. Weil, Biometrics, Vol. 8, No. 1, March 1952, pp. 51-64, as implemented in a computer program (Stephan, C., 1970, personal communication).

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TABLE 1

METHYLENE CHLORIDE - TECHNICAL GRADE:
ACUTE TOXICOLOGICAL PROPERTIES

MORTALITY OF MALE RATS DOSED ORALLY WITH THE TEST MATERIAL

Dose (mg/kg)	#Dead/#Treated	Time of Death
630	0/6	
1300	0/6	
2500	3/6	2 rats - 2 hours 1 rat - Day 2
5000	0/6	4 rats - 2 hours 1 rat - 4.5 hours 1 rat - 6 hours

TABLE 2

METHYLENE CHLORIDE - TECHNICAL GRADE: ACUTE TOXICOLOGICAL PROPERTIES

OBSERVATIONS OF MALE RATS DOSED ORALLY WITH THE TEST MATERIAL

Dose (mg/kg)	Signs of Toxicity	#Affected/#Treated	Approximate Time of Onset
630	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 2 hours
	Blackened tip of tail	1/6	1 rat - Day 3
	Exudate staining around nares	1/6	1 rat - Day 3
	Sloughing of distal 1/4 of tail	1/6	1 rat - Day 11
1300	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 2 hours
	Palpebral closure	6/6	6 rats - 2 hours
	Rapid, shallow breathing	6/6	6 rats - 2 hours
	Blackened tip of tail	2/6	2 rats - Day 3
	Sloughing of distal 1/4 of tail	2/6	2 rats - Day 11
2500	Lack of physical coordination	6/6[a]	6 rats - 15 minutes
	Lethargy	4/4[a]	4 rats - 2 hours
	Palpebral closure	4/4[a]	4 rats - 2 hours
	Rapid, shallow breathing	4/4[a]	4 rats - 2 hours
	Dark periocular staining	4/4[a]	4 rats - Day 2
	Blackened tip of tail	2/4[a]	2 rats - Day 3
	Sloughing of distal 1/4 of tail	2/4[a]	2 rats - Day 11
5000	Lack of physical coordination	6/6[a]	6 rats - 15 minutes
	Lethargy	2/2[a]	2 rats - 2 hours
	Palpebral closure	2/2[a]	2 rats - 2 hours
	Rapid, shallow breathing	2/2[a]	2 rats - 2 hours

[a]Data presented as number affected/number surviving.

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TOXICOLOGY RESEARCH LABORATORY
THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN

ACUTE ORAL TOXICITY

DOW CONFIDENTIAL

SPECIES ☐ RAT ☐ GUINEA PIG ☐ RABBIT ☐ MOUSE SEX ☐ ♂ ☐ ♀

☐ SOLUTION ☐ SUSPENSION
☐ WATER ☐ CORN OIL
☐ LIPOID

CAGE	DATE FED	ANIMAL MARKING	WEIGHT GM	DOSE GM./KG.	DOSE CC.	ANIMAL WEIGHT (G)			DATE
1		7117	202		0.10	185	192	187	
		7118	190		0.09	200	200	272	
		7124	202		0.10	215	227	229	
4		7126	168		0.08	161	178	211	
		7101	162		0.08	170	190	204	
		7102	189		0.09	193	223	250	
5		7103	185		0.09	195	212	236	
		7104	190		0.10	192	214	240	
		7105	171		0.08	169	186	220	
6		7106	171		0.08	175	176	222	
		7107	207		0.10	204	230	262	
		7108	172		0.09	168	195	237	
7		7109	182		0.09	200	216	256	
		7110	195		0.10	194	196	232	
		7152	194		0.10				
8		7153	170		0.08				
		7154	168		0.08	164	170	214	
		7131	174		0.09	174	187	220	
9		7155	200		0.10				
		7132	193		0.10				
		7134	204		0.10				
10		7158	194		0.10				
		7159	176		0.09				

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METHODOLOGY

Animals used for acute oral toxicity tests are fed by single-dose gavage using a calibrated syringe and a blunt feeding needle (rats, mice, guinea pigs or cattlecar (rabbits). The animals are fasted overnight (except rabbits) and are weighed before dosing, the day after dosing, and at 1 and 2 weeks after dosing. The animals are observed closely the day of treatment, then periodically for the 2-week observation period. Unless otherwise noted, 6-8 week old Sprague-Dawley rats, 6-8 week old CF$_1$ mice, 6-6 week old Hartley guinea pigs, or 3-3 1/2 month old New Zealand albino rabbits are used. For range studies, 2 or 3 animals of one sex per dose level for 2 to 6 levels are used; the third animal (if used) is sent to pathology for a gross examination at 24 hours after treatment. For LD50 studies, 5 animals per sex per dose level for 2 to 6 levels are used; all survivors are submitted for a gross pathological examination after the 2-week weighing. Non corrosive liquids are dosed as received, corrosive liquids are diluted in water or corn oil. Pastes and solids are prepared as solutions or suspensions in water, corn oil, or 0.5% methocel

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Dept/Div

P.O. Box | Street No./Name

City: MIDLAND | State: MI | Zip: 48640 | Country

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Contractor

Doc Type: • RI • UP • HEASD 8D SU HS FN

Doc Title: METHYLENE CHLORIDE - AEROTHENE GRADE: ACUTE ORAL TOXICITY

Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-09-2

1A

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METHYLENE CHLORIDE - AEROTHENE GRADE: ACUTE ORAL TOXICITY

RECEIVED

Reported By:

R. E. Carreon

Reviewed By:

P. G. Watanabe and K. S. Rao

Toxicology Research Laboratory
Health and Environmental Sciences, U.S.A.
Dow Chemical, U.S.A.
Midland, Michigan 48640

September 8, 1981

SUMMARY

The single-dose oral LD_{50} for Methylene Chloride - Aerothene Grade for male r ts was 1530 mg/kg (1159-2224 mg/kg, 95% confidence interval). Rats were administered 630, 1300, 2500 or 5000 mg/kg of the undiluted test material. The following in-life signs of toxicity were observed in test animals (dose groups effected are in parentheses): lack of physical coordination, lethargy, rapid shallow breathing, body tremors (all), rough hair coats (630, 1300, 5000 mg/kg), palpebral closure and/or watery eyes (630 and 5000 mg/kg), dark staining around the nares and a blackening of the tip of the tail along with a slight sloughing of up to 1/4 of the distal end of the tail (2500 mg/kg). All rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats, there was some evidence of gastrointestinal irritation, characterized by pale and roughened areas of the stomach wall, along with slight ulceration observed in two rats and in one rat fibrous adhesions of the pancreatic tissue to the stomach wall.

INTRODUCTION

A sample of Methylene Chloride - Aerothene Grade also known as Aerosol Grade Methylene Chloride or Aerothene MM and identified as Lot #63b2770 was submitted to the Toxicology Research Laboratory for assessment of acute oral toxicity. The source of this clear liquid was the Inorganic Chemicals Laboratory.

To assist the reader in evaluating the acute toxicological properties of this formulation, copies of the original laboratory work sheets are appended to this report.

Test Animals

Acute oral toxicity tests of this material were conducted on male CDF (Fischer 344-derived) rats (Charles River Breeding Laboratories, Portage, Michigan), weighing 133-178 g. All animals were maintained on a 12 hour light and dark cycle. They were supplied with commercial certified laboratory chow (Ralston Purina Company, St. Louis, Missouri) and water ad libitum. All animals were acclimated to the laboratory environment at least 1 week prior to testing, and were housed 2 or 3 per cage. Each animal was uniquely identified by an individual metal eartag.

ACUTE ORAL TOXICITY

Method

Four groups of male rats, each consisting of 6 rats/dose level, were fasted overnight prior to dosing. The following morning, each group received 630, 1300, 2500 or 5000 mg/kg/rat of the test material by single-dose gavage. Each rat was weighed immediately prior to treatment, the day after treatment and weekly thereafter for a 2 week post-treatment observation period. The rats were observed periodically during this time for signs of toxicity. All surviving rats were submitted for a gross pathological examination 2 weeks post-treatment. The acute oral

median lethal dose was calculated by the moving average method (Thompson and Weil, 1952). The "slope" was calculated as 2/log (LD_{84}/LD_{16}).

Results

Results of acute oral toxicity tests are given in Table 1. The single-dose oral LD_{50} for male rats was 1630 mg/kg (1169-2224 mg/kg, 95% confidence interval), with an approximate slope of 4.6. Results of animal observation during the 2 week post-treatment observation period are given in Table 2. In-life signs of toxicity observed in rats tested included: lack of physical coordination, rapid shallow breathing, body tremors, palpebral closure and a blackening of the tip of the tail. All rats which survived the 2 week post-treatment observation period recovered as evidenced by a weight gain during that period. Upon gross necropsy examination of surviving rats, there was some evidence of gastrointestinal irritation, characterized by pale and roughened areas of the stomach wall (630 and 1300 mg/kg), along with slight ulceration and fibrous adhesions of the pancreatic tissue to the stomach wall (2/5-1300 mg/kg).

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Thompson, W. R. and C. S. Weil, Biometrics, Vol 8, No. 1, March 1952, pp. 51-64, as implemented in a computer program (Stephan, C., 1978, personal communication).

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TABLE 1

METHYLENE CHLORIDE - AEROTHENE GRADE
ACUTE ORAL TOXICITY

MORTALITY OF MALE RATS DOSED ORALLY WITH THE TEST MATERIAL

Dose (ml/kg)	#Dead/#Treated	Time of Death
630	0/6	
1300	1/6	1 rat - Day 2
2500	6/6	4 rats - Day 2 1 rat - Day 3 1 rat - Day 5
5000	6/6	1 rat - 2.5 hours 1 rat - 4.5 hours 1 rat - 6 hours 2 rats - Day 2 1 rat - Day 3

TABLE 2

METHYLENE CHLORIDE - AEROTHENE GRADE: ACUTE ORAL TOXICITY

OBSERVATIONS OF MALE RATS DOSED ORALLY WITH THE TEST MATERIAL

Dose (mg/kg)	Signs of Toxicity	#Affected/#Treated	Approximate Time of Onset
630	Lack of physical coordination	6/6	6 rats - 15 minutes
	Lethargy	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
	Rough hair coats	6/6	6 rats - 2.5 hours
1300	Lethargy	6/6	6 rats - 15 minutes
	Lack of physical coordination	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
	Rough hair coats	6/6	6 rats - 2.5 hours
2800	Lethargy	6/6	6 rats - 15 minutes
	Lack of physical coordination	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
	Palpebral closure	6/6	6 rats - 6 hours
	Dark staining around nares	1/1[a]	1 rat - Day 3
	Darkened distal 1/4 of tail	1/1[a]	1 rat - Day 3
	Watery eyes	1/1[a]	1 rat - Day 4
6000	Lethargy	6/6	6 rats - 15 minutes
	Lack of physical coordination	6/6	6 rats - 15 minutes
	Rapid, shallow breathing	6/6	6 rats - 15 minutes
	Total body tremors	6/6	6 rats - 15 minutes
	Palpebral closure	3/3[a]	3 rats - 6 hours
	Rough hair coats	3/3[a]	3 rats - 6 hours

[a]Data presented as number affected/number surviving

TOXICOLOGY RESEARCH LABORATORY
THE DOW CHEMICAL COMPANY
OLAND, MICHIGAN

ACUTE ORAL TOXICITY

CHEMICAL: AEROTHENE MM

SPECIES: RAT ☒ RAT ☐ RABBIT ☐ MOUSE ☐ SEX: ☐ ☐

cc SYRINGE
EMPTY SYRINGE
cc
SP GRAVITY

☐ SOLUTION ☐ SUSPENSION
WATER ☐ CORN OIL
OTHER

CAGE	DATE PEG	ANIMAL MARKING	WEIGHT GR.	DOSE GM./KG.	DOSE CC.	ANIMAL WEIGHT (GM.) DATE		
						2-17-61	2-21-61	
1		7541	195	.02	0.07	169	200	222
		7542	157		0.07	164	197	182
		7543	147		0.07	155	187	215
2		7544	162		0.08	177	212	240
		7545	193		0.08	138	170	195
		7546	157	↓	0.07	163	191	212
3		7547	165	.03	0.10	173	207	226
		7548	160		0.10	165	198	216
		7549	160		0.10	DEAD	2-19-61	
4		7550	166		0.10	171	202	228
		7551	167		0.10	133	135	212
		7552	163	↓	0.10	173	203	224
5		7553	183	.04	0.15	DEAD	2-19-61	
		7554	161		0.10	154	DEAD	2-19-61
		7555	134		0.05	DEAD	2-19-61	
6		7556	155		0.10	155	DEAD	2-19-61
		7557	175		0.11	DEAD	2-19-61	
		7558	163	↓	0.21	DEAD	2-19-61	
7		7559	191	.04	0.12	DEAD	2-19-61	
		7560	164		0.12	DEAD	2-19-61	
		7561	150		0.12	DEAD	2-19-61	
8		7562	157		0.12	DEAD	2-19-61	
		7563	157		0.12	170	2-19-61	
		7564	157	.04	0.12	DEAD	2-19-61	

OBSERVATIONS:

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METHODOLOGY

Animals used for acute oral toxicity tests are fed by single-dose gavage using a calibrated syringe and a blunt feeding needle (rats, mice, guinea pigs) or catheter (rabbits). The animals are fasted overnight (except rabbits) and are weighed before dosing, the day after dosing, and at 1 and 2 weeks after dosing. The animals are observed closely the day of treatment, then periodically for the 2-week observation period. Unless otherwise noted, 6-8 week old Sprague-Dawley rats, 6-8 week old CF_1 mice, 6-8 week old Hartley guinea pigs, or 3-3 1/2 month old New Zealand albino rabbits are used. For range studies, 2 or 3 animals of one sex per dose level for 2 to 5 levels are used; the third animal (if used) is sent to pathology for a gross examination at 24 hours after treatment. For LD50 studies, 6-6 animals per sex per dose level for 2 to 6 levels are used; all survivors are submitted for a gross pathological examination after the 2-week weighing. Non-corrosive liquids are dosed as received, corrosive liquids are diluted in water or corn oil. Pastes and solids are prepared as solutions or suspensions in water, corn oil, or 0.5% methocel.

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Doc Title 23

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE; 24-HOUR EKG MONITORING

Chemical Name (500 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75-09-2

LA 6-11-93

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July 9, 1982

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: 24-HOUR EKG MONITORING

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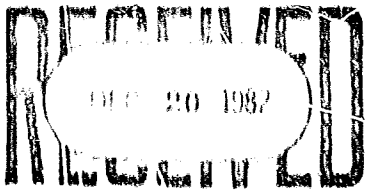

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24-HOUR EKG MONITORING

KEYWORDS

Holter Monitoring

Methylene Chloride

Carboxyhemoglobin

Occupational Exposure

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ABSTRACT

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: 24-HOUR EKG MONITORING

Ott MG, Skory LK, Holder BB, Bronson JM, and Williams PR,

Reports concerning the potential of methylene chloride and related chemicals to increase cardiac sensitivity to epinephrine and concerns stemming from the metabolism of methylene chloride to form carboxyhemoglobin prompted our interest in electrocardiographic monitoring of men with methylene chloride exposure. Fifty employees of two fiber production plants were selected for study, 24 of whom were occupationally exposed to methylene chloride. All participants were white males between the ages of 37 and 63. Eleven of these men had reported a history of heart disease.

Under the conditions of this study, neither increase in ventricular or supraventricular ectopic activity nor episodic ST-segment depression were associated with exposures to methylene chloride that ranged from 60 ppm time-weighted-average to approximately 475 ppm time-weighted-average.

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: 24-HOUR EKG MONITORING

This is the fourth in a series of health surveillance reports on fiber production employees exposed to solvents in two separate plants. In one of the plants, methylene chloride ($MeCl_2$) was a major component of the solvent system used to dissolve cellulose triacetate. In this paper, we present the results of 24-hour electrocardiographic (EKG) monitoring of 50 white male employees from the two plants.

A number of commonly used industrial solvents, including both unsubstituted and halogenated hydrocarbons, have been shown to increase cardiac sensitivity to epinephrine.(3,12) Other solvents, such as ethyl ether, ethyl alcohol and acetone have shown comparatively little, if any, activity in increasing the electric irritability of cardiac muscle.(7) In regard to $MeCl_2$, cardiac sensitization was observed in one dog study at concentrations of 2.4% in air but was not seen in a second study at concentrations of about 2% $MeCl_2$.(4,12)

An additional concern stems from the presence of increased blood carboxyhemoglobin from $MeCl_2$ metabolism. In monkeys, increased vulnerability to induced fibrillation has been demonstrated with carbon monoxide exposures sufficient to produce

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sufficient to produce 5 to 10% carboxyhemoglobin saturations.[6] Similar effects may extend to man.[14] Knelsen reported abnormal electrocardiograms in 7 of 26 men, aged 41 to 60, upon exposure to 100 parts-per-million (ppm) carbon monoxide (5-9% carboxyhemoglobin).[8] Arrhythmias were observed in two of these men, but not in 12 men under age 40. Furthermore, two experimental studies have been reported in which low levels of carboxyhemoglobin (2 to 5%) derived from direct carbon monoxide exposure, were associated with decreased time to onset and increased duration of angina during exercise.[1,2] ST-segment depression was also frequently observed in connection with the onset of angina. It should be noted that all the patients participating in these studies had pre-existing stable angina pectoris.

The occurrence in a 66 year old man of a fatal acute myocardial infarction associated with paint stripping has also been reported.[13] The formulation used to remove the paint was labelled as containing 80% $MeCl_2$; however, no data were given from which to estimate exposure.

The objective of this study was to determine whether or not occupational exposure to $MeCl_2$ in the present work setting resulted in increased ventricular or supraventricular ectopic activity or ST-segment depression.

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METHODS

The 24-hour EKG monitoring was conducted using two Avionics Mini Holter Recorders, Model 445. The men (24 from the study plant and 26 from the comparison plant) were a subset of the 51 white men in the study plant and 114 white men in the comparison plant who were 35+ years of age and participated in the prior health evaluation.[11] Preference was given to selecting older individuals and those with a history of heart disease under the presumption that these individuals would be most likely to demonstrate positive findings.

Based on environmental surveys reported earlier,[11] one individual was employed in a work area in which typical $MeCl_2$ exposure was 60 ppm time-weighted-average for an 8-hour day (TWA), 10 men were employed in areas in which typical exposure was 140 ppm TWA, four men were employed in work areas in which typical exposure was 280 ppm TWA, and nine men were employed in work areas in which typical exposure was 475 ppm TWA.

The procedure for carrying out the monitoring consisted of attaching and testing the recorder in the Medical Department, after having instructed the employee in the purposes and procedures of the study. The recorder was calibrated prior to each use with an Avionics 1-Millivolt Calibrator, Model 356B. Each volunteer was asked to keep a diary of his activities during the

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24-hour period and record any pain, shortness of breath or other discomfort experienced during the day. In two of three instances in which tapes did not record properly, a second monitoring session was scheduled and acceptable tapes were obtained. The 24-hour tape and diary were sent to Cardio-Bionic-Scanning-Inc. for computer analysis of the tape, visual scanning by a technician, and interpretation by a cardiologist. Copies of the subsequent reports provided the basic information for our analyses. Ventricular ectopic activity (VEA) was classified according to the Lown grading system: no VEA; occasional, isolated ventricular premature beats (VPB); frequent VPB; multiform VPB; and repetitive VPB.(9) Supraventricular ectopic activity was classified as to the presence or absence of atrial premature beats, atrial couplets and atrial tachycardia. ST-T wave changes were classified as ischemic if there was $\geq$1 mm of horizontal or down-sloping ST-segment depression for $\geq$0.08 sec. in at least three consecutive beats.(5) Analysis of the hourly rate of VEA relative to solvent exposure was also performed, in essence, using the individual as his own control and comparing VEA during work hours versus non-work hours.

CHARACTERISTICS OF THE POPULATION

Characteristics of white male participants from both the study and comparison plants are summarized in Table 1. Although the age range of participants was nearly the same for the two

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plants, there was a four-year difference in average age, with the comparison plant employees being older. There were also more men from the comparison plant with either a positive smoking history, or a reported history of heart disease. There were no statistical differences in height/weight distribution, blood pressure, alcohol history, or cholesterol between the two populations.

RESULTS

For the combined group of 50 participants, the percentage of men with VEA increased with age and history of heart disease. Under age 45, 33% (2 of 6) of the men experienced VEA, whereas, over age 44, 77% (34 of 44) of the men experienced VEA. All eleven men with a history of heart disease were found to have VEA. These men also had a greater likelihood of being classified as having multiform or repetitive VPB (4 of 11 vs. 4 of 39 among men not reporting a history of heart disease). Comparison of VEA by plant indicated a similar distribution for the total groups as well as for the subgroups of those persons not reporting a history of heart disease (see Table 2).

Comparison of supraventricular ectopic activity also did not reveal differences between the two plants. Of the fifty men studied, only four comparison plant and two study plant employees did not show at least one isolated supraventricular ectopic beat during the monitoring period. Supraventricular couplets were

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observed among 6 of the 26 comparison plant employees and 6 of the 24 study plant employees. Supraventricular tachycardia including junctional tachycardia was observed in four study plant and six comparison plant participants.

Table 3 summarizes the distribution of VEA per hour during work, sleep and non-work awake hours for the combined group and for the study and comparison groups separately. The three time periods were of approximately eight hours duration each. A Wilcoxon Rank Sum test was used to compare VEA (work minus non-work awake hours and work minus sleep hours) between the two plants, each man thus serving as his own control. In neither comparison was the relative difference between the study and referrent group statistically different from zero ($p>.1$, two-sided test).

For three of the 50 individuals, positive ST-T changes were reported. Of these positives, which were suggestive of ischemic heart disease, two were in the comparison plant group and one was in the study plant group. The individual with ST-segment depression from the study plant had had no VEA. The ST-segment depression occurred during a period of sinus tachycardia while the person was riding a bicycle. For one of the two persons in the comparison plant with ST-segment depression, the events occurred during periods of sinus tachycardia, once with chest

pain reported after walking up steps. During a second episode, no symptoms were reported. The second individual from the comparison plant experienced ST-T wave changes throughout a period of supraventricular tachycardia.

DISCUSSION

The rationale for choosing continuous ambulatory monitoring was that it might detect electrocardiographic changes associated with particular exposures whenever they occur, and however short-term. However, use of this method is not without limitations. For example, marked variability in ventricular premature depolarizations between days in the same individual and between individuals may decrease the sensitivity of the procedure.(10) Thus, there were sample size limitations to the study both in terms of the number of men monitored and in terms of the number of monitoring sessions per individual.

Given these limitations, neither increased ventricular or supraventricular ectopic activity, nor increased episodic ST-segment depression were found to be associated with $MeCl_2$ exposure. This lack of positive findings is consistent with cardiac sensitization studies, in that present $MeCl_2$ exposures were much lower than those shown experimentally to sensitize the myocardium.(4,12)

The present study did not apparently have the resolving power of investigations conducted with angina patients subjected to controlled exercise regimens. In at least two such studies, low level carbon monoxide exposure led to electrocardiographic abnormalities, earlier onset of angina, and ST-segment depression.[1,2] For many employees, the carboxyhemoglobin saturations, derived from $MeCl_2$ exposure in the present work environment, were comparable to or greater than those associated with EKG changes in the controlled studies.[1,2] This is true not even considering the carbon monoxide derived from cigarette smoke. However, in our study, no attempt was made to control exercise level or to exclude employees without known ischemic heart disease. It appears then that the critical factors in obtaining positive responses are the choice of subjects (i.e. inclusion of patients with pre-existing coronary disease), the exercise regimen, and the tighter controls possible with laboratory studies.

Acknowledgement

The authors thank C. S. Strande, M.D. for his contribution in the area of clinical toxicology during the study and analysis of the data.

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T A B L E 1

CHARACTERISTICS OF WHITE MALE PARTICIPANTS
FROM STUDY AND COMPARISON PLANT

Characteristics of Population	Study Plant Participants	Comparison Plant Participants
Number of Men	24	26
Age Mean	51.5	55.4
Range	37 - 62	38 - 63
Smoking Hx		
Never Smoked	5	1
Past Smoker	9	10
Current Smoker		
<1 pk/day	1	1
1+ pks/day	9	14
Diastolic BP >100 mm Hg	4	4
Reported History of Heart Disease	3	8
Taking Medications for Cardiovascular Disease or Hypertension	5	6

TABLE 2

SUMMARY OF VENTRICULAR ECTOPIC ACTIVITY (VEA) BY STUDY GROUP

	Study Plant		Comparison Plant	
Grading System	Nbr	Percent	Nbr	Percent
No VEA	8	33%	6	23%
<30 (Maximum VPB within any one hour)	11	46%	14	54%
30+ (Maximum VPB within any one hour)	0	0%	1	4%
Multiform VPB	4	17%	4	15%
Repetitive VPB	1	4%	1	4%
Excluding Men with a Hx of Heart Disease				
No VEA	8	38%	6	33%
<30 (Maximum VPB within any one hour)	10	48%	9	50%
30+ (Maximum VPB within any one hour)	0	0%	1	6%
Multiform VPB	2	10%	2	11%
Repetitive VPB	1	5%	0	0%

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TABLE 3

DISTRIBUTION OF VENTRICULAR ECTOPIC BEATS BY DAILY ACTIVITY (WORK, NON-WORK AWAKE, SLEEP)

Ventricular Ectopic Beats	Activity					
	Work		Non-Work Awake		Sleep	
	Nbr	Percent	Nbr	Percent	Nbr	Percent
Combined Group						
0	21	42%	17	34%	27	54%
<1/hr	13	26%	15	30%	11	22%
1-9/hr	8	16%	12	24%	7	14%
10-19/hr	2	4%	0	0%	2	4%
20+/hr	6	12%	6	12%	3	6%
Study Group						
0	11	46%	11	46%	14	58%
<1/hr	5	21%	6	25%	4	17%
1-9/hr	5	21%	4	17%	3	13%
10-19/hr	0	0%	0	0%	2	4%
20+/hr	3	13%	3	13%	1	4%
Comparison Group						
0	10	38%	6	23%	13	50%
<1/hr	8	31%	9	35%	7	27%
1-9/hr	3	12%	8	31%	4	15%
10-19/hr	2	8%	0	0%	0	0%
20+/hr	3	12%	3	12%	2	8%

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DETERMINATION OF TOTAL OXIDANT IN A PHOTOCHEMICAL SMOG CHAMBER: METHYL-CHLOROFORM, TRICHLOROETHYLENE, PERCHLOROETHYLENE, AND METHYLENECHLORIDE

Chemical Name (200 per name)	25	CAS No. (10)	24
METHYLENE CHLORIDE		75-09-2	

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Figure 3-7. Global Indexing Form

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DETERMINATION OF TOTAL OXIDANT IN A PHOTOCHEMICAL SMOG CHAMBER: METHYL-CHLOROFORM, TRICHLOROETHYLENE, PERCHLOROETHYLENE, AND METHYLENECHLORIDE

J. P. Arrington, M. J. Minta

Special Assignments
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The Dow Chemical Company
Midland, Michigan

A method has been devised to determine the total oxidant concentration resulting from simulated atmospheric photolysis of the above chlorocarbons. The amount of oxidant formed, the rate of disappearance of organic, and the effect of recent pollution control regulations on the use of these compounds will be discussed.

I. INTRODUCTION

On Jan 30, 1971, a proposed set of National Ambient Air Quality Standards were published in the Federal Register. These standards stipulate that an air pollution alert will be declared when "oxidant" reaches a 0.1 ppm 1-hour average and an air pollution warning will be declared when "oxidant" reaches a 0.4 ppm 1-hour average. Thus those solvents which contribute markedly to oxidant formation will ~~accordingly~~ be subject to stringent controls.

The goal of this project was to devise a method to determine "total oxidant" formation, using a small (< 10 liter capacity) photoreactor already available, and to apply this method to a study of Dow's chlorinated solvents (methylchloroform, trichloroethylene, perchloroethylene, methylene chloride, chloroform) and related materials. All of these studies were to be carried out in a manner that would simulate atmospheric conditions as closely as possible. in a small chamber

II. HISTORICAL

A. Background

As a result of widespread public concern over air pollution, especially "photochemical" smog, Los Angeles enacted Rule 66 in 1966. This rule, designed to regulate emission of reactive organic compounds into the atmosphere, rated and controlled these compounds based on several criteria: eye irritation, photochemical reactivity and, to a lesser extent, "total oxidant" formation. These criteria were derived from empirical observations in studies carried out by the Los Angeles County Air Pollution Control District (LACAPCD), which measured characteristics of photochemical smog induced by various compounds.

Rule 66 was, in a sense, a landmark. Many communities across the country showed interest in it, and San Francisco soon enacted the similar Regulation 3. Unfortunately, some of these later groups have shown a lesser under-

standing of the situation than has LACAPCD, since representatives of this group have repeatedly felt it necessary to warn that atmospheric contaminants may be very different in different cities. Their recommendations have been that each case should be studied individually. As an example of the possible differences, consider Los Angeles and London. The smog in Los Angeles is oxidizing, with a larger concentration of ozone than sulfur dioxide, whereas London smog is characterized by a higher sulfur dioxide concentration, making it reducing. Many other contaminants also vary greatly, depending upon local usage, weather conditions and other factors. Making the situation still more complicated is the fact that the emphasis in regulation has begun to shift more heavily toward control of "total oxidant" levels. This is particularly true of the 1971 National Air Quality Standards, and will probably be true of many local ordinances.

The restriction of trichloroethylene by Rule 66 was felt by some to be based on questionable studies (the criticism was that the concentrations used in the study did not fairly represent atmospheric conditions). Under the auspices of the Manufacturing Chemists Association (MCA), the Stanford Research Institute (SRI) conducted a rather complete study of the smog-forming potential of trichloroethylene. The results of this study have now been published[1], and provide us with a large body of data that enhance our understanding of the smog-forming mechanism, as well as providing a basis for rational regulation. The results of this study will be summarized below in the discussion of the smog-forming mechanism.

Battelle Research Institute recently compiled the results of a comprehensive study[2] of a wide variety of organic compounds for the National Paint, Varnish, and Lacquer Association (NPVLA), which "tend to confirm the data upon which Rule 66 and Regulation 3 were derived." The Battelle report emphasized a point that is worth repeating: there is no clear correlation among oxidant formation, eye irritation, and reactivity. This not only makes regulation on the basis of any single criterion nearly impossible, but also makes rational regulation difficult. How does one assign a weight to each of the criteria? These, then, are some of the complex problems that make a complete, thorough understanding of the chemical processes involved so important to the question of control. The

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following section discusses what is currently known about photochemical smog formation.

D. Photochemical Processes in Polluted Air

For the purpose of this discussion, "photochemical smog" will be defined broadly to arise from all photochemical processes which occur in the atmosphere. Some of these processes occur in non-polluted air as well, but a survey of them is a requisite to an adequate understanding of the smog-forming process.

One of the most complicated, and yet one of the most fundamental, processes that occurs in polluted atmospheres is the accumulation of ozone. The formation of ozone has long been recognized as being an integral part of the nitric oxide-nitrogen dioxide equilibrium, but in the absence of organics, the concentration rapidly reaches a very low steady-state value. The "normal" process is thought to occur as shown in the following equations:[3,4]

$$NO_2 \xrightarrow{h\nu} NO + O\cdot \qquad (1)$$

$$O\cdot + O_2 + M \longrightarrow O_3 + M \qquad (2)$$

$$O\cdot + NO_2 \longrightarrow NO + O_2 \qquad (3)$$

$$O_3 + NO \longrightarrow NO_2 + O_2 \qquad (4)$$

$$O_3 \xrightarrow{h\nu} O_2 + O\cdot$$

Under normal conditions, at low concentrations, nitric oxide greatly predominates.

The addition of many organic compounds to the atmosphere disrupts this equilibrium and may lead to any (or all) of the following phenomena:[5]

(1) The organic compounds are oxidized and disappear.

(2) Reaction products such as aldehydes, nitrates, PAN's*, are formed.

(3) Nitric oxide is oxidized to nitrogen dioxide.

(4) When nitric oxide disappears ozone begins to form and can reach dangerous levels.

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*Peroxyacylnitrites and Nitrates.

The mechanism by which organic additives influence the nitric oxide-nitrogen dioxide equilibrium is not clear, but has been suggested[3] to occur by way of a chain mechanism illustrated below. This scheme is admittedly quite speculative, but it appears to be a reasonable beginning.

$$Ha + O\cdot \longrightarrow HaO\cdot\ (RO\cdot) \qquad Ha = \text{aromatic, olefin} \qquad (6)$$

$$Ha + O\cdot \longrightarrow R\cdot + OH \qquad Ha = \text{aliphatic} \qquad (7)$$

$$R\cdot + O_2 \longrightarrow RO_2\cdot \qquad (8)$$

$$RO\cdot + O_2 \longrightarrow RO_3\cdot \qquad (9)$$

$$RO_2\cdot + NO \longrightarrow RO\cdot + NO_2 \qquad (10)$$

$$RO_3\cdot + NO \longrightarrow RO_2\cdot + NO_2 \qquad (11)$$

$$RO_2\cdot + O_2 \longrightarrow RO\cdot + O_3 \qquad (12)$$

$$RO\cdot + NO_2 \longrightarrow RONO_2 \qquad (13)$$

The reaction (12) is thus responsible for the accumulation of ozone, and reactions 10-12 are the chain carriers. Although this mechanistic scheme accounts fairly well for most of the salient features of the "system," it is not completely satisfactory. It fails, for example, to offer a clue to the reason a mass balance in nitrogen is lacking in nearly all the studies carried out to date. Further, this mechanistic scheme is not sufficiently sophisticated to explain the dependence of the maximum ozone level upon both the ratio and absolute concentration of organic and nitrogen oxide, as was reported in the SRI study.[1]

The SRI results on trichloroethylene are certainly worthy of comment, particularly since they are so pertinent to this study. It was shown[1] that at a given ratio of trichloroethylene to nitrogen oxides, the mole yield of ozone increased as the absolute concentration of the reactants decreased. It was also found that photolysis at low ratios (8:1 or less) of trichloroethylene to nitrogen oxides generates smaller amounts of ozone than does irradiation at higher ratios (8:1). Both these findings are illustrated in Table I. It is unfortunate that a wider range of ratios was not studied, as this might lead to rather interesting changes. The SRI data also serve to illustrate the difficulty of basing regulations upon a single characteristic of smog and testing only at a given set of concentration and ratio parameters. Other factors, such as stabilizers and relative humidity, have also been found

TABLE I

Ozone Yield as a Function of Absolute Concentration and Concentration Ratio

Ratio(trichlor/NO_x)	[trichlor]ppm	Max[O_3]ppm	% O_3 Yield
8:1	8	0.8	10
8:1	0.8	0.17	21
2:1	4	<0.05	<1
2:1	2	<0.1	<5

to influence the reactivity of trichloroethylene, although the effect on oxidant formation was not investigated.

The Battelle group[8] examined a series of 45 individual organic-solvent materials in a study conducted for the NPVLA. Their study was exhaustive in the number of smog characteristics measured. Unfortunately, each of their experiments was done at a fixed ratio (2:1) of organic to nitrogen oxides, and it has already been shown that this doesn't always give the complete picture.

An excited state ($^1\Delta_g$) of molecular oxygen has recently been suggested[9] as a reactant in the photooxidation of nitric oxide to nitrogen dioxide. A mechanism by which this might occur is shown below.

$$O_2(^1\Delta_g) + RH \longrightarrow R\text{-}OOH \quad R = \text{olefin} \qquad (14)$$

$$ROOH \xrightarrow[\Delta]{} RO\cdot + OH \qquad (15)$$

$$RO\cdot + O_2 \longrightarrow RO_3\cdot \qquad (9)$$

$$RO_3\cdot + NO \longrightarrow RO_2\cdot + NO_2 \qquad (11)$$

This is simply one way of producing RO· without the necessity for invoking a reaction of the organic molecule with a free oxygen atom (both species are present at very low concentrations). A number of recent reviews[6-8] attest to the reactivity of singlet oxygen with a wide variety of unsaturated organic molecules. In fact, the usual reaction of singlet oxygen with olefins is illustrated by Rxn (14) above. From the point of view of reactivity, then, it is quite reasonable to assume that singlet oxygen plays a part in the oxidation of nitric oxide to nitrogen dioxide in polluted atmospheres. The formation of singlet oxygen in polluted air is also quite credible. Many organic molecules are known to sensitize the conversion of the ground state ($^3\Sigma$) of oxygen to its excited stage ($^1\Delta$). Almost any carbonyl-containing compound or aromatic hydrocarbon is capable of sensitizing this transformation quite efficiently, so the presence of some singlet oxygen in polluted atmospheres is quite possible. This reactive state of oxygen has also been suggested to arise

$$\text{Organic} \xrightarrow{h\nu} \text{Org.}\ ^{*1} \longrightarrow \text{Org.}\ ^{*3} \qquad (16)$$

$$\text{Org.}^{*3} + O_2(^3\Sigma) \longrightarrow \text{Org.} + O_2\ (^1\Delta g) \qquad (17)$$

from other reactions such as the photolysis of ozone,[9,10] and bimolecular

reaction between ozone and nitric oxide (reaction 4).[11] Although the formation of singlet oxygen and its participation in the conversion of nitric oxide to nitrogen dioxide in polluted atmospheres seems plausible, it has not yet been demonstrated to occur in the lower atmosphere. Among others, Pitts' group at Riverside, California, is actively studying this problem.

The accumulation of ozone in polluted atmospheres is thus a result of a disruption of the nitric oxide-nitrogen dioxide-ozone equilibrium by the addition of certain organic molecules. Some conclusions can be drawn from the proposed mechanism, even though it may not be complete. The first is that compounds that do not readily react with oxygen atoms (benzene, paraffins) should exhibit a low rate of ozone formation. It is also clear that nearly all of these reactions are initiated from the photodissociation of nitrogen dioxide to nitric oxide and an oxygen atom, and everything else occurs in a dark, or secondary reaction. Further, in the complete absence of either added organics or nitrogen oxides, the symptoms of photochemical smog endemic to Los Angeles would be removed. Since it is unlikely that we shall ever attain "zero" concentrations of either organics or nitrogen oxides, it is important to determine the degree to which each organic material contributes to the symptoms of photochemical smog, and to enact regulations on this basis. It is on this premise that Rule 66 was enacted and the work described below was initiated.

III. RESULTS AND DISCUSSION

A. General Comments

As discussed above, many workers[3-9] have reported that ozone does not begin to appear until the nitric oxide has been converted to nitrogen dioxide. It is also clear from these data that the same maximum ozone level is attained no matter which of these two nitrogen oxides is part of the starting mixture. In order to avoid this "induction period" and to thereby shorten the analysis time, nitrogen dioxide was used in all of these experiments. The concentration of the organic used was often as high as 50 ppm; this was done in order to facilitate gc monitoring (response of flame ionization detectors to chlorocarbons is very poor). A ratio of 4:1 for organic/NO_2 was chosen for use in each of the experiments. The range for most of the earlier work was from 2:1 to 6:1; it was felt that 4:1 is a good compromise.

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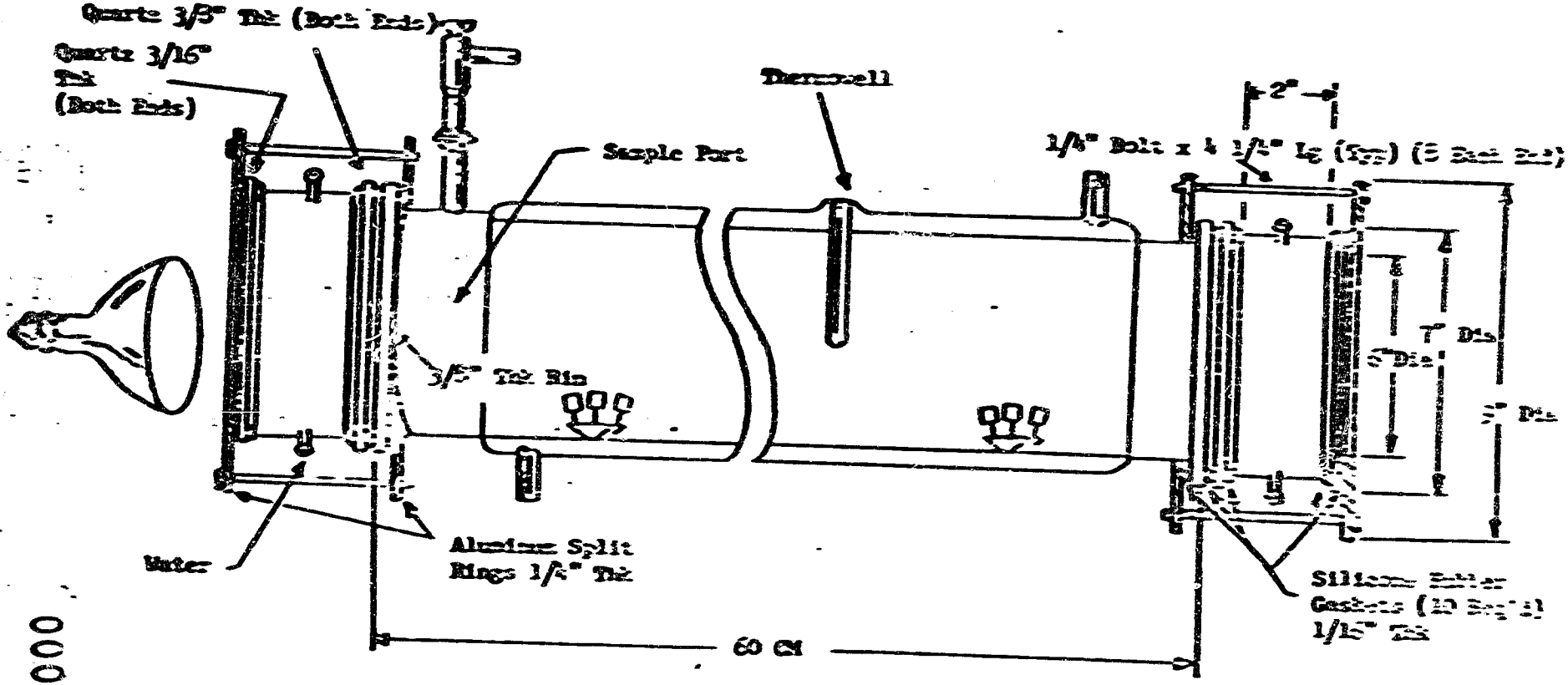
Figure 1

Photochemical Reactor Design[7]



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The precision with which oxidant could be measured in these experiments suffers from several factors. The major one is, of course, the fact that each reading comes from what is actually a separate experiment. Other factors are the difficulty of introducing nitrogen dioxide reproducibly and the large volume of air being flushed through the absorbing reagent. A rough estimate of the precision in oxidant measurement would be on the order of ± 10-15%. While this is far from being as quantitative as one might wish, the data obtained still seem to give quite adequate representation of ozone formation, particularly when one is concerned with relative characteristics of various compounds.

B. Method for Oxidant Measurement

A number of problems were encountered in devising a method to measure ozone at levels below 1 ppm in the photochamber. A method which seems to be quite satisfactory was devised, however, and it is briefly described below.

The contents of the chamber were flushed with nitrogen through a neutral-buffered 1% KI solution[12] in a gas absorbing tower of the type described by Smith.[13] The optimum conditions were a flow rate of 1 l/min with 22 ml of the absorbing reagent; smaller quantities of KI and/or higher flow rates gave consistently low readings, as determined by titration with thiosulfate. The validity of the method was tested by generating small quantities of ozone with an electric discharge in a flask, and removing two aliquots by syringe. One was injected into the photochamber and flushed through a KI solution as just described; the other was directly bubbled slowly through an equivalent volume of KI solution. Titration of each solution (several runs) indicated that over 95% of the ozone in the chamber could be accounted for.

The next step was to compare results obtained from an actual irradiation with literature values. Toluene was chosen to serve as a comparison, since it had been studied by other groups[8] who found it to lead to "moderate" quantities of ozone at a relatively rapid rate. Thus, the study of toluene can function as a benchmark for the work reported here and help to assess the validity of the overall method being used.

C. Toluene

The photolysis of toluene was carried out at 16 ppm in the presence of 4 ppm nitrogen dioxide. Figure 2 shows the results of this irradiation. It can be seen that the maximum oxidant concentration (0.8 ppm) was reached after

4 hr of irradiation. Battelle reported[2] that a maximum ozone concentration of 0.49 ppm was attained after 3.3 hr, starting with 4 ppm toluene and 8 ppm nitric oxide. In earlier work at Dow, Bradaway found the half-life for disappearance of toluene was 6.75 hr, starting with 10 ppm toluene and 3 ppm nitric oxide, whereas the data obtained in this study indicate a half-life of about 4 hr. Bearing in mind the differences in starting concentrations, light intensities, and reaction times for nitric oxide versus nitrogen dioxide, the agreement in data seems quite satisfactory.

It should be mentioned that it is not possible to assess separately the quantity of nitrogen dioxide as compared to ozone from the data obtained in this study. Although it is simple to irradiate nitrogen dioxide in the absence of organics (see Figure 3) and to follow its rapid rate of disappearance (nitrogen dioxide reacts with aqueous potassium iodide, although non-stoichiometrically), the ozone concentration cannot be obtained by substracting the nitrogen dioxide curve from the "total oxidant" curve with the difference being ozone. This is because the addition of organic can drastically alter the nitric oxide-nitrogen dioxide equilibrium. The only manner in which we were able to determine ozone by itself would be to run a separate series of experiments to determine nitrogen dioxide in the presence of organic and ozone. Because of the lack of precision and the time involved when this particular chamber is used, it is probably not worthwhile to separate "total oxidant" into its various components.

D. Methylchloroform

The photolysis of methyl chloroform was studied at two concentrations, 50 ppm and 10 ppm. The results are illustrated in Figures 5 and 6. The data at 10 ppm indicate that oxidant level (which includes a contribution from nitrogen dioxide) rapidly decreases until it approaches zero. (The lowest level that could be measured is about 0.05 ppm). The curve for the 50 ppm reaction again rapidly decreases, but asymptotically approaches 0.1 ppm, which is very low, considering the high initial concentration of methylchloroform. As before, it is not clear whether the "total oxidant" is due to ozone or to residual nitrogen dioxide. Since methylchloroform is itself unreactive, and no oxidant (or almost none) is formed from its photolysis, it can be expected that methylchloroform will be exempt from air pollution-control regulations that are concerned with the standard symptoms of photochemical smog (eye irritation, oxidant formation, reactivity).

E. Trichloroethylene

Trichloroethylene is by far the most reactive of the compounds studied for this report, and this fits in well with other studies.[1] The data (see Figure 4) for photolysis of 50 ppm trichloroethylene also indicate significant oxidant formation, again in agreement with the SRI study.[1] The rapidity with which oxidant forms is perhaps surprising. That it is real, however, is borne out by the fact that most of the points in Figure 4 were obtained in duplicate. Again, however, some of the oxidant may be due to a slower decrease in the nitrogen dioxide concentration.

F. Perchloroethylene

Perchloroethylene was found to be rather reactive from the standpoint of disappearance of organic, dropping from 50 ppm to $\leq$ 3 ppm in 4 hours. In this respect it is quite similar to trichloroethylene; however, perchloroethylene is rather dramatically different from trichloroethylene with respect to oxidant formation. A comparison of Figures 8 and 9 with Figure 3 shows that photolysis of perchloroethylene leads to a slight oxidant formation initially but that the final oxidant levels reached after 13 hours of irradiation are lower than those for the blank. This same behavior was borne out in a lower concentration experiment where total oxidant with perchloroethylene decreased from 0.66 ppm to 0.17 ppm in 4.36 hours compared to a final level of 0.52 ppm for the blank (the initial organic and NO_2 were 12.5 ppm and 2.74 ppm respectively).

G. Methylene Chloride

Methylene chloride behaved in an identical manner as methylchloroform under simulated atmospheric photolysis at both 50 and 12.5 ppm. It produced oxidant levels essentially identical with background and showed no disappearance of organic in 13 hours or irradiation. Thus methylene chloride should be given a clean bill of health by control legislation which emphasizes either chemical reactivity and/or oxidant formation in evaluating atmospheric pollutants.

H. General Discussion

The value for total oxidant is plotted as though it were entirely due to ozone, which reacts stoichiometrically and completely with neutral-buffered 1% potassium iodide.[14] At the early stages of the irradiation, however, this obviously is not true, as the major portion is due to nitrogen dioxide. Nitro-

gen dioxide reacts with potassium iodide, but at less than 1:1 stoichiometry. A generally accepted value is about 50%. Thus, if we have 5 ppm nitrogen dioxide, we could expect approximately the same value under these conditions as 1 ppm ozone. The points at the early stages of the reaction should therefore be considered as "effective oxidant concentration."

While it is not surprising that methylchloroform is unreactive under these conditions, this obviously raises some problems. What happens to methylchloroform in air? Methylchloroform absorbs in the uv at wavelengths < 200 nm and under these conditions C-Cl bond cleavage might be predicted, eventually leading to phosgene, formaldehyde and HCl, among others. It is difficult to believe, however, that a sufficient number of photons of high enough energy would be found in the lower atmosphere to cause this reaction to occur to any significant extent, so we are still faced with the same problem.

Methylene chloride presents a similar problem to that encountered with methyl chloroform; we still do not know its fate in air. Further studies are currently in progress to establish whether either material is susceptible to oxidative photolysis in the upper atmosphere where higher energy radiation is available or whether decomposition at sea level is real but simply too slow to be detected on our experimental time scale.

IV. CONCLUSIONS

A method has been developed to measure "total oxidant" formation resulting from irradiation of mixtures of organic solvents and nitrogen dioxide in a small (10 liter) photochamber. The precision of this method suffers from the size of the chamber since each point obtained requires a separate experiment.

Methylene chloride has been found to be unreactive when subjected to simulated atmosphere photolysis ($\lambda > 295$ m), with no oxidant formation observed at initial concentrations of either 50 or 12.5 ppm. Methylchloroform has been found to be quite unreactive, and no oxidant could be detected at a level of 10 ppm methylchloroform and 2.5 ppm of nitrogen dioxide. Even at 50 ppm, only a very small amount of oxidant could be detected, where the detectable limit is approximately 0.05 ppm. Perchloroethylene was found to be quite

reactive, disappearing at about the same rate as trichloroethylene, but producing only a small amount of oxidant. In confirmation of earlier work, both trichloroethylene and toluene were found to be moderately reactive and to lead to significant oxidant formation. It can be reasonably assumed that methylchloroform and methylene chloride will be exempt from future pollution-control restrictions based on photochemical smog symptoms. ~~Is it in likely that perchloroethylene will also be exempt if oxidant formation is a primary criterion.~~

V. LIST OF FIGURES

Figure 1	Photochemical Chamber Design
Figure 2	Photooxidation of Toluene with Nitrogen Dioxide
Figure 3	Irration of Nitrogen Dioxide
Figure 4	Photooxidation of Trichloroethylene with Nitrogen Dioxide
Figure 5	Photooxidation of Methylchloroform (50 ppm) with Nitrogen Dioxide
Figure 6	Photooxidation of Methylchloroform (10 ppm) with Nitrogen Dioxide
Figure 7	Photooxidation of Methylene Chloride with Nitrogen Dioxide
Figures 8,9	Photooxidation of Perchloroethylene with Nitrogen Dioxide

VI. REFERENCES

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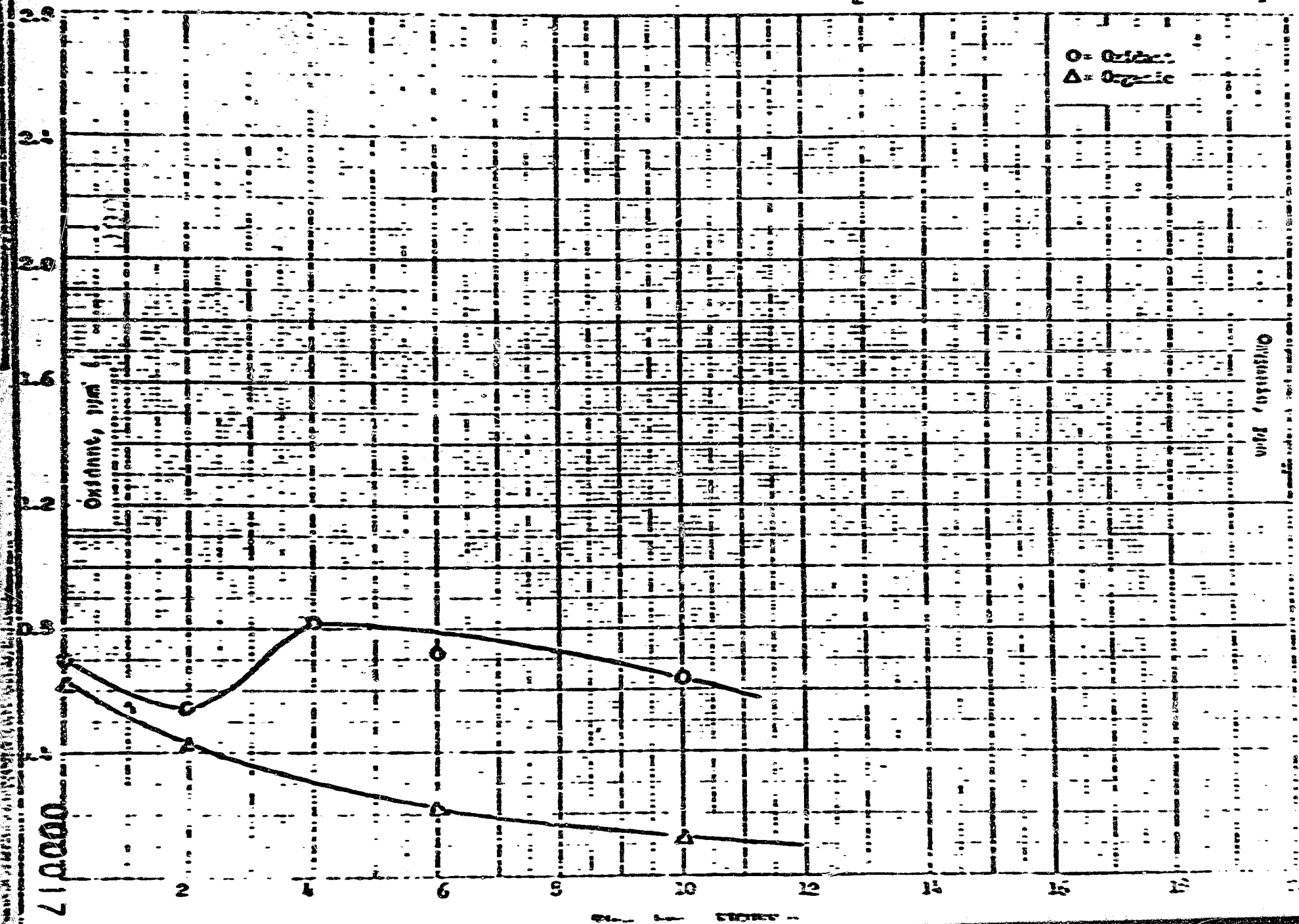
Photooxidation of Toluene (16 ppm) with NO_2 (4 ppm)



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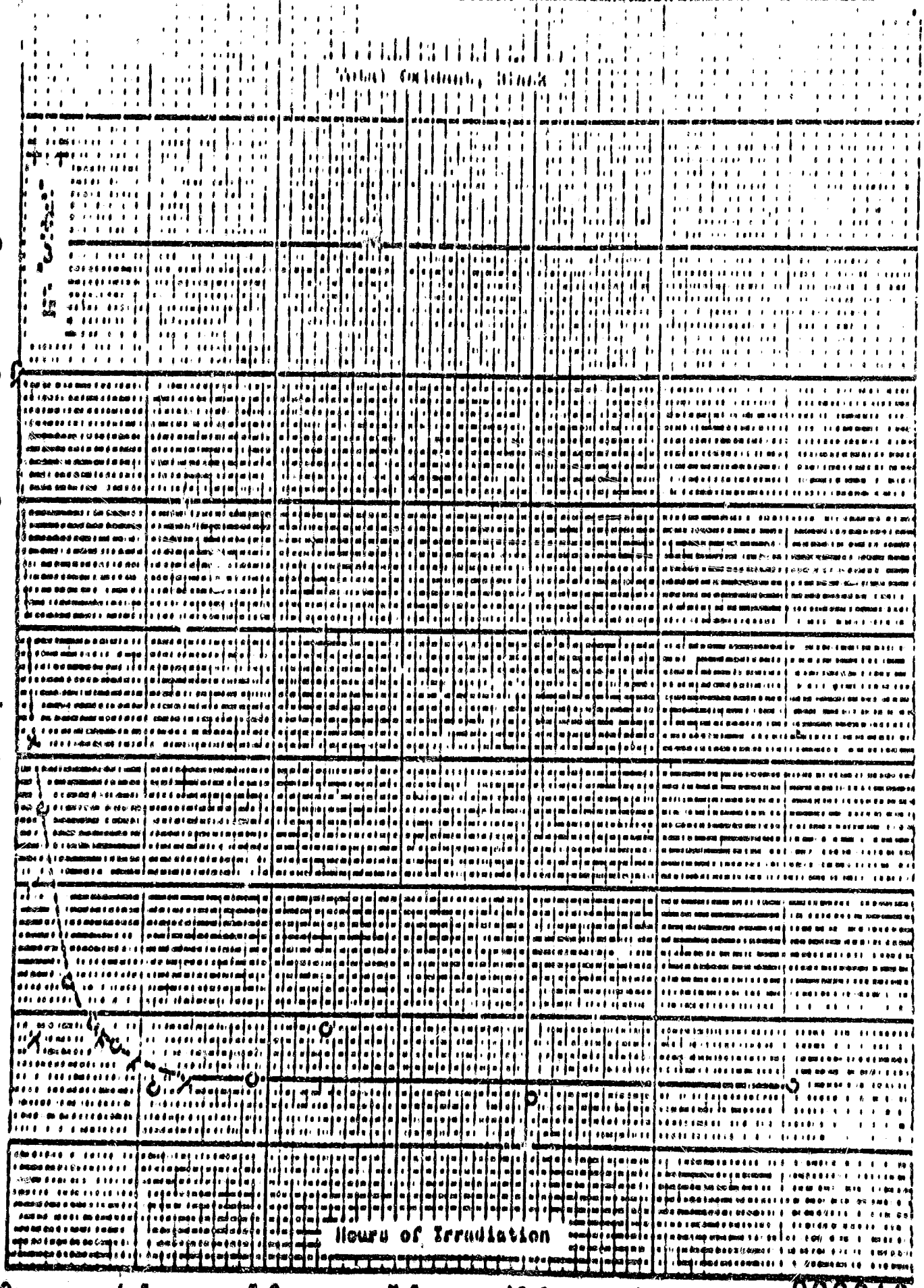

Figure 5

Photooxidation of Trichloroethylene (50 ppm) with NO_2 (12 ppm)

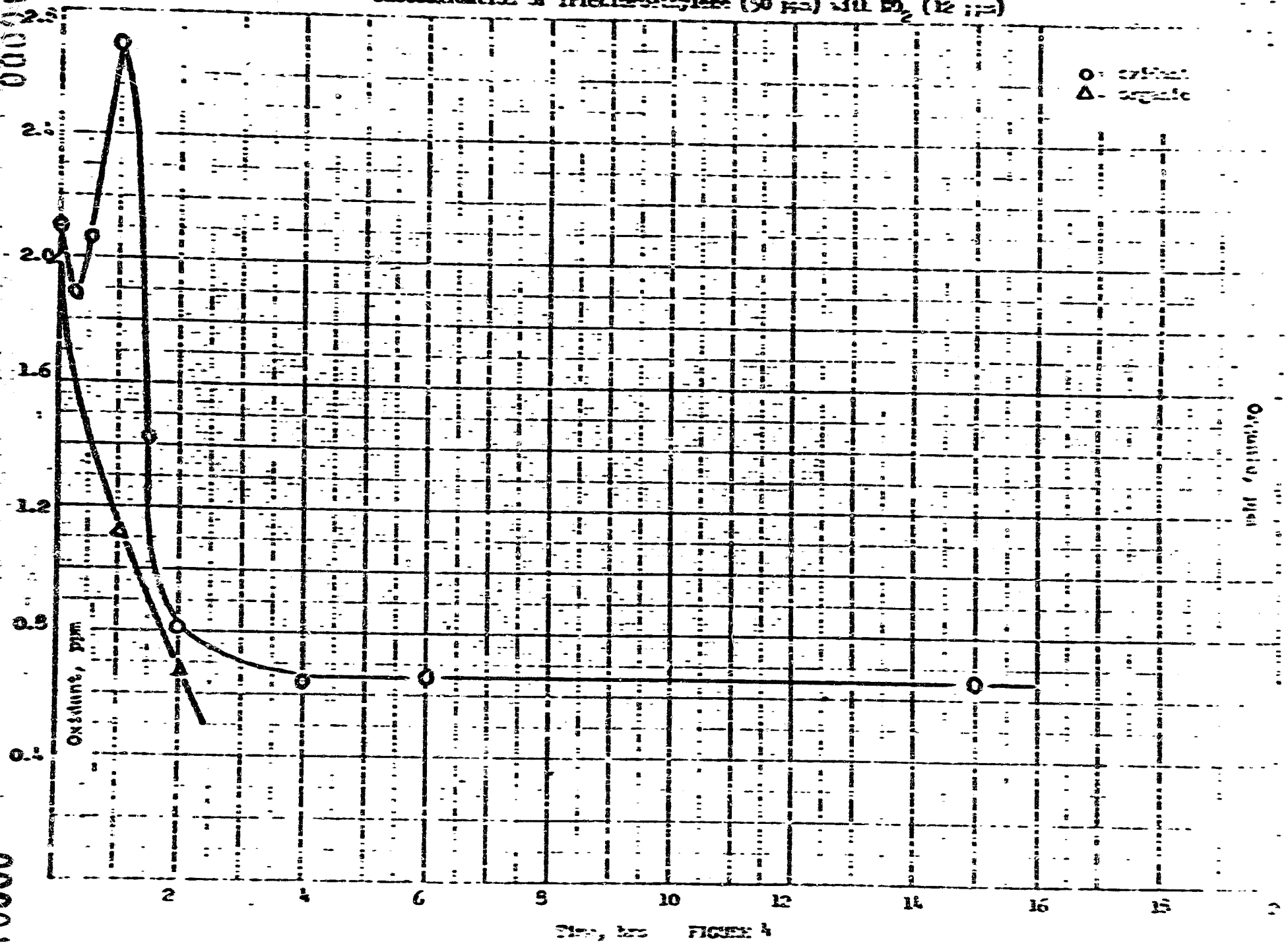

FIGURE 4

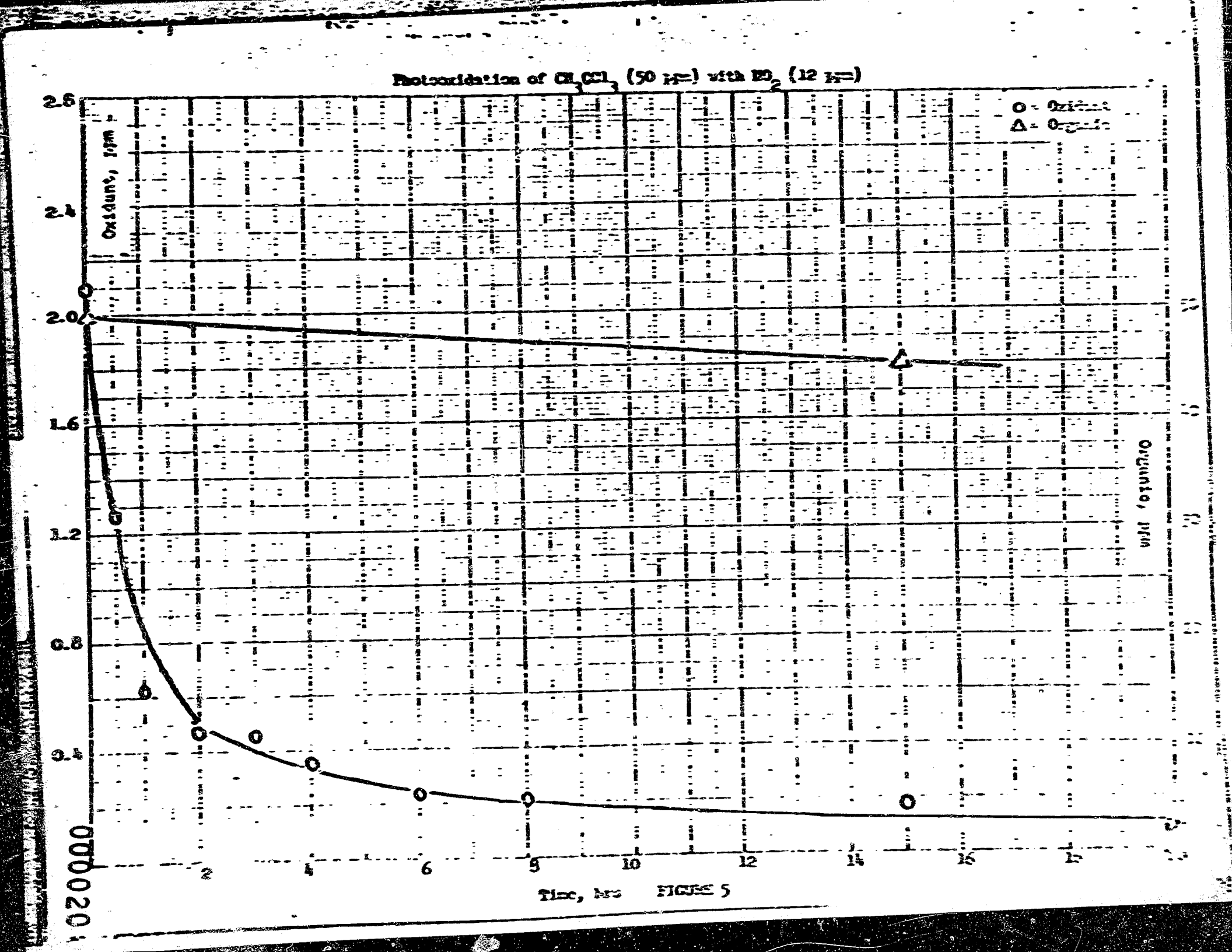
Photooxidation of CH_3CCl_3 (50 ppm) with NO_2 (12 ppm)
O - Oxidant
Δ - Organic
Oxidant, ppm
Organic, ppm
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
2
4
6
8
10
12
14
16
Time, hrs

FIGURE 5

Photoxidation of CH_3CCl_3 (10 ppm) with NO_2 (2.5 ppm)

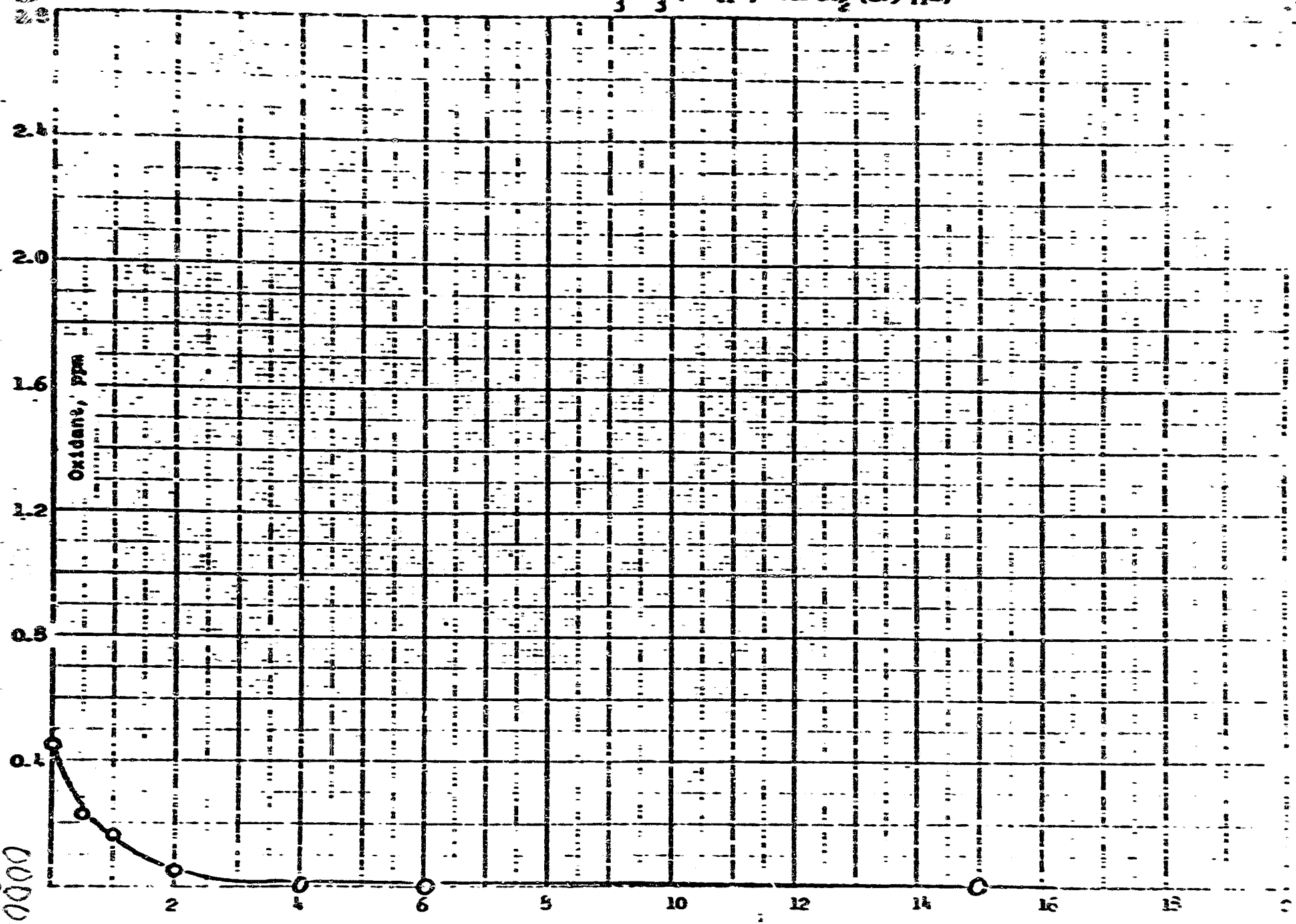

FIGURE 6

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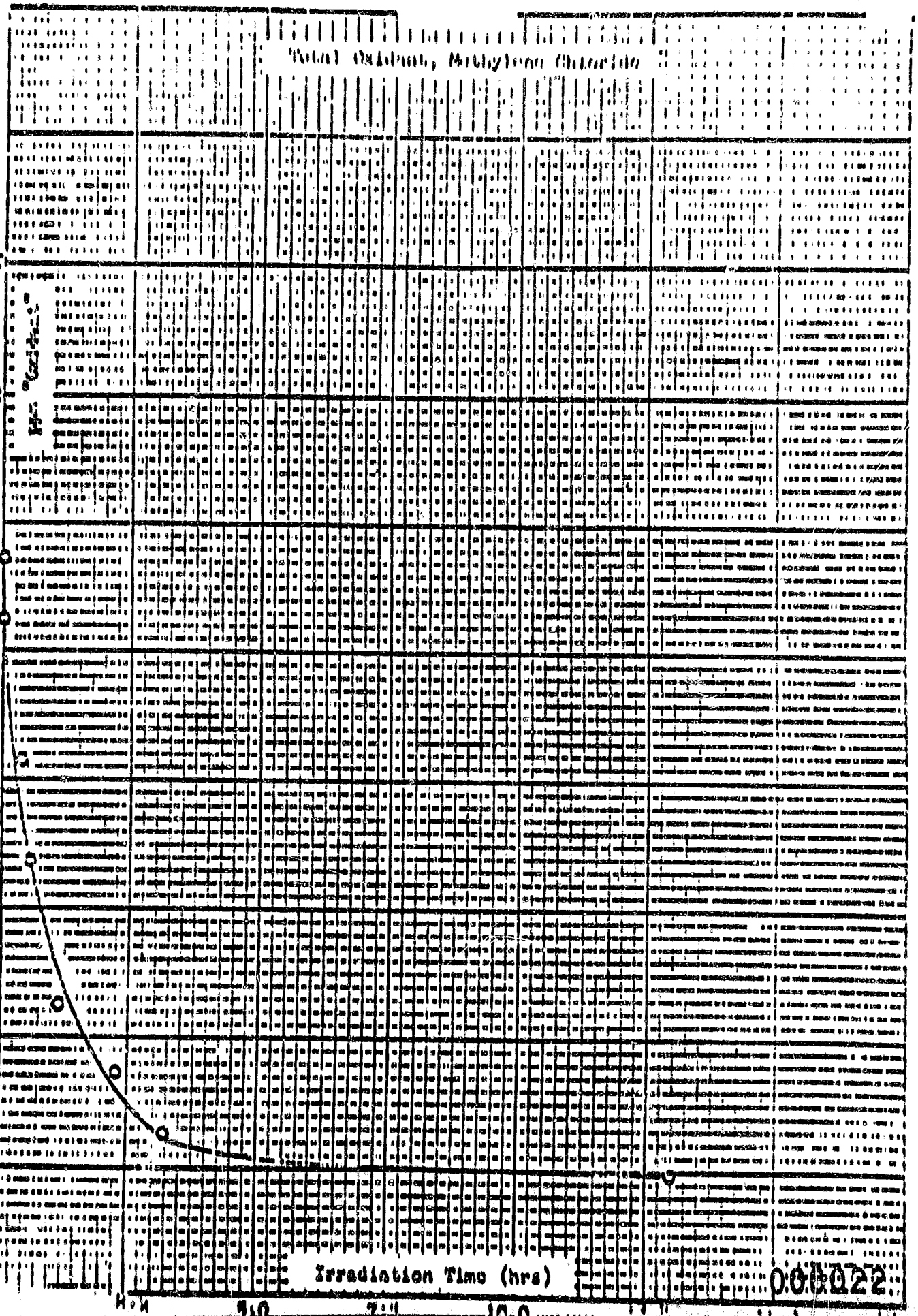
Total Oxidants, Acetylene Chloride
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3.0
2.5
2.0
1.5
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Figure 7

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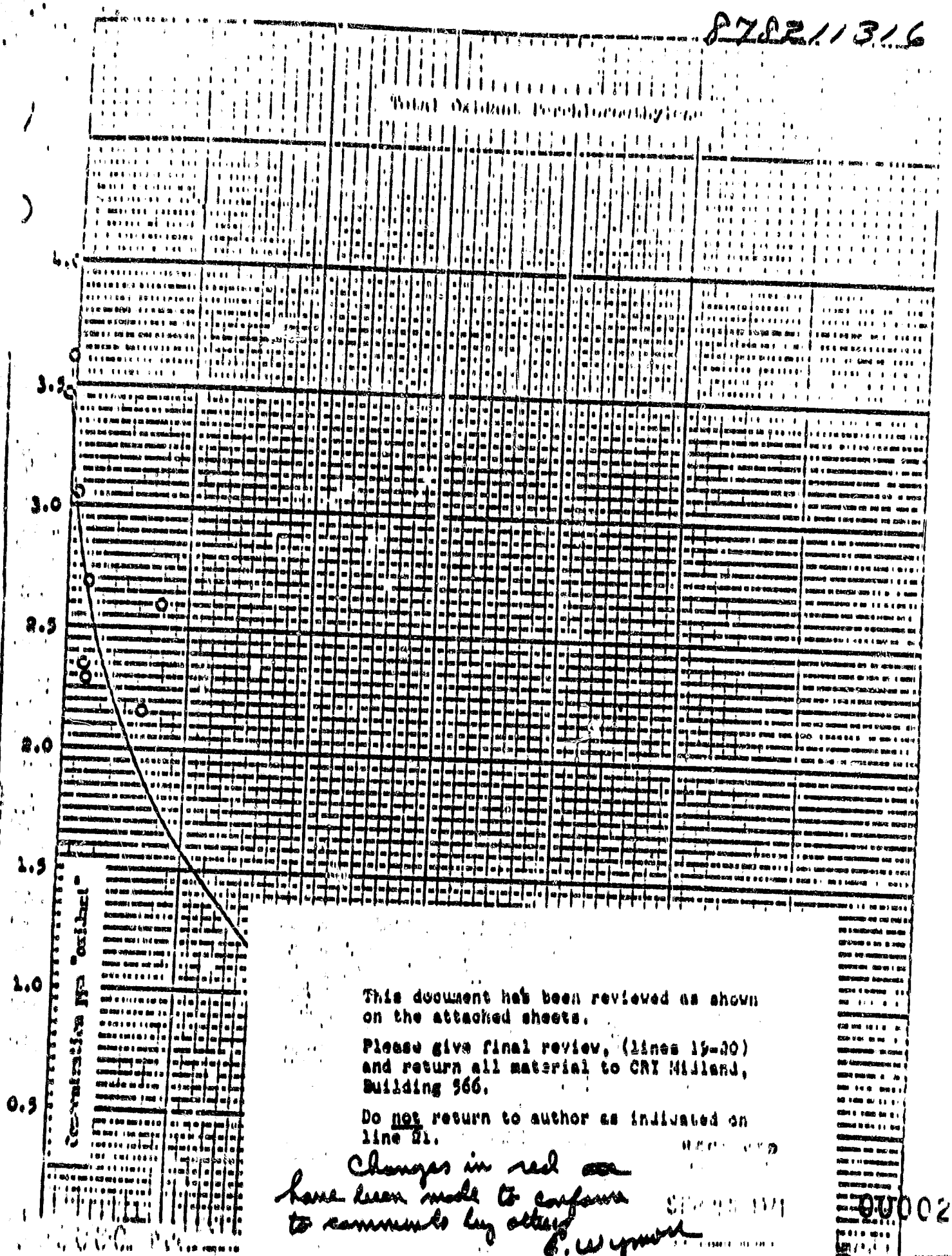

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Contractor 21

Doc Type 22

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Doc Title 23

ENVIRONMENTAL FATE AND EFFECT OF CHLORINATED SOLVENTS

Chemical Name (300 per name)	25	CAS No. (10)	26
METHYLENE CHLORIDE		75-09-2	

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D-568

8782113171-I

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ENVIRONMENTAL FATE AND EFFECT OF CHLORINATED SOLVENTS

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The Dow Chemical Company, Midland, Michigan 48640

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A seminar on the environmental fate and effect of chlorinated solvents will be based on the slides shown on the following pages. The verbal presentation will be based on the four papers which are reproduced on the pages following the slides.

SLIDES

US Production of Chlorinated Solvents in 1972

CH_2Cl_2	471,000,000 lbs.
$CHCl_3$	235,000,000
CH_3CCl_3	441,000,000
$CHCl=CCl_2$	440,000,000
$CCl_2=CCl_2$	735,000,000

US Tariff Commission Report, March 1973.

Distribution of Chlorinated Solvents in the Environment

Atmosphere

Water

Soil

$CHCl_3$, CCl_4, $CFCl_3$, $CHCl=CCl_2$, $CCl_2=CCl_2$

Found in the atmosphere and natural water bodies

[0.03 - 70 ppt (10^{12})]

Murray and Riley, 1973; Lovelock, Maggs, and Wade, 1973; Kleopfer and Fairless, 1972.

Compound	Solubility in water (25°C)	Vapor pressure (25°C)	Partition coefficient $K = C_a/C_w$ Calcd	Found
CH_2Cl_2	19,800 ppm	426 mm	0.10	0.11
$CHCl_3$	7,950	200	0.16	0.13
CH_3CCl_3	1,300	123	0.68	
$CHCl=CCl_2$	1,100	74	0.48	
$CCl_2=CCl_2$	400	19	0.41	0.50

Brown, 1973.

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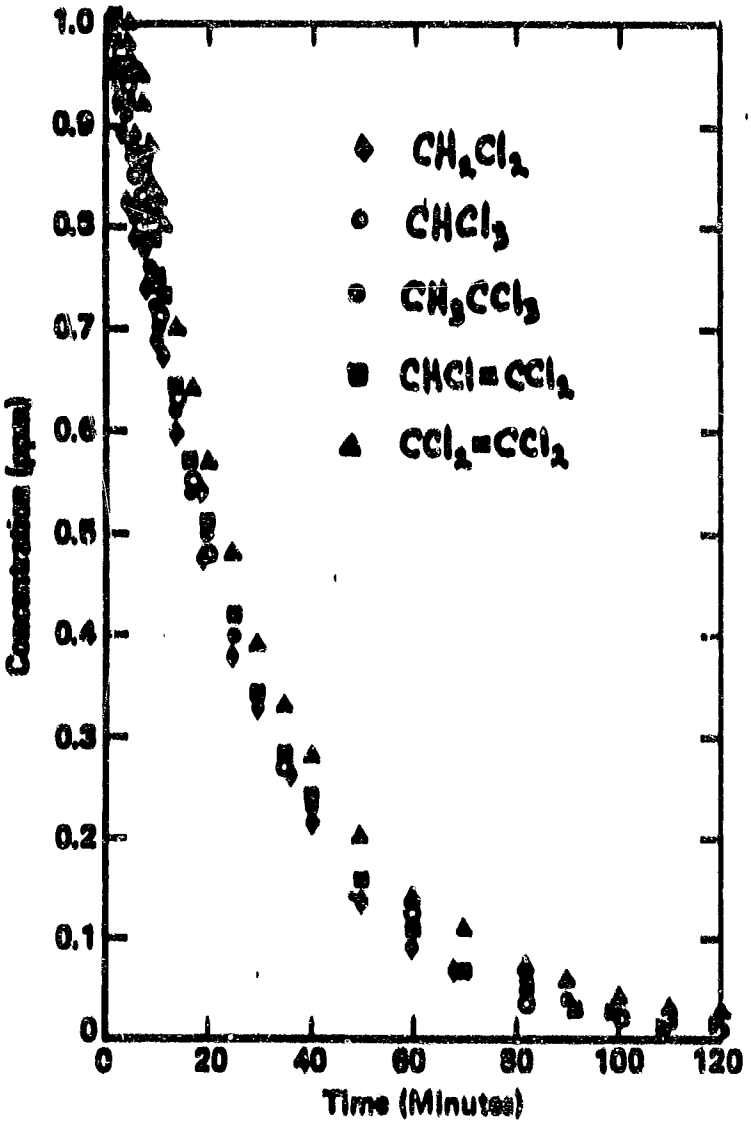

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Concentration (ppm)
0
20
40
60
80
100
120
Time (Minutes)
CH_2Cl_2
$CHCl_3$
CH_3CCl_3
$CHCl=CCl_2$
$CCl_2=CCl_2$

$$NO_2 \xrightarrow{h\nu} NO + O$$

$$O + O_2 + M \longrightarrow O_3 + M$$

$$O + NO + M \longrightarrow NO_2 + M$$

$$O + NO_2 \longrightarrow O_2 + NO$$

$$O_3 + NO \longrightarrow O_2 + NO_2$$

$$O_3 \xrightarrow{h\nu} O_2 + O$$

$$2\,NO + O_2 \longrightarrow 2\,NO_2$$

$$NO_2 + O_2 \underset{}{\overset{h\nu}{\rightleftharpoons}} NO + O_3$$

Addition of organic compounds –

1) Disrupts NO, NO_2, O_2, O_3 equilibrium.

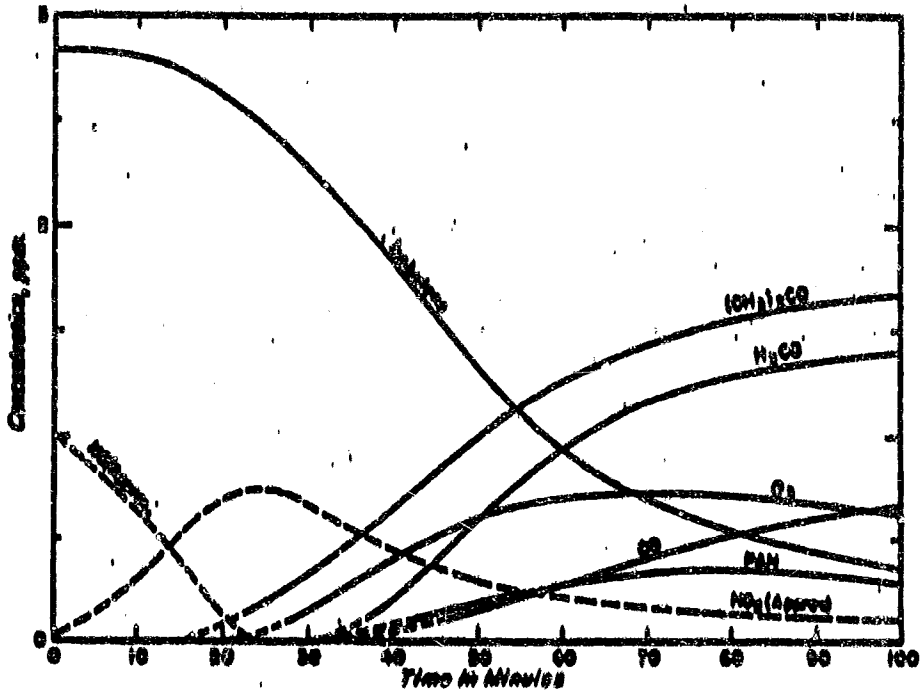
a) NO concentration decreases.

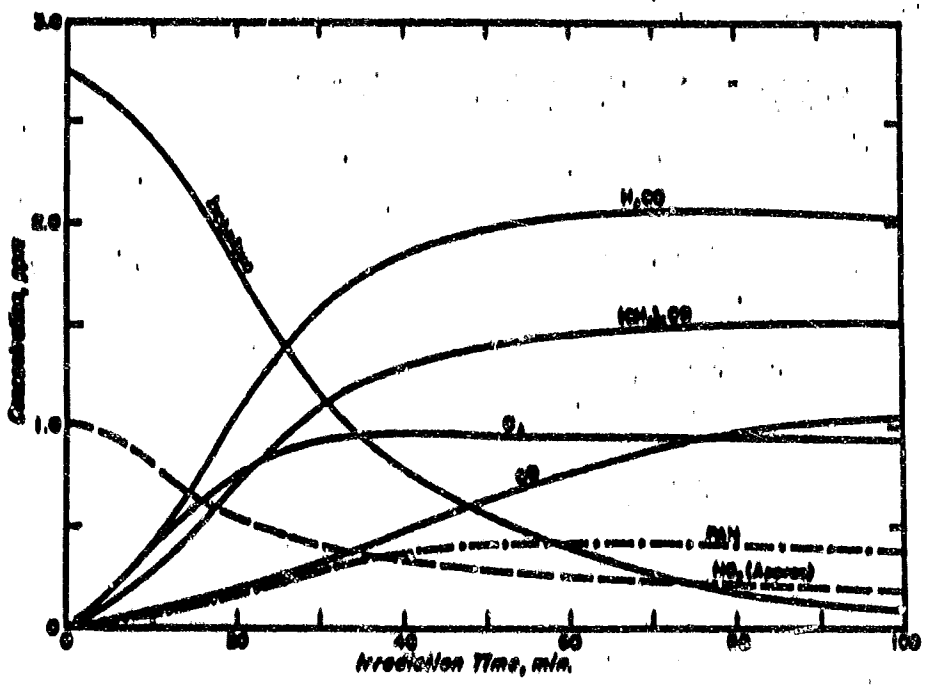
b) NO_2 concentration increases and then decreases.

c) O_3 concentration increases after most of NO has disappeared.

2) Organic compounds disappear.

3) Reaction products are formed [$RCHO$, R_2CO, $RONO$, RCO_3NO_2 (PAN), CO, etc.].





Leighton, 1961.

$$H_2O + NO_2 + NO \longrightarrow 2\ HONO$$

$$HONO \xrightarrow{h\nu} HO\cdot + NO$$

$$RCH{=}CHR + \cdot OH \longrightarrow R\overset{OH}{\overset{|}{C}}H{-}\dot{C}HR$$

$$R\overset{OH}{\overset{|}{C}}H{-}\dot{C}HR + O_2 \longrightarrow R\overset{OH}{\overset{|}{C}}H{-}\overset{OO\cdot}{\overset{|}{C}}HR$$

$$R\overset{OH}{\overset{|}{C}}H{-}\overset{OO\cdot}{\overset{|}{C}}HR + NO \longrightarrow R\overset{OH}{\overset{|}{C}}H{-}\overset{O\cdot}{\overset{|}{C}}HR + NO_2$$

$$R\overset{OH}{\overset{|}{C}}H{-}\overset{O\cdot}{\overset{|}{C}}HR \longrightarrow R\dot{C}HOH + RCHO$$

Kerr, Calvert, Demerjian, 1972; Altshuller, Bufalini, 1965, 1971.

$$RCHO + \cdot OH \longrightarrow RCO\cdot + H_2O$$

$$RCO\cdot + O_2 \longrightarrow RCO_3\cdot$$

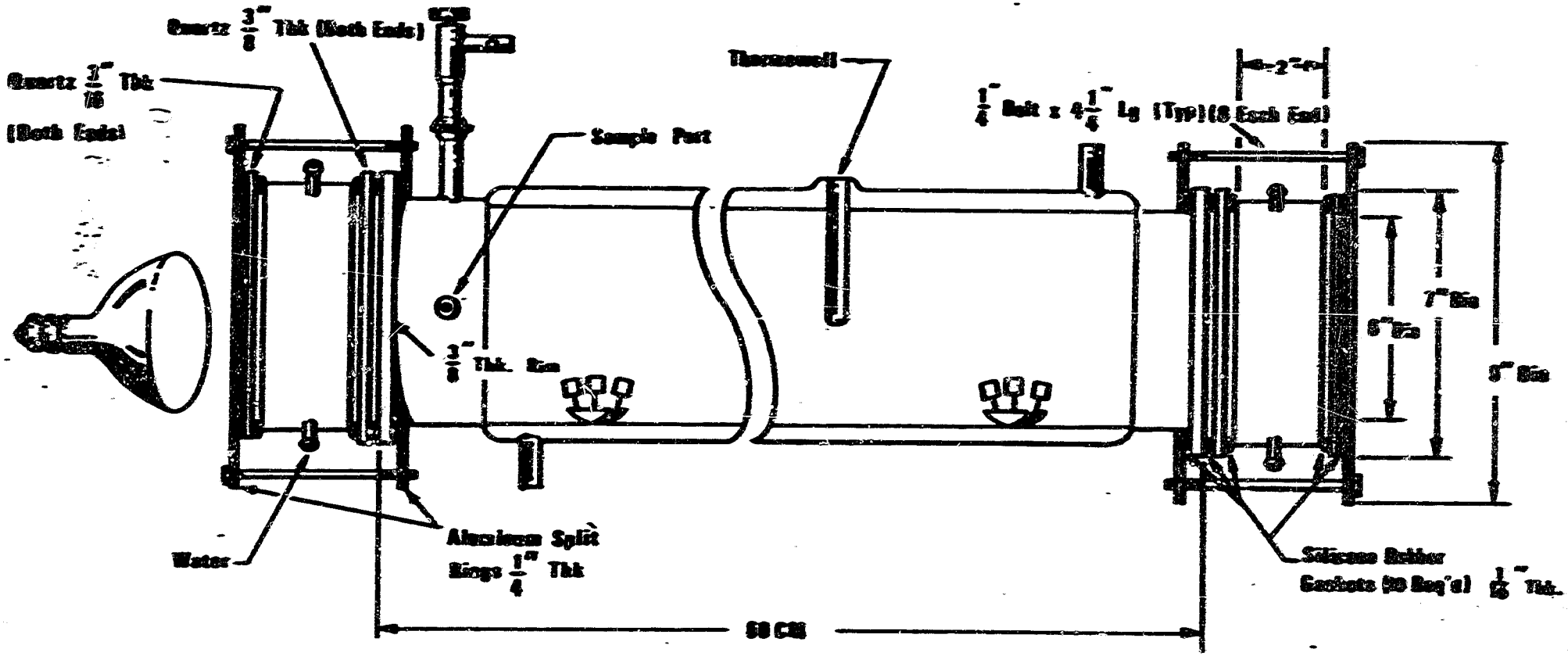
$$RCO_3\cdot + NO_2 \longrightarrow RCO_3NO_2 \text{ (PAN)}$$

$$RH + \cdot OH \longrightarrow R\cdot + H_2O$$

$$R\cdot + O_2 \longrightarrow RO_2\cdot$$

$$RO_2\cdot + NO \longrightarrow RO\cdot + NO_2$$

$$RO_2\cdot + O_2 \longrightarrow RO\cdot + O_3$$


Quartz 3/8" Thk (Both Ends)
Quartz 1/16" Thk (Both Ends)
Thermowell
1/4" Bolt x 4 1/4" Lg (Typ) (8 Each End)
2"
Sample Port
3/8" Thk. Min
7" Min
5" Min
Water
Aluminum Split Rings 1/4" Thk
Silicone Rubber Gaskets (O Ring) 1/16" Thk.
60 CM

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Usual Reaction Conditions

10 ppm organic compound

5 ppm NO (or NO_2)

35% relative humidity

UV light intensity ~2.5 x bright sunlight
(medium pressure Hg arc, $\lambda > 290$ nm)

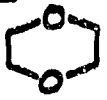
26-28° C

Absolute and Relative Photoreactivities of Standard Compounds Determined at Different Laborato

Compound	Stanford Research Institute	General Motors Corporation	Battelle Memorial Institute	National Air Pollution Control Administration	This work
	50% (18 hr.) 0.42	18% (6 hr.) 0.40	~42% (5 hr.) ~0.5	—	50% (6.8 hr.) 0.43
PhMe	—	39% (6 hr.) 0.87	~50% (5 hr.) ~0.6	—	50% (6.8 hr.) 0.43
$CH_2=CH_2$	50% (7.5 hr.) 1.0	45% (6 hr.) 1.0	~50% (3 hr.) 1.0	50% (3.7 hr.) 1.0	50% (2.9 hr.) 1.0
$CHCl=CCl_2$	50% (7.0 hr.) 1.1	—	—	50% (2.6 hr.) 1.4	50% (3.5 hr.) 0.83
	50% (0.44 hr.) 17	85% (6 hr.)	~50% (0.2 hr.) ~15	—	50% (0.23 hr.) 13

Effect of Light Intensity

Compound	Relative light intensity	$t_{1/2}$	Relative reactivity
	1.00	11 minutes	1.00
	0.50	23	0.48
	0.25	40	0.26
$CH_2=CH_2$	1.00	2.8 hr.	1.00
	0.50	5.2	0.52
	0.25	10.0	0.28
$CHCl=CCl_2$	1.00	3.5 hr.	1.00
	0.50	6.3	0.56
	0.25	9.5	0.37

Compound	$t_{1/2}$ With NO	$t_{1/2}$ With NO_2
CH_3CCl_3	23.5 hr. (<5%)	28 days (<5%)
CH_2Cl_2	21.0 (<5%)	6 (<5%)
t-BuOH	34.5	
	16.0	
$CH_2ClCHCl_2$	15.9	
	15.9	15.2 hr.
EtOAc	14.6	
$CCl_2=CCl_2$	14.2	8.3
MeCOEt	9.8	
$MeNO_2$	9.2	
PhCl	8.7	
	6.9	6.5
PhMe	6.8	
n-BuOH	6.5	
PhEt	5.0	
	4.7	
$CH_2=CHCl$	4.3	
s-BuOH	4.0	
$CHCl=CCl_2$	3.5	2.9
MeCO t-Bu	3.5	
t-BuOH	3.5	
	3.4	
$MeOCH_2CHOHMe$	3.1	
	3.1	
	2.9	
	3.0	3.0
	2.9	2.8

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$CH_2=CH_2$	2.9	2.5
$CH_2=CCl_2$	2.1	
	0.87	0.19
t-Bu $CH_2C(Me)=CH_2$	0.60	
	0.30	0.17
	0.16	

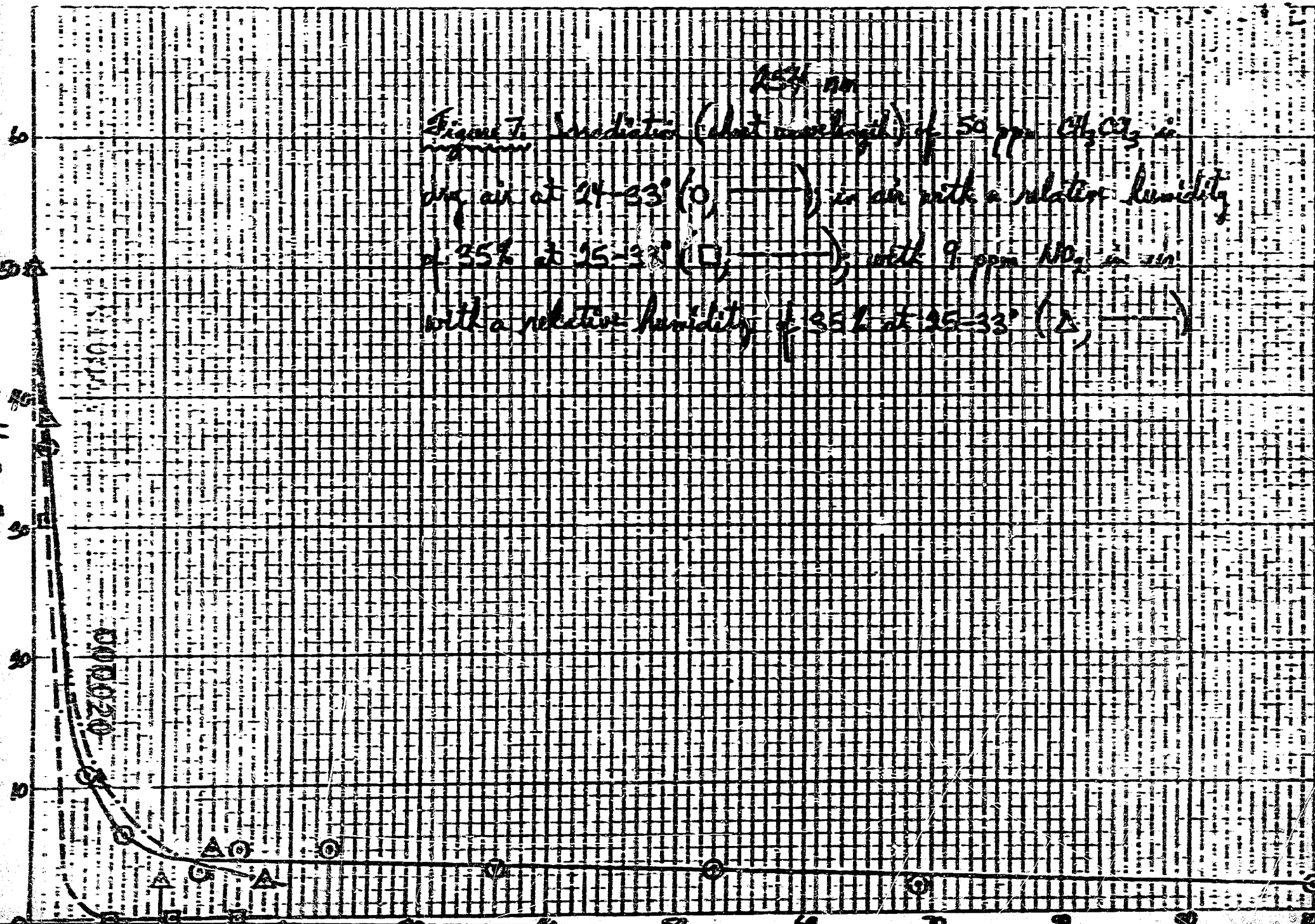


Figure 7. Irradiation (short wavelength) of 50 ppm CH_3CO_3 in dry air at 24-33° (○, ——); in air with a relative humidity of 35% at 25-33° (□, ——); with 9 ppm NO_2 in air with a relative humidity of 35% at 25-33° (△, ——)

Effect of Concentration

Compound	Initial concentrations (ppm) Compound	NO	$t_{1/2}$	Relative reactivity
	10	5	26 minutes	1.0
	20	10	23	1.2
	40	20	17	1.5
	100	50	13	2.0
$CHCl=CCl_2$	10	5	3.5 hr.	1.0
	20	10	3.0	1.2
	40	20	1.5	2.3
	100	50	1.0	3.5
$CCl_2=CCl_2$	10	5	11.2	1.0
	40	20	7.0	1.6
	100	50	3.8	3.0

Effect of NO Concentration

$CHCl=CCl_2$ - 10 ppm

NO Concentration	$t_{\frac{1}{2}}$
0 ppm	10.9 hr.
2.5	1.8
5	2.0
10	2.5
20	2.5
40	2.5

Effect of Relative Humidity

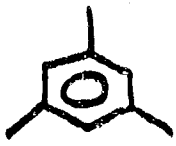
$CHCl=CCl_2$ - 10 ppm

Relative Humidity	NO Concentration	$t_{\frac{1}{2}}$
0 %	5 ppm	2.0 hr
35	5	2.1
70	5	3.0
0	0	5.0
35	0	10.9

Competition Reactions

Relative reactivity with NO_2:

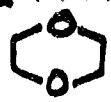
reactivity increased in the presence of

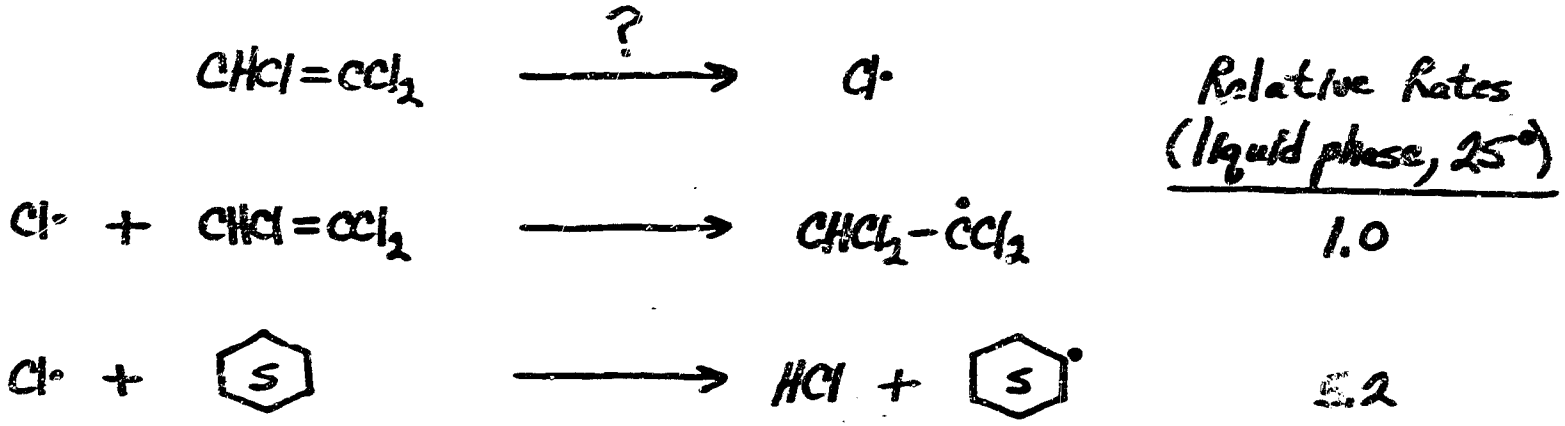
reactivity decreased in the presence of 

"Leveling Effect"

Butalin and Altshuller, 1967.

Photoreactivity of $CHCl=CCl_2$ in Competition Reactions

Compound	$t_{1/2}$ $CHCl=CCl_2$	$t_{1/2}$ Compound Alone	$t_{1/2}$ Compound in Mixture
—	3.5 hr	—	—
CH_3CCl_3	3.6	$\gg$ 24 hr.	$\gg$ 24 hr.
	7.0	15.9	6.4
EtOAc	6.1	14.6	12.5
$CCl_2=CCl_2$	2.8	14.2	5.0
MeCOEt	5.8	9.8	7.4
	8.7	7.6	4.9
PhMe	7.6	6.8	5.2
n-BuOH	8.5	6.5	3.0
t-BuOH	7.8	3.5	3.3
	6.0	3.4	2.4
$MeOCH_2CHOHMe$	10.5	3.1	2.5
$CH_2=CH_2$	4.8	2.9	2.5
	6.1	0.87	0.82
	8.3	0.30	0.16



Poutsma and Hinman, 1964.

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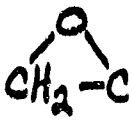
Photoreactivity of (S) in Competition Reactions

Compound	$t_{1/2}$ (S)	$t_{1/2}$ Compound Alone	$t_{1/2}$ Compound in Mixture
—	6.9 hr	—	—
CH_3CCl_3	9.0	$\gg$ 24 hr	$\gg$ 24 hr
CH_2Cl_2	6.8	$\gg$ 24	$\gg$ 24
$CH_2=CHCl$	5.4	4.3	5.5
$CHCl=CCl_2$	4.9	3.5	8.7
$MeCO_2$-Bu	8.3	3.5	3.9
	5.4	3.0	13.1
$CH_2=CH_2$	6.1	2.9	4.1
$CH_2=CCl_2$	5.0	2.1	3.8

Photoreactivity of $CH_2=CH_2$ in Competition Reactions

Compound	$t_{\frac{1}{2}}$ $CH_2=CH_2$	$t_{\frac{1}{2}}$ Compound Alone	$t_{\frac{1}{2}}$ Compound in Mixture
———	2.9 hr.	———	———
CH_3CCl_3	2.9	$\gg$ 24 hr.	$\gg$ 24 hr.
	4.0	15.9	12.4
	4.1	6.9	6.1
n-BuOH	3.7	6.5	3.8
$CHCl=CCl_2$	2.6	3.5	4.8
	4.1	0.87	0.73
	3.2	0.30	0.37

Photoreactivity of in Competition Reactions

Compound	$t_{1/2}$	$t_{1/2}$ Compound Alone	$t_{1/2}$ Compound in Mixture
—	0.30 hr.	—	—
CH_3CCl_3	0.24	$\gg$ 24 hr.	> 24 hr.
CH_2Cl_2	0.26	$\gg$ 24	> 24
n-BuOH	0.32	6.5	4.3
$CHCl=CCl_2$	0.16	3.5	8.3
$CH_2=CH_2$	0.37	2.9	3.2
	0.37	0.87	0.37

Photoreactivities in a Multicomponent System

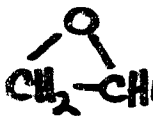
Compound	$t_{1/2}$ In Mixture	$t_{1/2}$ Alone
	12.9 hr.	15.0 hr.
$CHCl=CCl_2$	11.1	3.5
$CH_2=CH_2$	3.5	2.9
	0.35	0.87
	0.35	0.30

Effect of $CHCl=CCl_2$: (S) Ratio

Concentrations		Ratio	NO	$t_{1/2}$	
$CHCl=CCl_2$	(S)	$\frac{[CHCl=CCl_2]}{[S]}$	Concentration	$CHCl=CCl_2$	(S)
10 ppm	0 ppm	∞	5 ppm	3.5 hr	—
10	0.01	1000	5	3.3	—
10	0.1	100	5	2.7	~0.9 hr
10	0.2	50	5	3.5	1.3
10	2	5	5	5.6	2.2
10	10	1	5	>16	5.5
2	10	0.2	5	>20	6.7
0	10	0	5	—	6.9
10	2	5	10	6.6	2.8
10	10	1	10	8.7	4.9

Effect of $CHCl=CCl_2$: CH_2-CHEt Ratio

Concentrations		Ratio	NO	$t_{1/2}$	
$CHCl=CCl_2$	CH_2-CHEt	$\frac{[CHCl=CCl_2]}{[CH_2-CHEt]}$	Concentration	$CHCl=CCl_2$	CH_2-CHE
10 ppm	0 ppm	∞	5 ppm	3.5	—
10	2	5	5	4.2	4.1
10	10	1	10	7.0	6.4
0	10	0	5	—	15.9

Compound	$t_{1/2}$ NO (5 ppm)	$t_{1/2}$ NO_2 (5 ppm)	$t_{1/2}$ O_3 (15 ppm)	Relative Reactivity NO	Relative Reactivity NO_2	Relative Reactivity O_3
	15.9 hr.	15.2 hr.	0.83 hr.	0.23	0.19	0.51
	6.9	7.5	1.0	0.51	0.38	0.42
$CHCl=CCl_2$	3.5	2.9	0.42	1.0	1.0	1.0
$CH_2=CH_2$	2.9	2.5	—	1.2	1.2	—
	0.87	0.19	—	4.0	5.1	—
	0.30	0.17	—	11.7	6.9	—

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Competition Experiments with NO, NO_2, O_3

Compound Pair (10 ppm each)	$t_{1/2}$ NO (10 ppm)	$t_{1/2}$ NO_2 (10 ppm)	$t_{1/2}$ O_3 (10 ppm)	Reactivity Ratios NO	Reactivity Ratios NO_2	Reactivity Ratios O_3
	4.9 hr.	5.0 hr.	0.73 hr.	1.8	2.1	1.2
$CHCl=CCl_2$	8.7	10.5	0.87			
	6.4	8.0	0.5	1.1	1.3	1.0
$CHCl=CCl_2$	7.0	10.5	0.5			
	0.37	0.13	—	1.0	1.1	—
	0.37	0.15	—			

Possible Oxidation Products and Reactive Intermediates from $CHCl=CCl_2$

$$\left.\begin{array}{l} COCl_2 \\ CHCl_2COCl \\ CCl_3CHO \\ Cl_2 \end{array}\right\} \longrightarrow Cl\cdot,\ \cdot CCl_3,\ R\dot{C}O$$

$$CBrCl_3 \longrightarrow \cdot CCl_3$$

$$MeCHO \longrightarrow Me\dot{C}O$$

Photoreactivity of $CHCl=CCl_2$ with Possible Oxidation Products and Other Co-reactants

Co-reactant	$t_{\frac{1}{2}}$
NO (5 ppm)	3.5 hr
CCl_3CHO (2)	3.2
CCl_3CHO (5)	0.93
CCl_3CHO (10)	0.45
MeCHO (10)	15.7
MeCHO (10) + NO (5)	10.7
$CBrCl_3$ (10)	5.0
$CBrCl_3$ (10) + NO (10)	1.3
Cl_2 (1)	3.1
Cl_2 (1) + NO (5)	0.43
Cl_2 (2)	0.18
Cl_2 (5)	< 0.05
Cl_2 (10)	< 0.05
$COCl_2$ (5)	5.8
$COCl_2$ (5) + NO (5)	2.3

Photoreactivity of CH_2-$CHEt$ with Possible $CHCl=CCl_2$ Oxidation Products

Co-reactant	$t_{\frac{1}{2}}$
NO (5 ppm)	15.9 hr.
NO_2 (5)	15.2
$CHCl=CCl_2$ (10) + NO (10)	6.4
CCl_3CHO (10)	3.3
CCl_3CHO (10) + NO (10)	3.1
CCl_3CHO (10) + NO_2 (10)	3.1
$CHCl_2COCl$ (10) + NO (10)	9.1

Compound	$t_{1/2}$ NO (5 ppm)	MeCHO (10 ppm)	MeCHO + NO (10 ppm) (5 ppm	CCl_3CHO (10 ppm)
	15.9 hr.	—	—	3.3 hr.
	6.9	33 hr.	22 hr.	3.4
$CHCl=CCl_2$	3.5	15.6	15.3	0.45
$CH_2=CH_2$	2.9	—	10.6	3.1
	0.87	12.2	0.50	1.8
	0.30	6.3	0.22	2.3

Competition Experiments with Cl_2 (5 ppm)

Compound Pair (10 ppm each)	$t_{\frac{1}{2}}$
$CHCl=CCl_2$	<0.12 hr. (100%)
$CCl_2=CCl_2$	<0.12 (100%)
$CH_2=CH_2$	0.37
$CCl_2=CCl_2$	1.7
$CH_2=CH_2$	0.57
$CHCl=CCl_2$	0.58

Relative Rates for $R_2C=CR_2 + Cl_2 \xrightarrow[27°C]{\text{Gas Phase}} R_2CClCClR_2$

$CH_2=CH_2$	$CHCl=CCl_2$	$CCl_2=CCl_2$
20,000	4	1

P. Goldfinger, et. al., 1963.

Other Possible Reactive Intermediates

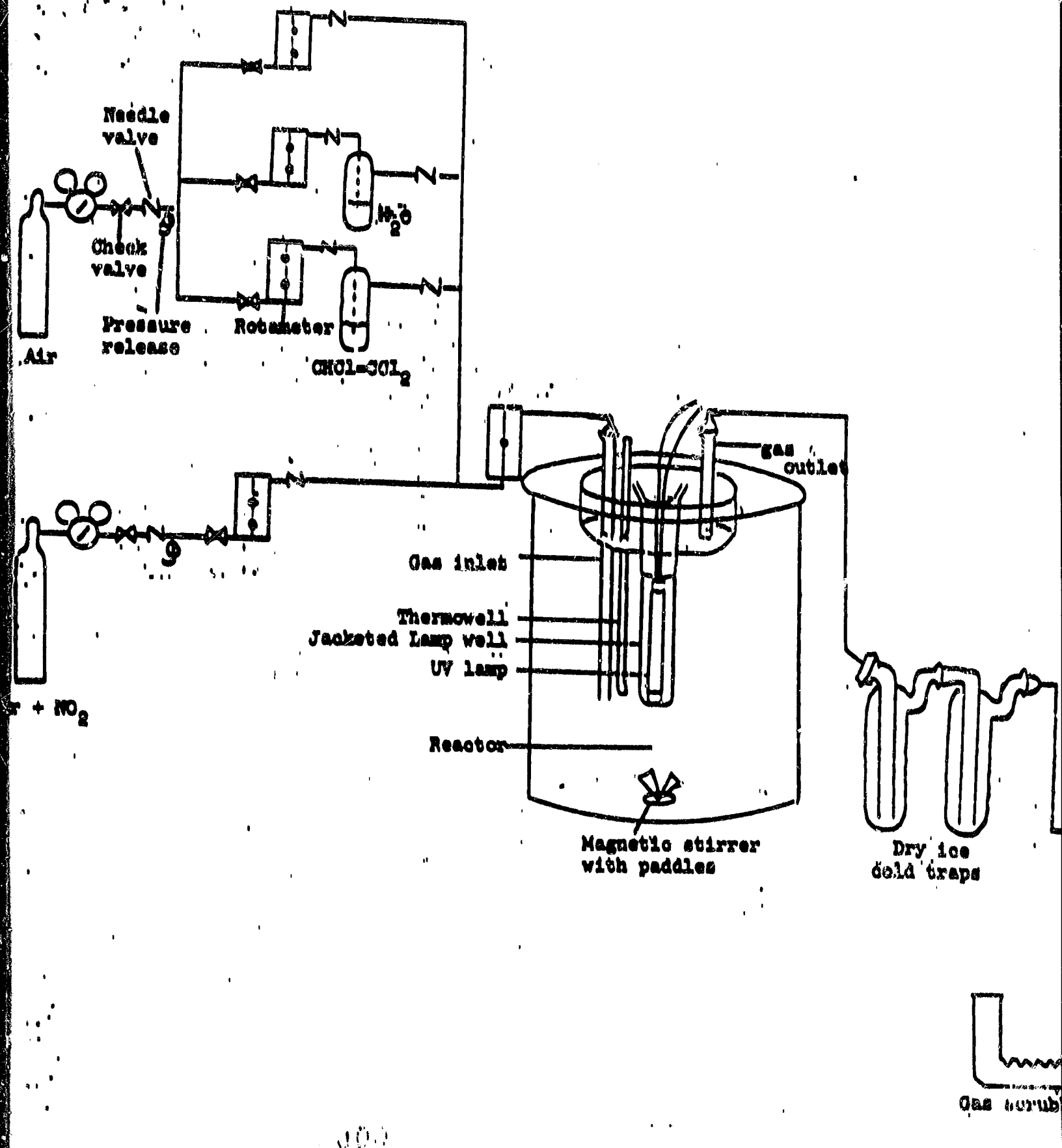
O_2 ($^1\Delta$ and $^1\Sigma$)

HO·

ClO·

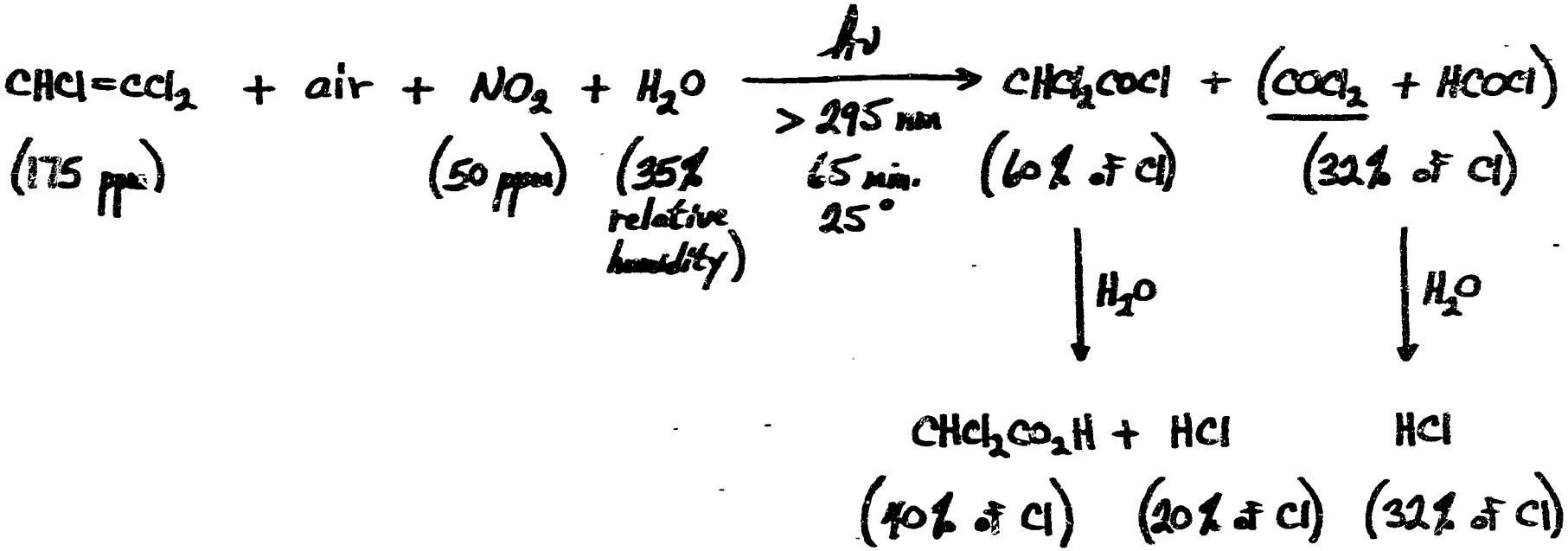
ClOO·

Pitts, et. al., 1969; Stedman, et. al., 1970; Kiryushin and Poluektov, 196
Johnston, et. al., 1969; Pierrard, 1969.

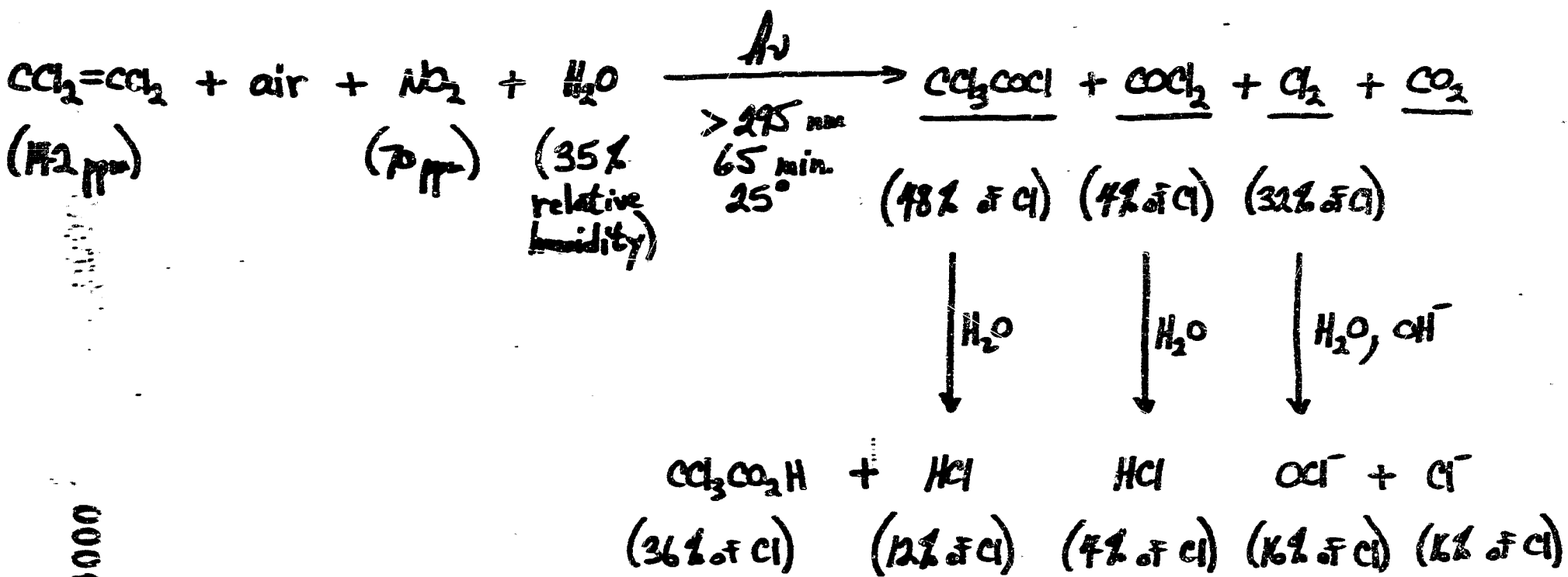

Needle valve
Check valve
Pressure release
Rotameter
Air
H_2O
$CHCl=CCl_2$
gas outlet
Gas inlet
Thermowell
Jacketed Lamp well
UV lamp
Reactor
Magnetic stirrer with paddles
Dry ice cold traps
+ NO_2
Gas scrub

$$CH_2Cl_2 \text{ (155 ppm)} + \text{air} \xrightarrow[22 \text{ min., } \sim 25^\circ]{h\nu,\ 254\ nm} HCl\ (9\%) + Cl_2\ (16\%) + HClO_3\ (8\%) + HClO_4\ (> 30\%)$$

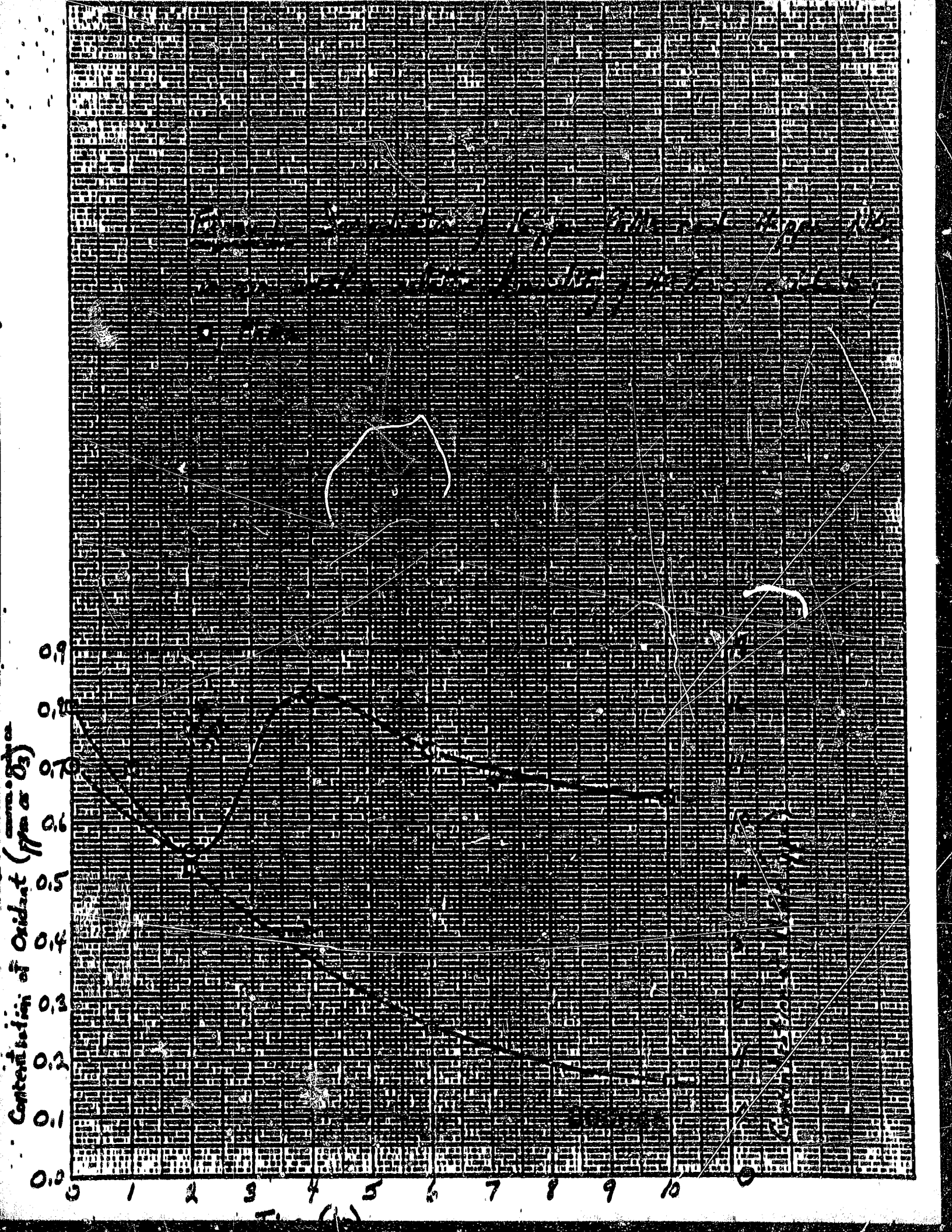
$$CH_3CCl_3 \text{ (169 ppm)} + \text{air} \xrightarrow[22 \text{ min., } \sim 25^\circ]{h\nu,\ 254\ nm} HCl\ (20\%) + Cl_2\ (39\%) + HClO_3\ (3\%) + HClO_4\ (> 24\%) + HOAc\ (10\%) + CH_2ClCO_2H$$

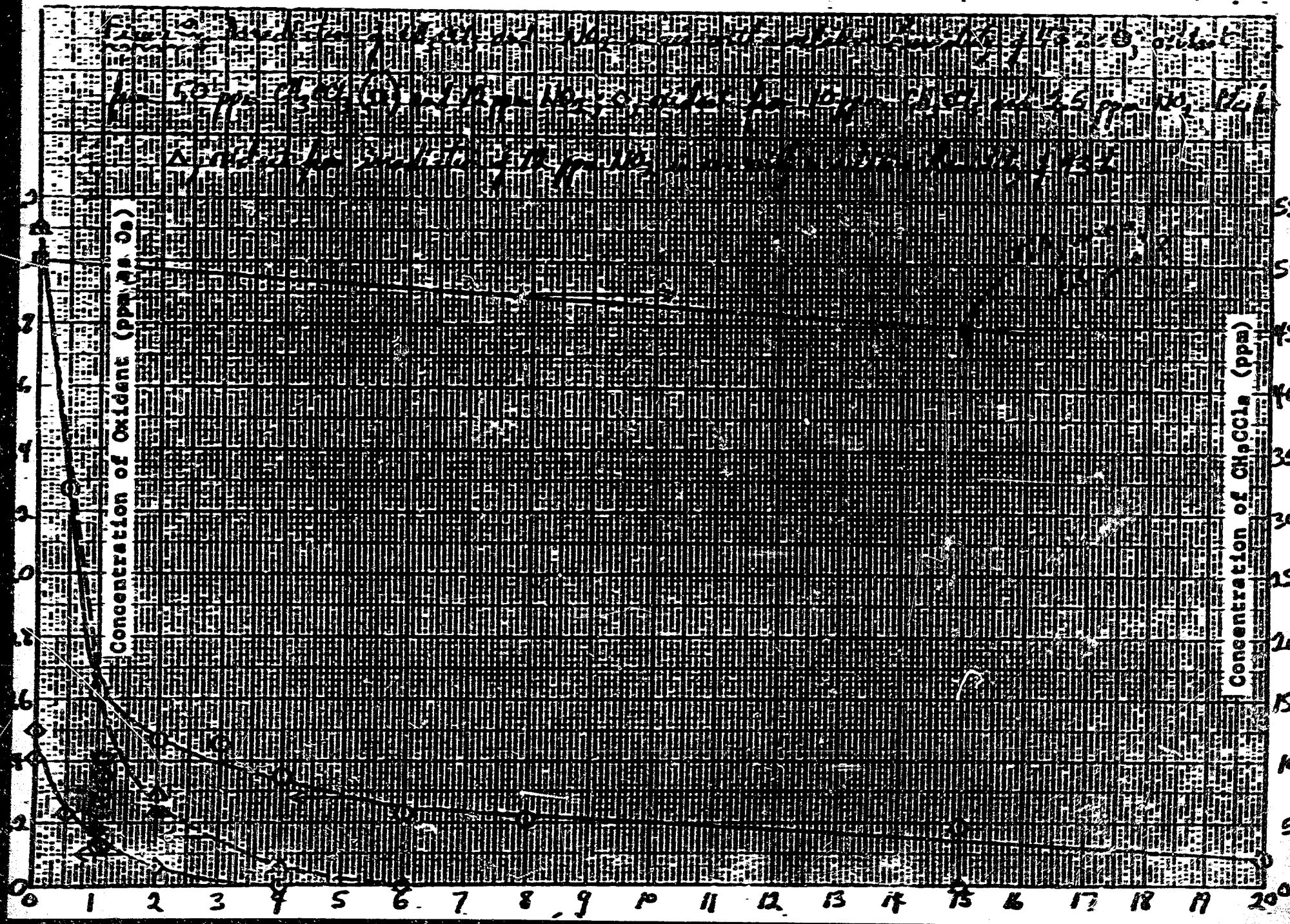

CHCl=CCl2 + air + NO2 + H2O
(175 ppm)
(50 ppm)
(35% relative humidity)
hν
> 295 nm
15 min.
25°
CHCl2COCl + (COCl2 + HCOCl)
(60% of Cl)
(32% of Cl)
H2O
H2O
CHCl2CO2H + HCl
HCl
(40% of Cl)
(20% of Cl)
(32% of Cl)

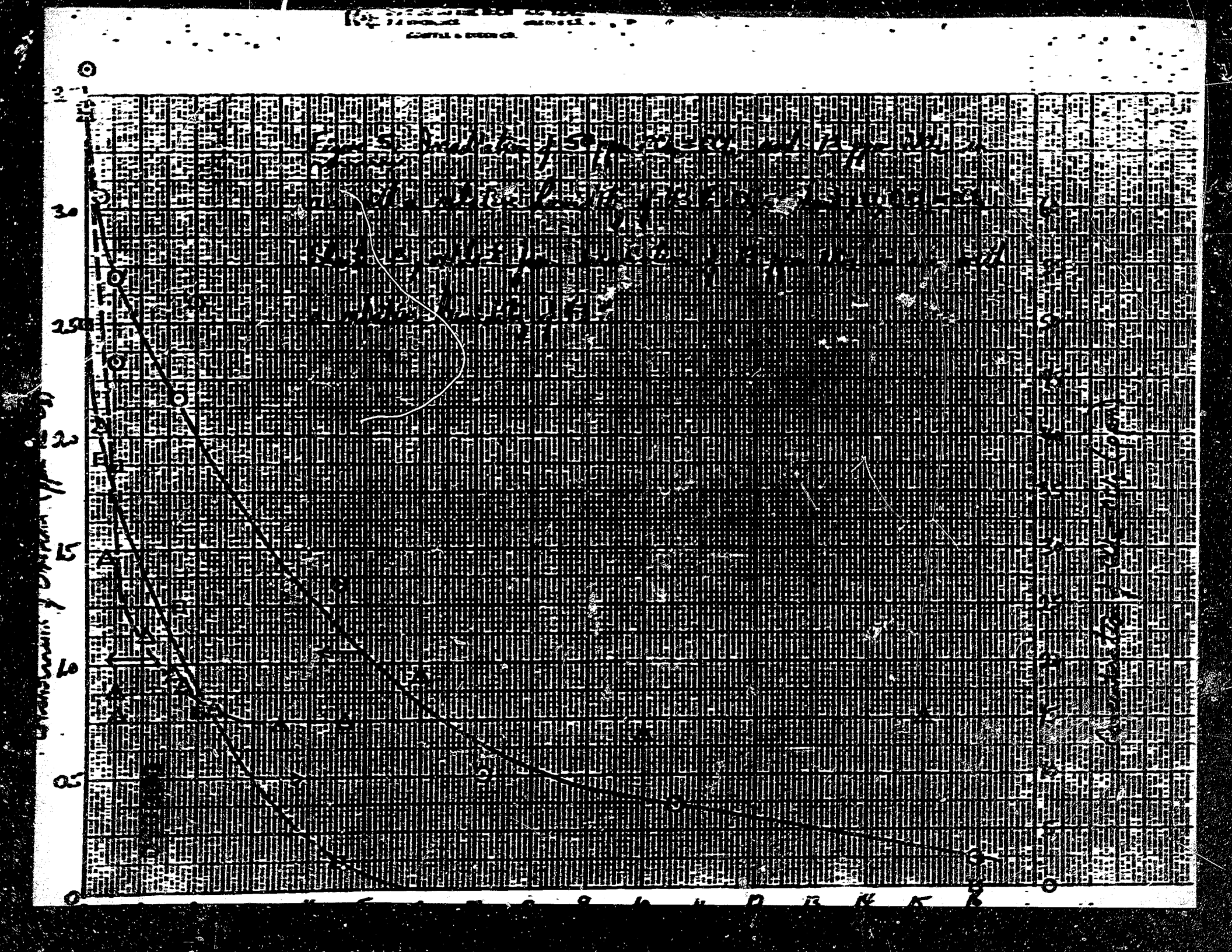
000044

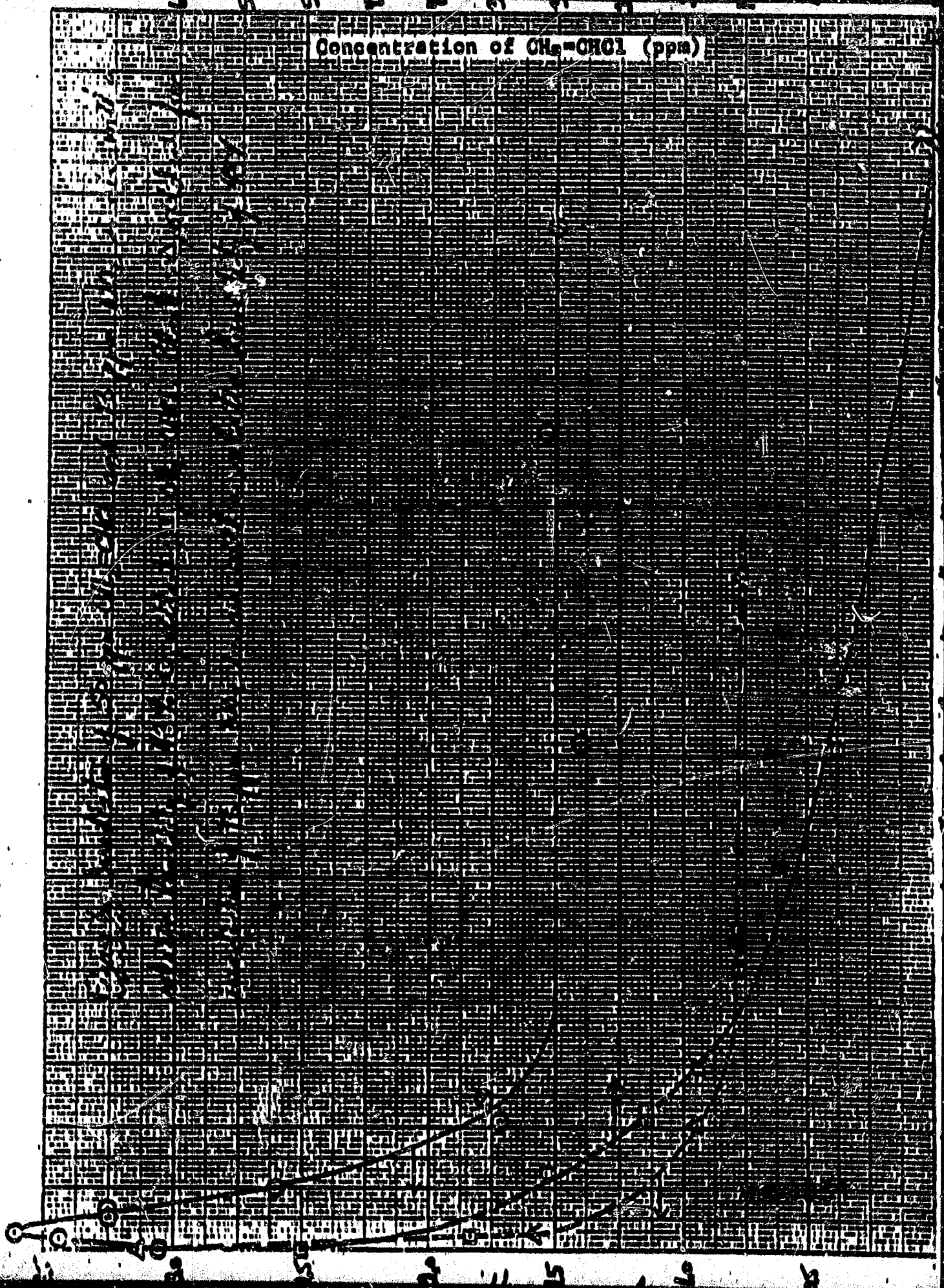

CCl2=CCl2 + air + NO2 + H2O
(ppm)
(ppm)
(35% relative humidity)
hν
>295 nm
65 min.
25°
CCl3COCl + COCl2 + Cl2 + CO2
(48% of Cl)
(4% of Cl)
(32% of Cl)
H2O
H2O
H2O, OH⁻
CCl3CO2H + HCl
HCl
OCl⁻ + Cl⁻
(36% of Cl)
(12% of Cl)
(4% of Cl)
(16% of Cl)
(16% of Cl)

000045


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10
Time (hr)
Concentration of Oxidant (ppm as O3)


Concentration of Oxidant (ppm as O3)
Concentration of CH3CCl3 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
55
50
45
40
35
30
25
20
15
10
5
0




Concentration of $CH_2=CHCl$ (ppm)

Summary

Compound	Smog potential as judged by reactivity and oxidant formation
CH_2Cl_2	~ none
CH_3CCl_3	~ none
$CHCl=CCl_2$	moderate
$CCl_2=CCl_2$	slight

Acknowledgments

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Dr. Michael J. Mintz

Dr. Jack P. Arrington

Mrs. Nancy B. Tefertiller

Mrs. Violeta L. Stevens

Mr. George J. Kalles

PAPERS

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Organic Photochemistry. XIII.[1] Environmental Fate and Effect of Chlorinated Solvents. II.[2] Simulated Atmospheric Photoreactivity of Methylene Chloride, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, and Other Chlorinated Hydrocarbons

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Chemicals Research, Dow Interdisciplines Group, and Chemicals Laboratory, The Dow Chemical Company, Midland, Michigan 48640

Abstract

In order to estimate the reactivity of chlorinated solvents in the atmosphere, laboratory studies were carried out on the photolysis rates of four common chlorinated solvents, six other chlorinated hydrocarbons, and 23 other organic compounds under simulated atmospheric conditions. Most reactions included nitric oxide or nitrogen dioxide, and water vapor in the air; the rates of disappearance of the organic compounds were measured. Under simulated sea level atmospheric conditions ($\lambda > 290$ nm), vinylidene chloride, cis- and trans-dichloroethylene, trichloroethylene, and vinyl chloride were moderately reactive when compared with the reactivity of a series of standard hydrocarbons. Chlorobenzene, tetrachloroethylene, and 1,1,2-trichloroethane

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[1] Part XII: (Dilling et al., 1973).

[2] Part I: (Dilling et al., 1974a).

[3] Chemicals Research.

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[5] Chemicals Laboratory.

had a low reactivity. Methylene chloride and 1,1,1-trichloroethane were nonreactive under sea level conditions, but were rapidly decomposed under simulated high altitude conditions (λ 254 nm principally).

The fate and effect of chlorinated solvents which may be discharged to the environment (Murray and Riley, 1973a; Murray and Riley, 1973b; Farber, 1973) are important from an ecological standpoint. In the preceding paper (Dilling et al., 1974a) we showed that the major portions of several chlorinated solvents evaporated relatively rapidly from dilute aqueous solutions under simulated environmental conditions. We therefore undertook a study of the reactivity of several of the more important chlorinated solvents in use today, methylene chloride (CH_2Cl_2), 1,1,1-trichloroethane (CH_3CCl_3), trichloroethylene ($CHCl=CCl_2$), and tetrachloroethylene ($CCl_2=CCl_2$), and also several other related chlorinated compounds under simulated atmospheric conditions.

To the best of our knowledge no studies have been reported on the photoreactivity (rate of disappearance) of CH_2Cl_2 and CH_3CCl_3 under simulated atmospheric conditions. However it has been reported (Altshuller and Bufalini, 1971) that chlorinated saturated hydrocarbons were virtually unreactive. An indication of the expected low reactivity of CH_2Cl_2 and CH_3CCl_3 can be found in other chemical studies on these compounds. Liquid CH_2Cl_2 was unreactive toward oxygen even at temperatures up to 140° (Carlisle and Levine, 1932). CH_3CCl_3 in the liquid phase was very resistant to oxidation with oxygen under relatively mild conditions (Archer, 1970), but did react under high pressure (Young, 1956). The quantum yield for the chlorine initiated photooxidation of CH_3CCl_3 in the vapor phase was much lower than was the quantum yield for the photooxidation of $CCl_2=CCl_2$ (Bertrand et al., 1971).

Considerably more work has been done on the behavior of $CHCl=CCl_2$ under simulated atmospheric conditions than on the other chlorinated solvents considered in this paper. There is reasonably good agreement on the effect of $CHCl=CCl_2$ based on some criteria. Based on other criteria the agreement is not

as good. In general $CHCl=CCl_2$ is of intermediate reactivity when compared with other common atmospheric contaminants (Schuck and Doyle, 1959; Hamming, 1967; Wilson et al., 1969c; Altshuller and Bufalini, 1971). The data were variable and easily effected by changes in experimental conditions (Hamming, 1967; Wilson et al., 1969a; Wilson et al., 1969b). The rates of formation of smog components and their maximum observed concentrations fell rapidly as the $CHCl=CCl_2$:nitric oxide (NO) ratio decreased; at ratios less than one, the photoreactivity of $CHCl=CCl_2$ was completely inhibited (Wilson et al., 1969b; Wilson, 1969). At any fixed $CHCl=CCl_2$:NO ratio the reactivity of $CHCl=CCl_2$ decreased as the absolute concentrations fell as measured both by $CHCl=CCl_2$ disappearance and appearance of smog components (Wilson, 1969).

$CHCl=CCl_2$ was moderately reactive under simulated atmospheric conditions as judged by its rate of disappearance (Schuck and Doyle, 1959; Wilson, 1969; Wilson et al., 1969b; Wilson et al., 1969c; Altshuller and Bufalini, 1971). It was less reactive than propylene ($MeCH=CH_2$) (Schuck and Doyle, 1959; Wilson et al., 1969c) but there was disagreement concerning its reactivity relative to the standard coupound ethylene ($CH_2=CH_2$). Some workers found $CHCl=CCl_2$ disappeared more slowly than $CH_2=CH_2$ (Wilson, 1969; Wilson et al., 1969b; Wilson et al., 1969c) while others found $CHCl=CCl_2$ reacted more rapidly than $CH_2=CH_2$ (Schuck and Doyle, 1959; Altshuller and Bufalini, 1971). The effect of several other variables on the rate of disappearance of $CHCl=CCl_2$ have been determined. These variables include $CHCl=CCl_2$:NO ratio and their absolute concentrations, and replacement of NO with nitrogen dioxide (NO_2) (Wilson et al., 1969c).

In general $CCl_2=CCl_2$ had a relatively low reactivity under simulated atmospheric conditions (Schuck and Doyle, 1959; Hamming, 1967; Altshuller and Bufalini, 1971).

Experimental

Short Term (≤24 Hr) Sea Level Simulated Photolyses. Reactor Design and Operation. The reactor (9.98 liters) is shown in Figure 1. All materials in the reactor to which the reaction mixture was exposed were constructed of glass, quartz or Teflon-except for two silicone rubber gaskets for seals at the ends. The reactor and light sources were enclosed in a wooden box so that there would be little exposure of the untraviolet light to the chemist and for protection in case of implosion of the reactor while under vacuum.

The reactor temperature (26-28°) was controlled by a constant temperature bath which circulated water through the jacket surrounding the reactor as well as through the 2 inch cylinders attached to the ends of the reactor. The cooling water in the cylinders at the ends was necessary to remove the heat from the sunlamps.

In order to continuallly circulate the contents within the reactor, an internal, leak-proof and inert circulation system was designed. Stirrers (Figure 2) were used; they were constructed from watch glasses, Teflon blades, and bar magnets, and were rotated by external magnetic stirrers. The effectiveness of these stirrers was demonstrated by the addition of a sample of cyclohexane ($c\text{-}C_6H_{12}$) to the reactor. Uniform mixing occurred in less than one minute with the stirrers on, while it took over 14 minutes with the stirrers off (Table I). See below for reactor loading and sampling techniques.

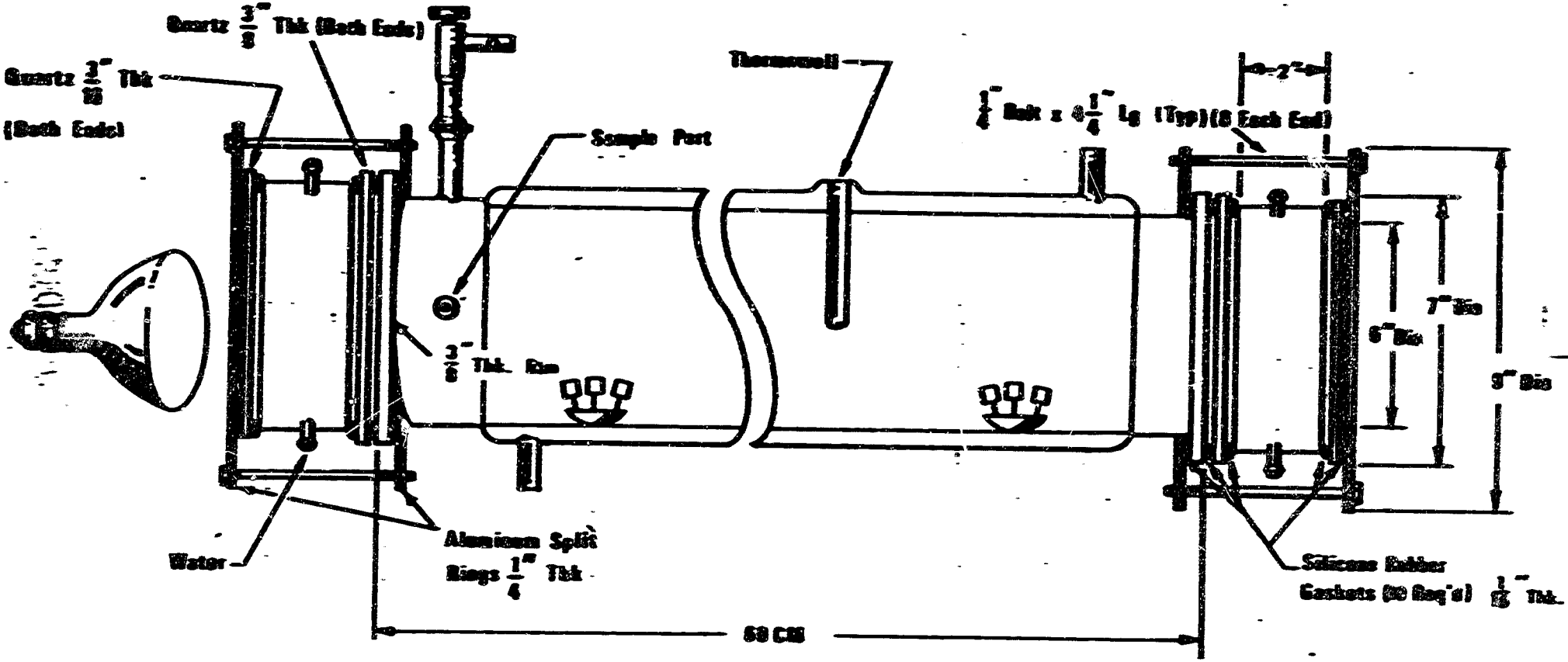

Figure 1. Short Term Sea Level Simulated Photolysis Reactor

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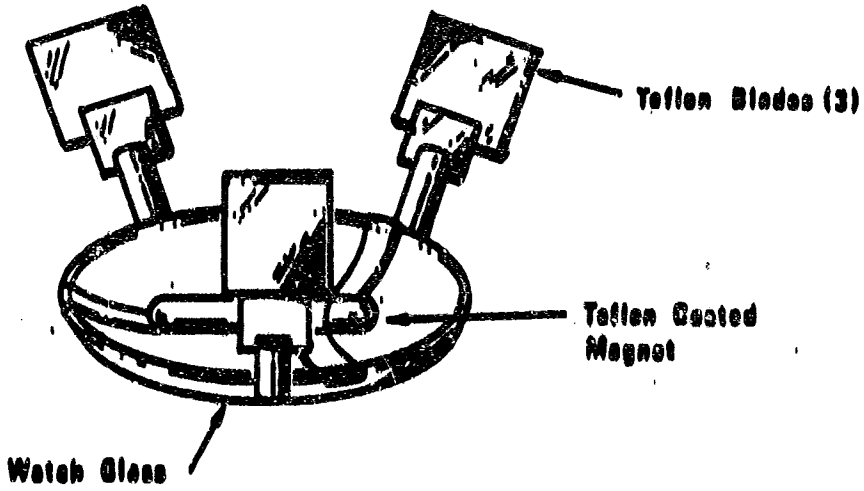

Figure 2. Stirring Devices For Photochemical Reactor

Table I. Effectiveness of Air Circulation

Time	g-C_6H_{12} as peak height
With stirrers on	
0 seconds	g-C_6H_{12} added
60	74
200	75
340	73
With stirrers off	
0 seconds	g-C_6H_{12} added
90	180
4 minutes	184
7	150
11	124
14	112
16	Stirrers turned on
17	82
19	83
23	82

The ultraviolet light sources were two General Electric 275 watt reflector sunlamps (Koller, 1967b) whose short wavelength cut off was 290 nm as determined with an International Light Inc. IL600 Research Photometer and an IL660 Photomultiplier Power Supply. The 290 nm cut off is about the same cut off as that of sunlight at the earth's surface (Koller, 1965d). These lamps were used since to a rough approximation they simulate sunlight (Micksic et al., 1954; Koller, 1965a) and are easily maintained; the output in the ultraviolet range is approximately that of sunlight, and the lamp design was convenient for use with our reactor. A General Electric ultraviolet meter, model 80W40Y17, was used to compare the ultraviolet light output of the sun with that from the sunlamps. Figure 3 shows the ultraviolet light output of two sunlamps. The data were obtained by leaving one end of the reactor open while the lamp and cooling jacket at the other end were in operation. The light intensity was determined by taking readings within the reactor at various distances from the lamp. The process was repeated with the second lamp in place of the first one. The upper curve in Figure 3 is the combined intensity of both lamps. An average of ~90 E-vitons/cm^2 is estimated. The intensity of natural sunlight at noon one summer day in Freeport, Texas, was 35 E-vitons/cm^2. Thus, the ultraviolet light intensity within the reactor varied from about 2 to 4 times that of natural sunlight; the estimated average is ~2.6 times. This higher intensity does not affect our conclusions to any significant extent since all the reaction rates were compared in a relative manner. It should be noted that the contents within the reactor received approximately two times more ultraviolet radiation than if these same contents were to occupy a similar volume in space between the lamps if the reactor were not there. The increased light within the reactor very likely resulted from the

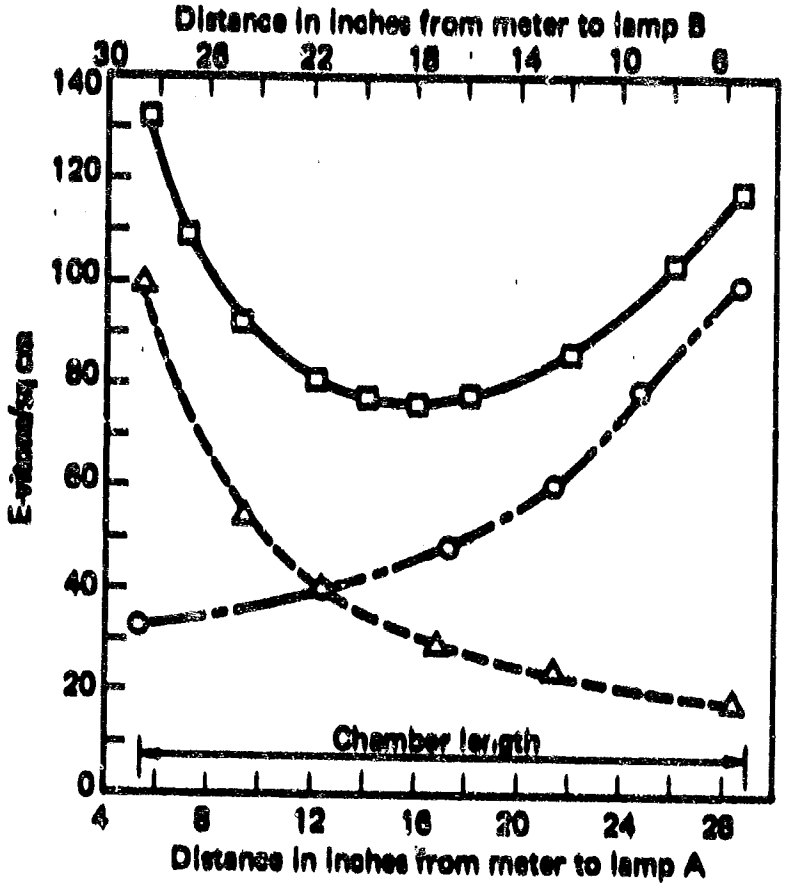

Figure 3. Ultraviolet light intensity inside reactor; intensity from lamp A (- - - -); intensity form lamp B (—— - ——); combined intensity from lamps A and B (————).

000064

multiple reflections within the reactor walls. Since the sunlamps gradually deteriorated, they were periodically replaced. To determine when this was necessary, a series of standard materials were periodically run and compared. A constant voltage transformer was used to stabilize the output from the sunlamps. There should be essentially no loss of ultraviolet light from the sunlamp which passes through 2 inches of distilled water and two thicknesses (3/16 and 3/8 inch) of 101 quartz (Koller, 1965e).

The reactor leak rate or rate of loss of contents through glass wall adsorptions, absorption in the Teflon, or permeation through the silicone rubber gaskets was minimal, too low to determine. The loss of sample through "leakage" was probably less than the experimental error in sampling and analysis. It is estimated that there was less than 5% loss in an 8 hr run, and certainly not greater than 10% in a 24 hr run.

The reactor was cleaned by evacuating to ~0.2 mm for an hr or more. The cleanliness of the reactor was periodically checked by filling with pure air and analysing the air by gc for any residual solvent or impurities. No evidence of contaminant buildup was found during this work.

Sampling Techniques and Analyses. The rate of solvent disappearance in the reactor was determined with a hydrogen flame ionization gas chromatograph (F&M 5750). Initially, 1 cc samples were removed with a syringe from the sample port which was stoppered with a serum cap. These samples were then transferred to the gas chromatograph, and upon elution, the peaks were either measured by peak height or by peak area with an Infotronics CRS-100 Electronic Integrator which was connected to a Teletype Printer. All-glass hypodermic syringes, labeled B-D Yale, were used. Later, an automatic sampling device was incorporated into the analysis. This device gave excellent reproducibility while the syringe

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technique was sometimes erratic and required several sample injections in order that a data point could be determined with confidence. A small diaphragm pump (Universal Electric Co., Model 143-005) was used to circulate the reactor contents through a Beckman gas sampling valve. All tubing between the reactor, pump and sampling valve was stainless steel (SS) 0.045 inch ID with a capacity of ~0.3 cc/ft. A four-way Asco solenoid valve (Model 8345A) was used to change the valve positions on the gas sampling valve. All of the operations connected with the analysis by gc were completely automated by a multiple timing device.

The gc conditions were as follows: helium flow through column, ~25 cc/minute (25 psi); hydrogen flow at the flame head, ~10 cc/minute (4 psi); air flow at the flame head, ~300 cc/minute (33 psi); detector block temperature, 280°; injection port temperature, usually near the column temperature; column temperature, variable but usually less than 150°. All analyses were carried out under isothermal conditions except when low and high boiling compounds were investigated together. The columns used were a 12 ft x 1/8 inch SS packed with Poropak Q, 7 ft x 1/8 inch SS packed with 20% Carbowax, and 6 ft x 1/8 inch SS packed with 10% UC-W98 (silicone rubber). Table II shows the relative response of various chlorinated compounds along with some hydrocarbons. The lower limits of detectability for most of these materials were not determined, but in most cases were probably 0.1 to 1 ppm or less.

To check the reproducibility of the addition of materials to a constant volume of air and the linearity of the analytical method, liquid cis-CHCl=CHCl was added in incremental volumes from a one microliter syringe to 10 liters of nitrogen in a Saran® bag. After each solvent addition, the air was analyzed by gc. The following data were obtained (volume of cis-CHCl=CHCl added and gc peak height given): 0.15 µl, 14.3 units; 0.30 µl (~10 ppm), 31.5 units; 0.60 µl,

Table II. Response to Various Chlorinated Compounds by Flame Ionization Detector GC

Compound[a]	Column	Area	Relative response
CH_2Cl_2	Orinite	191	1.0
$CH_2=CH_2$	Poropak Q	303	1.6
Vinyl chloride ($CH_2=CHCl$)	Orinite	397	2.1
Vinylidene chloride ($CH_2=CCl_2$)	Orinite	455	2.4
cis-Dichloroethylene (cis-CHCl=CHCl)	UC-W98	465	2.4
trans-Dichloroethylene (trans-CHCl=CHCl)	Orinite	619	3.2
$CHCl=CCl_2$	Orinite	432	2.3
$CCl_2=CCl_2$	Orinite	442	2.3
CH_3CCl_3	UC-W98	625	3.3
trans-2-Butene (trans-MeCH=CHMe)	Orinite	920	4.8
n-C_6H_{12}	Orinite	937	4.9

[a]Sample size was 1 cc and all areas were based on a sensitivity of 1 x 4 with a compound concentration of 10 ppm in air.

60.2 units; 0.90 µl, 86.3 units. In another experiment, 0.29 µl cis-CHCl=CHCl was added to the reactor (9.58 liters) and gc analysis gave a peak height of 31.75 units. The excellent agreement between the reactor and the Saran® bag indicated that there were no peculiar wall effects or adsorption effects with either container.

Materials. Ultra high purity air (synthetic, 20% O_2, 80% N_2) obtained from Matheson showed no impurities by gc. NO and NO_2 were from Matheson and were used as received. Water was redistilled. CH_2Cl_2 (uninhibited) was redistilled, and the center cut was used. CH_3CCl_3 (uninhibited) was redistilled, and the center cut, bp 74°, was used. $CHCl=CCl_2$ (MI-TRI®) which contained 20 ppm diisopropyl amine was used without purification unless otherwise stated. $CCl_2=CCl_2$ was redistilled. cis-CHCl=CHCl from Matheson was redistilled to give a center cut, bp 58-60°. trans-CHCl=CHCl from Eastman was redistilled to give a center cut, bp 48°. $CH_2=CH_2$ from Matheson and research grade trans-MeCH=CHMe from Phillips Petroleum were used as received. c-C_6H_{12} was redistilled. Other materials were either redistilled or shown to be pure by gc.

Example of a Typical Photolysis. After the reactor was evacuated to 0.2-0.5 mm for an hour or more, 47.9 µl of NO, taken from an exit tube of a cylinder with a syringe, was added to the reactor with the stirrers on to give a final concentration of 5 ppm. The NO was added at this point to prevent the premature oxidation of NO to NO_2 since NO is readily oxidized at "high" concentrations of NO in air. While the reactor was still under vacuum, 9½ µl of distilled water was added and evaporated with a heat gun from outside the chamber to give a relative humidity of 35-40%. Pure air was then added from a Saran® bag to the reactor until the pressure inside the chamber was equalized with atmospheric pressure. Finally, 0.34 µl of $CHCl=CCl_2$ was added to the reactor from a one

microliter syringe (type with plunger in needle of syringe) to give a concentration of 10 ppm (all concentrations on a molar or gas volume basis). The $CHCl=CCl_2$, as well as most other solvents except alcohols, evaporated from the end of the syringe needle in less than one minute. The reactor temperature was maintained at 26-28° throughout the reaction. Constant volume samples (1 cc) were analyzed by gc periodically as follows (time and peak height given): 0 minutes, 85 units; 17,78; 34, 73.5; 51, 70; 68, 66.5; 85, 61; 102, 57.5; 119, 53.5; 136, 52; 153, 49; 170, 46; 187, 44; 204, 42; 221, 40; 238, 40; 255, 37; 272, 35; 289, 34.5; 306, 33. The half life of the $CHCl=CCl_2$ was thus 3.3 hr. The first samples analyzed, before the lamps were turned on, were used as standards to which subsequent samples during the reaction were compared. The rate of compound disappearance during the reaction was then determined directly from the reduction in peak height (most of the peaks were highly symmetrical) or reduction in peak area. The compound half-life or the percent that reacted in 6 to 8 hr (for compounds which reacted slowly) was used as a measure of the reactivity of the compound. The 6-8 hours reaction time is equivalent to 2-3 days of intense noon-time sun for 6 hr each day. The chlorinated solvent concentration was generally chosen at 10 ppm in order to obtain reasonable gc responses throughout the run.

When NO_2 was introduced into the reactor in a manner similar to that described for NO above, the dimerization equilibrium was taken into account for purposes of calculating the correct volume of gas at atmospheric pressure.

Some of the reactions were run in duplicate or more. Average values are given in the tables; duplicate values obtained within several weeks generally agreed within ~10% of each other. The variation in compound half-lives among the different tables is probably due to variations in light intensity inside the

reactor. The reactivities listed within a given table are consistent within that table.

Long Term Sea Level Simulated Photolyses. Reactor Design and Operation. The photo reactor used in this work was designed to minimize the possibility of leakage over periods of several weeks. Consequently, there were only two non-glass seals, a 4 mm Teflon stopcock near one end and a capillary tubing sample port which was sealed with a silicone rubber septum near the other end. The reactor was a flat-ended pyrex cylinder, similar in shape and size to the one shown in Figure 1; the volume was 11.6 liters. At each end was positioned the same type of sunlamp described above. Midway between each lamp and the end of the reactor (16 cm) was a 3/8 inch thick quartz plate to reduce the warming of the photoreactor by the sunlamps; the reactor did not have a water jacket. The ends of the reactor were further cooled by forced air which had been passed over a cold water cooling coil. The entire system was placed in a hood to maintain adequate air flow to dissipate the heat. The temperature was measured by a thermocouple taped to the outside of the reactor near the center of the cylinder. The hood in which the apparatus was placed was covered by black cloth to eliminate the possibility of eye injury from the ultraviolet radiation.

The simulated atmosphere used in the photoreactions was obtained by premixing, in the desired proportions, a dry air stream with an air stream saturated with water and one containing a known amount of NO_2. The gas streams were monitored by flowmeters (Dilling and Tefertiller, 1974) and the photo reactor was flushed with the gas mixture for a period of time calculated to replace 99.9% of the ambient air in the photoreactor (Dilling et al., 1974b). A sample of the gas mixture was then removed and analyzed for NO_2 using a modified Saltzman (1954) procedure. This procedure involved withdrawing a 47.8 cc gas sample

into an evacuated tube which contained 10 ml of the absorbing reagent. The calibration curve was determined in the same manner with air-NO_2 mixtures prepared directly by pressure-volume measurements on a vacuum line. Finally a quantity of the chlorinated solvent calculated to give 50 ppm (on a molar basis) in air was injected by syringe through the septum into the reactor. The system was allowed to equilibrate overnight, and gas samples were removed for analysis before turning the lamps on.

Sampling Techniques and Analyses. Gas samples were removed at intervals with Hamilton gas-tight syringes and analyzed by gc.

Two gc systems were used to analyze for the chlorinated solvent concentration. The first system, which employed a thermal conductivity detector, was a F & M 500 instrument equipped with a 10 ft x 1/4 inch column packed with 20% Apiezon L on chromosorb WAW. The column temperature was 125°, the detector 300°, and the injection port 140°. The helium flow rate through the column was 43 cc/minute. A 10 cc gas sample was used. The peak areas were measured with a planimeter. A liquid standard of CH_3CCl_3 in $CHCl=CCl_2$ was used for calibration in experiments with the former compound.

The second and more precise gc system employed a hydrogen flame ionization detector. An Aerograph Hy-Fi Model 550-B, with a Bendix electrometer, was equipped with a 10 ft x 1/8 inch column packed with 20% N,N-bis(2-cyanoethyl)-formamide on chromosorb W. The column temperature was generally 57° and the carrier gas was nitrogen. A 2 cc gas sample was used. A gas standard was used to calibrate the gc. It consisted of 50 ppm of the chlorinated solvent in air in a 12.6 liter Pyrex glass sphere which had two openings, a 4 mm Teflon stopcock and a capillary sampling port closed by a silicone rubber septum. Samples from this bulb were reproducible for at least 6 weeks, indicating no leakage from the bulb.

Materials. NO_2 free from air (mass spectroscopy) was prepared by condensing material from Matheson at 0° and degassing to 10^{-2} mm in three freeze-pump-thaw cycles (-78° to 0°). NO_2 in air was obtained from Matheson; the NO_2 concentration was found to be 25 ppm by the modified Saltzman method. Dry air was obtained by passing Matheson dry cylinder air through a drying tower which contained phosphorous pentoxide dispersed on calcium sulfate. CH_2Cl_2 and carbon tetrachloride (CCl_4) were distilled in glass materials from Burdick and Jackson. The CH_3CCl_3 was the same as described above.

Analysis of CH_2Cl_2 Reaction Mixture. The contents of the reactor from the reaction shown in Figure 6 (after 13 days irradiation) were removed from the reactor by rinsing it with 20 ml of 0.01 N sodium hydroxide solution and 40 ml of water. The combined rinses were evaporated to a small volume, acidified with nitric acid, and titrated with 0.100 N silver nitrate solution (Dilling and Tefertiller, 1974). The titration required 0.36 ml to reach an end point. This represents 77% of the chlorine present in the initial CH_2Cl_2 (1.51 µl, 0.0470 mg atoms of chlorine).

High Altitude Simulated Photolyses. The photoreactor was a Pyrex sphere of 12.1 liters capacity having a 4 mm Teflon stopcock and a capillary tubing sample port closed by a silicone rubber septum. The reactor had a 60/50 silicone greased ground glass joint into which was inserted a low pressure immersion mercury arc lamp. Air blowers were directed at the top and bottom of the reactor to dissipate the small amount of heat generated by the lamp. The temperature of the reactor was measured by a thermocouple taped to the outside of the flask near the middle between the blowers.

The short wavelength light source was a 10 watt Hanovia low pressure mercury arc lamp which was in the form of a coil. The emission from this lamp was measured at a distance of 2.4 meters from the lamp with the International Light photometer described above. The intensity at various wavelengths were as follows: 254 nm, 3.5 µ watts/cm^2; 265, 0.016; 297, 0.032; 313, 0.11; 334, 0.0043; 366, 0.053; 406, 0.055; 436, 0.063; 546, 0.034. The total energy at 254 nm was

2.5 watts. Radiation of 185 nm may have been emitted (Calvert and Pitts, 1966a), but it was not measured. The intensity of short wavelength light outside the earth's atmosphere was calculated from data given by Koller (1965c). The total intensity in the 140-260 nm range was 0.287 milliwatts/cm^2, in the 140-280 nm range 0.714 milliwatts/cm^2, and in the 140-300 nm range 1.672 milliwatts/cm^2.

The materials used and the techniques employed for loading the reactor and for sampling and analysing the reaction mixtures were the same as described above for the long term photolyses.

CH_3CCl_3 (50 ppm) was stable for at least 4 days in the dark in the presence of ozone (2) which had been generated by photolysing air in the reactor.

No significant problems were encountered from adsorption of CH_3CCl_3 on the reactor walls as shown by measuring the amount of material present in the gas phase after multiple injections.

Results and Discussion

Short Term (≤ 24 Hours) Sea Level Simulated Photolyses. We chose to carry out this research with small photoreactors (~10 liters) because of certain disadvantages of large smog chambers (e.g. Dimitriades, 1967; Doyle, 1970) including the following. The chamber cannot be built completely out of an inert and impermeable material such as glass, since a rigid framework made of some kind of metal such as aluminum (Wilson et al., 1968) is needed for support. It cannot be evacuated for cleaning purposes. Cleaning must be done through a purging process which requires a lengthy time and large volumes of air. The ambient air must be purified at the chamber site through a complicated process to remove oxidants, oxides of nitrogen, any organic compounds, etc., since it is not practical to use purified cylinder air. Temperature control is more

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difficult than with a small reactor. However, large chambers have certain advantages which include the following. The construction of the lighting system has greater flexibility since a wide variety of lamps can be used to obtain better spectral distribution and intensity which simulates sunlight. Since relatively large volumes of air are available for analysis, wet chemical analysis can be obtained for NO, NO_2, O_3 , aldehydes, etc., which are important in evaluating photochemical smog production. However, we have been able to measure total oxidant formation with the small reactor (Dilling et al., 1974b). Eye irritation data can only be obtained by exposing a panel of human subjects to the contents of a large chamber through the use of eyeports.

Correlation of the results from one study to another is often difficult since many of the variables such as lighting, temperature, chamber construction materials, air purity,and other reaction conditions are difficult to evaluate and control. To best compare results from various reactors, standard materials are run which range from very slow reacting materials to compounds which are highly reactive. Table III shows a comparison from several different laboratories, of the absolute photochemical reactivities (rate of compound disappearance) and approximate relative reactivities of several standard hydrocarbons [c-C_6H_{12}, toluene (PhMe), trans-MeCH=CHMe] and of $CHCl=CCl_2$ compared with $CH_2=CH_2$. There is a relatively good correlation between the five sets of data. The variation in absolute reactivities between groups in Table III can be attributed largely to the differences in light intensity and reactant concentrations.

There is a good correlation between reactivity and light intensity (see below).

Table III. Absolute and Relative Photochemical Reactivities of Standard Hydrocarbons and $CHCl=CCl_2$ Determined at Different Laboratories

Compound	Stanford Research Institute[a]	General Motors Corporation[b]	Battelle Memorial Institute[c]	National Air Pollution Control Administration[d]	This work[e]
$c\text{-}C_6H_{12}$	50% (18 hr)[f]	18% (6 hr)[h]	~42% (5 hr)	—	50% (6.8 hr)
	0.42[g]	0.40	~0.5		0.43
PhMe	—	39% (6 hr)	~50% (5 hr)	—	50% (6.8 hr)
		0.87	~0.6		0.43
$CH_2=CH_2$	50% (7.5 hr)	45% (6 hr)	~50% (3 hr)	50% (3.7 hr)	50% (2.9 hr)
	1.0	1.0	1.0	1.0	1.0
$CHCl=CCl_2$	50% (7.0 hr)[i]	—	—	50% (2.6 hr)	50% (3.5 hr)
	1.1			1.4	0.83
trans-MeCH=CHMe	50% (0.44 hr)	85% (6 hr)	~50% (0.2 hr)	—	50% (0.23 hr)
	17		~15		13

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(footnotes continued on next page)

Footnotes to Table III.

[a]Data from Wilson et al. (1969a); initial concentrations (ppm) of organic compound and NO: c-C_6H_{12}, CH_2=CH_2, and CHCl=CCl_2, 8 and 2; trans-MeCH=CHMe; 3 and 1.3. [b]Data from Heuss and Glasson (1968a, 1968b); initial concentrations, 2 ppm organic compound, 1 ppm NO. [c]Data from Levy (1968); initial concentrations, 4 ppm organic compound, 2 ppm NO. [d]Data of Koposynski cited by Altshuller and Bufalini (1971). [e]Initial concentrations, 10 ppm organic compound, 5 ppm NO. [f]Data given as percent disappearance of organic compound in the indicated length of time. [g]Relative reactivity compared with CH_2=CH_2 as 1.0. [h]n-Hexane used instead of c-C_6H_{12}. [i]Extrapolated from data at different light intensity by assuming an inverse relationship between light intensity and half-life.

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Table IV shows a summary of the results of the photochemical reactivity of a variety of chlorinated compounds along with the standard hydrocarbons and other materials. All of the data are based on the rate of disappearance of the compound under the same reaction conditions. The compounds are listed in order increasing reactivity with NO.

Table IV. Photochemical Reactivity of Chlorinated Compounds, Standard Hydrocarbons, and Other Materials Under Simulated Atmospheric Conditions

Compound[b]	Time (hr) for 50% disappearance of compound unless otherwise indicated[a]	
	With NO[c]	With NO_2[d]
CH_3CCl_3	23.5 (< 5%)[e]	8.0 (< 5%)[e]
CH_2Cl_2	21.0 (< 5%)[e]	7.5 (< 5%)[e]
t-Butyl alcohol (t-BuOH)	15.2 (22%) [34.5][f]	
Epichlorohydrin [c-$CH(CH_2Cl)CH_2O$]	16.0	
1,1,2-Trichloroethane ($CH_2ClCHCl_2$)	7.0 (22%) [15.9][f]	
1-Butene oxide (c-$CHEtCH_2O$)	14.9 (47%) [15.9][f]	15.2[g]
Ethyl acetate (EtOAc)	7.0 (24%) [14.6][f]	
$CCl_2=CCl_2$	12.5 (44%) [14.2][f]	7.0 (42%) [8.3][f]
Methyl ethyl ketone (MeCOEt)	6.5 (33%) [9.8][f]	
Nitromethane ($MeNO_2$)	9.2	

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Table IV. continued ...

Chlorobenzene (PhCl)	7.5 (43%) [8.7][f]	
n-C_6H_{12}	6.9	6.5 7.5[g]
PhMe	6.8	
n-Butyl alcohol (n-BuOH)	6.5	
Ethyl benzene (PhEt)	5.0	
Trioxane [c-$(CH_2O)_3$]	4.7	
CH_2=CHCl	4.3	
s-Butyl alcohol (s-BuOH)	4.0	
CHCl=CCl_2	3.5	2.9
Methyl isobutyl ketone (MeCOi-Bu)	3.5	
Isobutyl alcohol (i-BuOH)	3.5	
Dioxane [o-$O(C_2H_4)_2O$]	3.4	
1-Methoxy-2-propanol ($MeOCH_2CHOHMe$)	3.1	

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Table IV. continued.....

p-Xylene[h] (p-$Me_2C_6H_4$)	3.1	
m-Xylene[h] (m-$Me_2C_6H_4$)	2.9	
cis-CHCl=CHCl	3.0	3.0
trans-CHCl=CHCl	2.9	2.8
$CH_2=CH_2$	2.9	2.5
$CH_2=CCl_2$	2.1	
Cyclohexene (c-C_6H_{10})	0.87	0.19[g]
Diisobutylene (t-$BuCH_2CMe=CH_2$)	0.60	
trans-MeCH=CHMe	0.30	0.17[g]
N-Methylpyrrole (c-C_4H_4NMe)	0.16	

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[a]All reactions carried out with 35% relative humidity. [b]All compounds at 10 ppm in air initially unless specified otherwise. [c]All initial concentrations 5 ppm. [d]All initial concentrations 16.8 ppm except as noted. [e]Corrected for reactor "leakage" which was estimated to be ~5% in 8 hr. [f]Estimated value for 50% disappearance of compound. [g]Initial NO_2 concentration 5 ppm. [h]p-$Me_2C_6H_4$ and m-$Me_2C_6H_4$ measured simultaneously at initial concentrations of 2 and 8 ppm respectively.

It is obvious that CH_3CCl_3 and CH_2Cl_2 are essentially unreactive for up to a day with either NO or NO_2 present. $CCl_2=CCl_2$ is less reactive than g-C_6H_{12} while the remaining chlorinated ethylenes including $CHCl=CCl_2$ are comparable to the reactivity of $CH_2=CH_2$. Thus, those chlorinated compounds which are less reactive that g-C_6H_{12}, especially CH_3CCl_3 and CH_2Cl_2, would not be expected to produce significant photochemical smog symptoms, whereas $CHCl=CCl_2$ and the remaining chlorinated ethylenes, may be expected to produce photochemical smog symptoms. In general the relative reactivities of the materials in Table IV roughly parallel what is known or expected for their reactions with electrophilic radicals.

In several reactions, light intensities were varied in order to determine the effect on the reaction rate since our reactions were carried out with a higher light intensity than real sunlight. The total light intensity was reduced by placing a 30 x 30 mesh SS screen between the sunlamp and the reactor. One screen reduced the light intensity by 50% whereas two screens set at a 45° angle to each other reduced the intensity by 75%. Table V shows a good linear correlation between reactivity and light intensity with the possible exception of $CHCl=CCl_2$ at the lowest light intensity. These results also suggest that it is unnecessary to duplicate the intensity of sunlight to study photochemical reactions related to air pollution. It should only be nexessary to know how the laboratory ultraviolet radiation intensity compares to that of sunlight and that a realistic wave length range is used. Thus, one could readily calculate what the reactivity of the compound would be in the real atmosphere at any time of the day barring any other complicating factors.

Table V. Effect of Light Intensity on Reaction Rates

Compound[a]	Relative light intensity	Time for 50% disappearance of compound	Relative reactivity
trans-MeCH=CHMe	1.00[b]	11 minutes	1.00
	0.50	23	0.48
	0.25	40	0.26
$CH_2=CH_2$	1.00[b]	2.8 hr	1.00
	0.50	5.2	0.52
	0.25	10.0	0.28
$CHCl=CCl_2$	1.00[b]	3.5 hr	1.00
	0.50	6.3	0.56
	0.25	9.5	0.37

[a]All reactions carried out with 10 ppm of the compound, 5 ppm NO, and 35% relative humidity. [b]Normal light intensity used in this work.

This linear correlation between light intensity and reactivity is also in agreement with the results observed by other workers (Schuck and Doyle, 1959; Tuesday, 1961; Bufalini and Altshuller, 1967). These workers found that the distribution of reaction products was nearly independent of the light intensity with the exception that O_3 had increased with increased light intensity. In our work no data were obtained on how the photochemical reaction is affected by a variation in the wave length distribution within the 300-400 nm range, but no major effects are expected (Laity, 1971).

Tuesday (1961, 1963) observed an increase in the rate of photooxidation of trans-MeCH=CHMe when NO_2 was used instead of NO. We also found that trans-MeCH=CHMe (and c-C_6H_{10}) were consumed faster when NO_2 was initially present. However, with slower reacting materials, the difference in reactivity with NO and NO_2 is insignificant except for $CCl_2=CCl_2$ (Table IV). Presumably, the reason is that NO must be first oxidized to NO_2 before the photooxidation process can begin (Altshuller and Bufalini, 1965). In the case of slow reactors, there is sufficient time for some of the oxidation of NO to occur, whereas in the case of trans- MeCH=CHMe and c-C_6H_{10}, the initial presence of NO_2 allows an immediate reaction to occur.

In the real atmosphere, the absolute concentrations of organic material and NO may be constantly changing, particularly as the components leave the source of emission and become diluted into the atmosphere. A change in the absolute concentrations of compound and NO from 10:5 to 100:50 (ppm) resulted in a 2 to 3.5-fold increase in reactivity (Table VI). By extrapolation, the reactivity probably would continue to decrease as the concentrations are decreased. Further data on the reactivity of CH_2Cl_2, CH_3CCl_3, $CHCl=CCl_2$, $CCl_2=CCl_2$,

Table VI. Effect of Concentration on Reaction Rates

Compound[a]	Initial concentrations (ppm)		Time for 50% disappearance of compound	Relative reactivity
	Compound	NO		
trans-MeCH=CHMe[b]	10	5	26 minutes	1.0
	20	10	22.5	1.2
	40	20	17	1.5
	100	50	13	2.0
$CHCl=CCl_2$	10	5	3.5 hr	1.0
	20	10	3.0	1.2
	40	20	1.5	2.3
	100	50	1.0	3.5
$CCl_2=CCl_2$	10	5	6.5[c] hr (29%) [11.2][d]	1.0
	40	20	7.0	1.6
	100	50	3.8	3.0

[a]All reactions carried out with 35% relative humidity. [b]Reactions with trans-MeCH=CHMe carried out with 0.50 relative light intensity. [c]Time required for 29% disappearance of $CCl_2=CCl_2$. [d]Estimated value for 50% disappearance of compound.

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and CH_2=$CHCl$ at concentrations of 50 ppm in the presence of 12 ppm NO_2 are given in a later paper of this series (Dilling et al., 1974b); these data were obtained in a study of oxidant formation in the presence of these compounds.

Table VII. Effect of $CHCl=CCl_2$/NO Ratio on Reaction Rate

Initial concentration (ppm)[a] $CHCl=CCl_2$	NO	Time (hr) for 50% disappearance of $CHCl=CCl_2$
10	0	7.0 (32%)[b] [10.9][c]
10	2.5	1.8
10	5	2.0
10	10	2.5
10	20	2.5
10	40	2.5

[a]All reactions carried out with 35% relative humidity. [b]Time required for 32% disappearance of $CHCl=CCl_2$. [c]Estimated value for 50% disappearance of $CHCl=CCl_2$.

Table VIII. Effect of Relative Humidity and Absence of NO on Reactivity of $CHCl=CCl_2$[a]

Relative humidity (%)	Initial NO concentration (ppm)	Time (hr) for 50% disappearance of $CHCl=CCl_2$
0	5	2.0
35	5	2.1
70	5	3.0
0	0	5.0
35	0	7.0 (32%)[b] [10.9][c]

[a]All reactions carried out with 10 ppm $CHCl=CCl_2$ initially. [b]Time required for 32% disappearance of $CHCl=CCl_2$. [c]Estimated value for 50% disappearance of $CHCl=CCl_2$.

Long Term Sea Level Simulated Photolyses. Since CH_2Cl_2 and CH_3CCl_3 were found to be essentially non reactive under the short term photolysis conditions described above, it was of interest to determine if these two important chlorinated solvents would undergo a very slow decomposition under simulated low altitude atmospheric conditions over an extended time period. Experiments were conducted in an essentially leak-free reactor for up to 4 weeks of continuous irradiation.

CH_3CCl_3 (50 ppm) was stable for at least 4 weeks when subjected to continuous irradiation in the presence of 10-11 ppm NO_2 in air with a relative humidity of 35% at 25-27° (Figure 4, data at 19 and 28 days were obtained with a less precise thermal conductivity gas chromatograph).

CH_2Cl_2 (50 ppm) was stable for 4-6 days when irrodiated in air with 10 ppm NO_2 at 25-28° and a relative humidity of 35% (Figures 5 and 6). Then within a few days the CH_2Cl_2 reacted completely. In the products of the reaction, from which the data in Figure 6 were obtained, it was possible to titrate 77% of the total chlorine charged (in the CH_2Cl_2) as inorganic chloride after washing the reactor with dilute sodium hydroxide solution.

CH_3CCl_3 would thus appear to stable in the lower atmosphere for a long time. CH_2Cl_2 may also he stable. The rather abrupt disappearance of the CH_2Cl_2 after several days irradiation is unexplained. It is possible that an impurity in the CH_2Cl_2 inhibited the reaction until the impurity itself had decomposed or that a product was formed at a low concentration which catalyzed the reaction. It is not particularly surprising that CH_3CCl_3 and CH_2Cl_2 were not reactive when irradiated with long wavelength ultraviolet light ($\lambda > 290$ nm) since their ultraviolet spectra show no absorption in that spectral region. Apparently these chlorinated solvents are unreactive toward the radicals and atoms derived from the photolysis of NO_2.

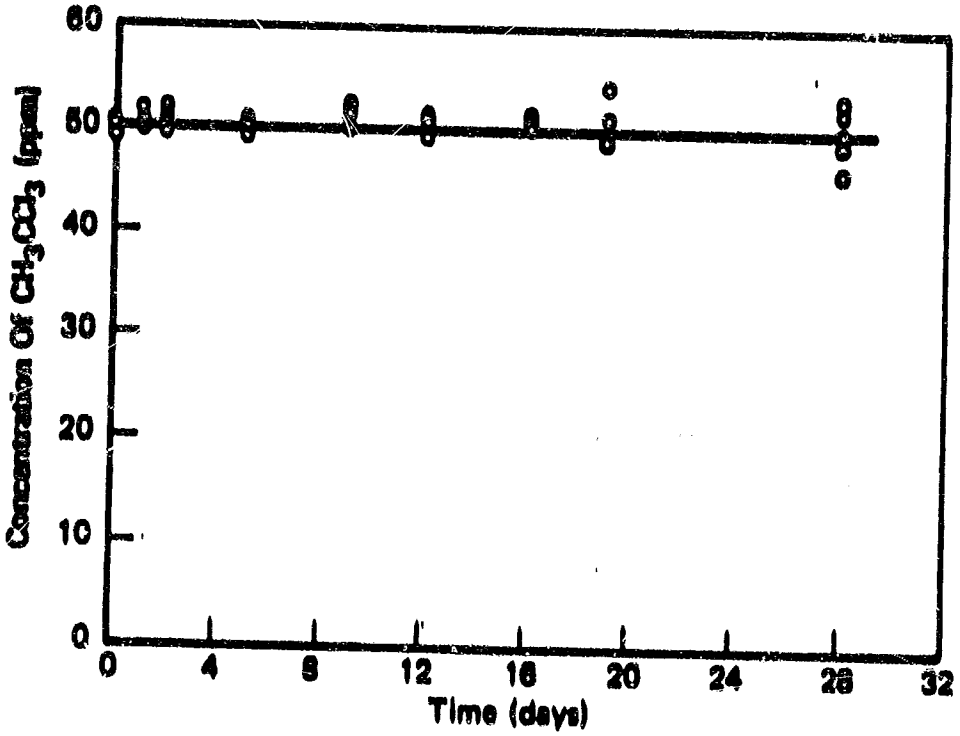

Figure 4. Irradiation of 50 ppm CH_3CCl_3 with 10-11 ppm NO_2 in air with a relative humidity of 32% at 25-27°.

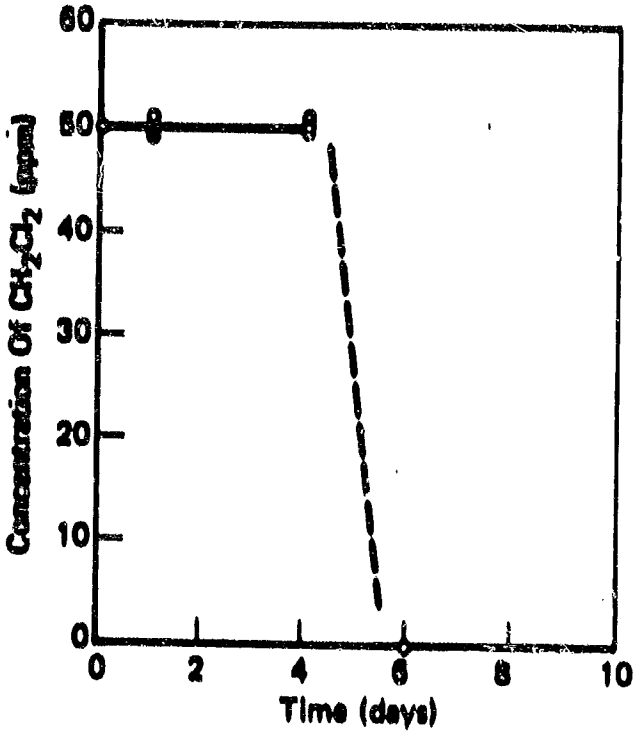

Figure 5. Irradiation of 50 ppm CH_2Cl_2 with 10 ppm NO_2 in air with a relative humidity of 35% at 25-26°.

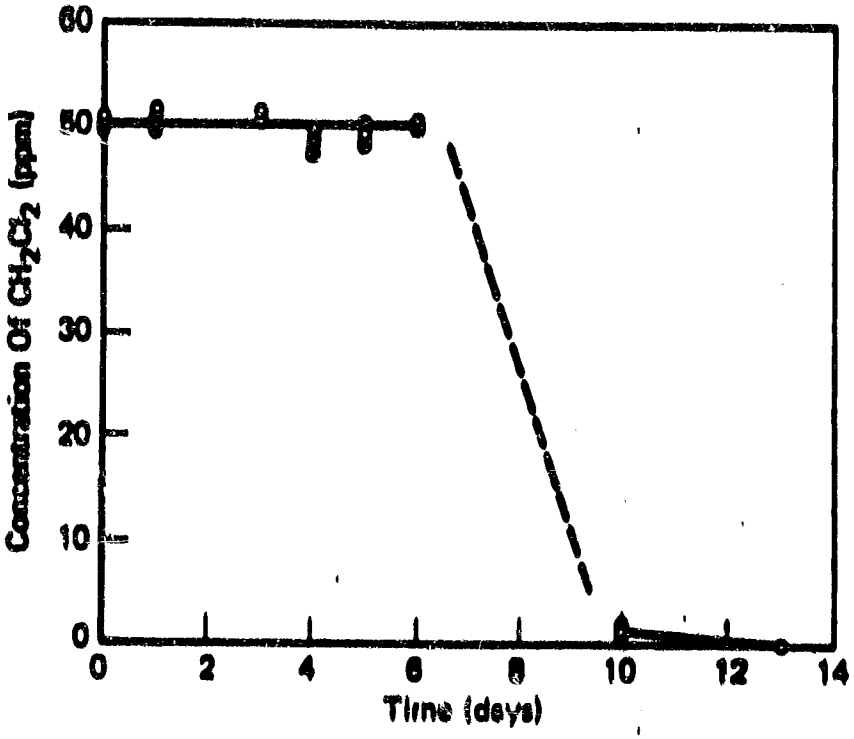

Figure 6. Irradiation of 50 ppm CH_2Cl_2 with 10 ppm NO_2 in air with a relative humidity of 35% at 25-28°.

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High Altitude Simulated Photolyses. Since CH_3CCl_3 and probably CH_2Cl_2 were stable for prolonged times under simulated low altitude atmospheric conditions and both chlorinated solvents evaporated rapidly from water, the question arises as to what is their ultimate fate. One possibility is diffusion into the upper atmosphere (O_3 layer) where short wavelength light (down to 140 nm) is present (Koller, 1965c). Diffusion between the troposphere and the stratosphere was predicted (Machta, 1958) and demonstrated (Manson et al., 1961). In order to qualitatively determine the reactivity of CH_3CCl_3 and CH_2Cl_2 under high altitude conditions we irradiated these materials with short wavelength light. CCl_4 was also studied under these conditions since this compound has been detected in the stratosphere (Lovelock et al., 1973) at an altitude of ~12 kilometers (Lovelock, 1973).

The short wavelength light source was a low pressure mercury arc lamp. The major portion of the emission from this type of lamp occurs at 254 nm (Calvert and Pitts, 1966b). There also may be radiation of 185 nm emitted from this source. The emission from this lamp at 254 nm is 10-40 times as intense as the sun's radiation just outside the earth's atmosphere in the 140-260 or 140-300 nm region. Just within the earth's atmosphere (~40 kilometers altitude) where the sunlight intensity is decreased to ~1/3 of its original intensity in the 200-280 nm range by absorption of its short wavelength radiation by O_3 (Chem. Eng. News, 1966), the lamp would be 30-120 times as intense as sunlight.

The temperature of the atmosphere at the altitude which was simulated is not known; at 11 km the temperature is -50 to -60°C and rises to about 0°C at 50 km (Chem. Eng. News, 1966). The photoreactions were run at 23-33°. The

photoreactions were run at 740-750 mm pressure; the pressure at high altitides is lower, 210 mm at 10 km, 0.75 mm at 50 km (Weast et al., 1964). Pressure probably has an effect on the rates of these photoreactions. The rate could be either increased or decreased with a decrease in pressure depending on the mechanism of the photodecompositions. There is relatively little water in the atmosphere at higher altitudes; most is found in the troposhere (< 11 km) (Chem. Eng. News, 1966).

Irradiation of CH_3CCl_3 in dry air or in air with a relative humidity of 35% with or without NO_2 under our simulated high altitide conditions caused a $\geq$ 90% decomposition of the CH_3CCl_3 within 10 minutes (Figure 7). The reason for the variation in the extent of reaction often 10-20 minutes for the various reaction conditions is not known. Injection of CH_3CCl_3 into the dark reactor which contained O_3 from a previous air photolysis did not lead to any measurable decomposition of the CH_3CCl_3 over a 4 day period.

Simulated high altitude irradiations of CH_2Cl_2 (Figure 8) and CCl_4 (Figure 9) in dry air brought about > 95% decompositions of both compounds within 10 minutes.

Assuming a linear relationship between light intensity and rate of photodecomposition, one can estimate that these three chlorinated compounds would be greater than 90% decomposed within several hours to a few days if the compounds do diffuse to the upper regions of the earth's atmosphere. Since these chlorinated compounds do absorb ultraviolet radiation at 254 nm and are unreactive in the presence of O_3 in the dark and in the presence of NO and NO_2 with longer wavelength light, we suggest that photodissociation is the initial reaction of these materials.

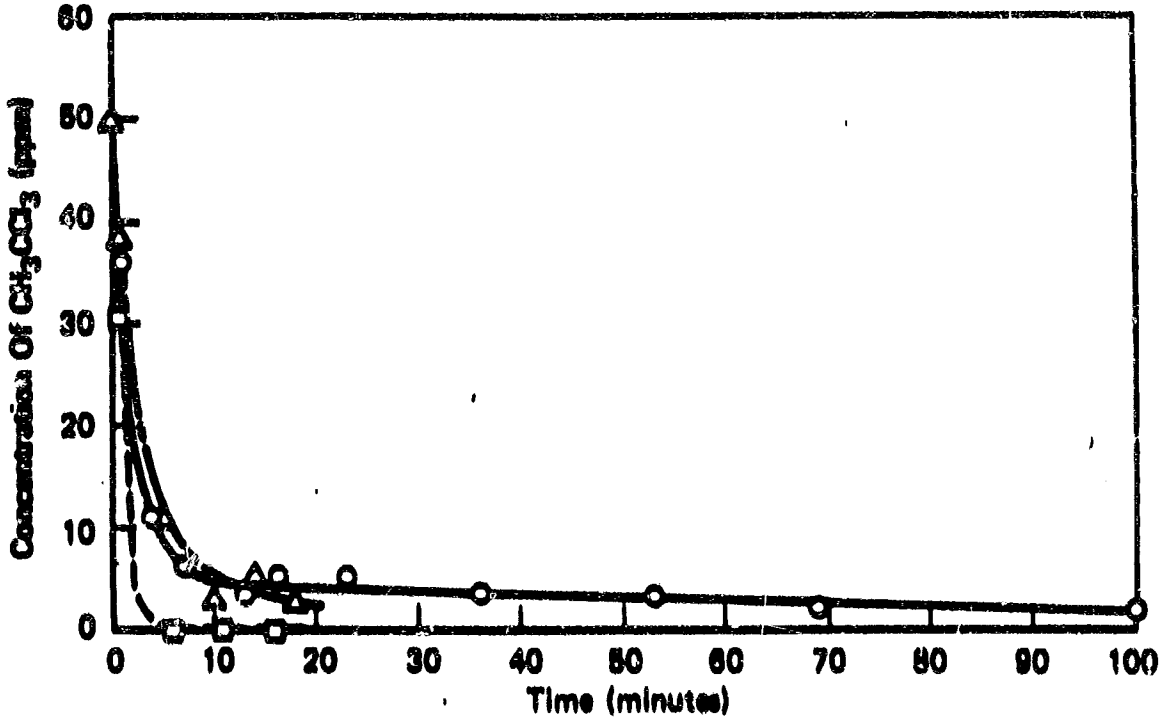

Figure 7. Irradiation (short wavelength) of 50 ppm CH_3CCl_3 in dry air at 24-33° (O,_____); in air with a relative humidity of 35% at 25-33° (□,— — — —); with 9 ppm NO_2 in air with a relative humidity of 35% at 25]33° (△, —·—·)

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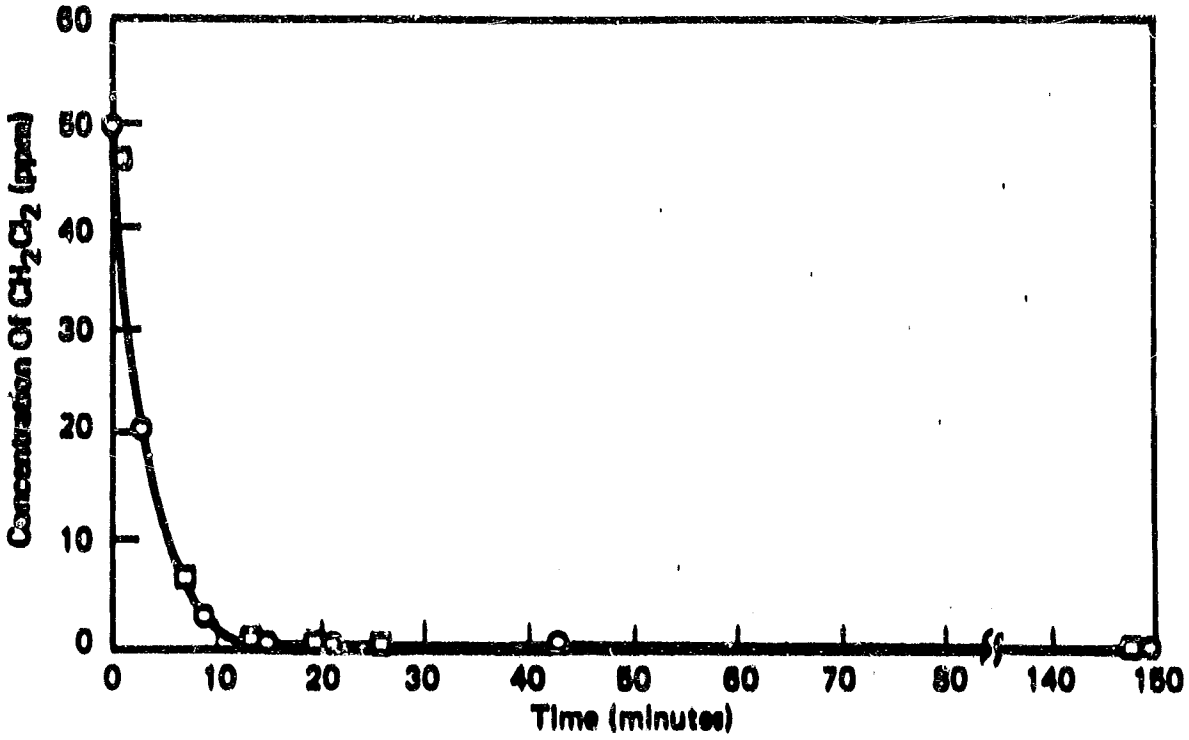

Figure 8. Irradiation (short wavelength) of 50 ppm CH_2Cl_2 in dry air at 23-33° (○ and □ for 2 identical runs)

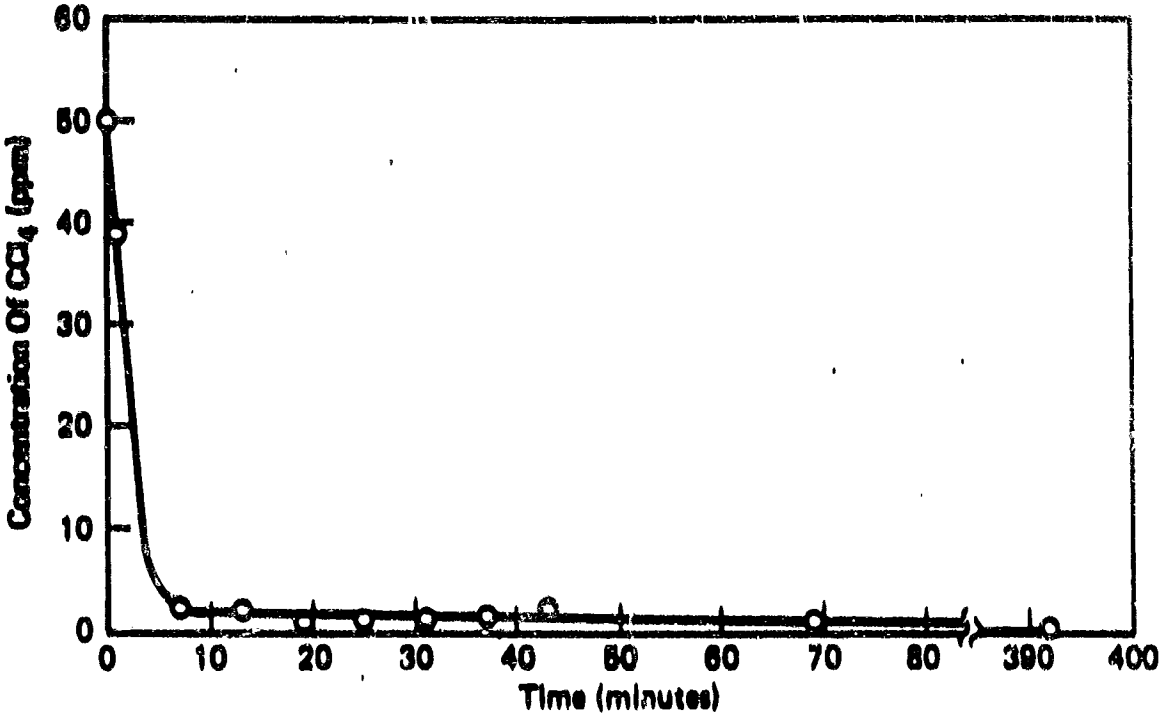

Figure 9. Irrdiation (short wavelength) of 50 ppm CCl_4 in dry air at 23-33°.

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Organic Photochemistry. XIV.[1] Environmental Fate and Effect of Chlorinated Solvents. III.[2] Simulated Atmospheric Photoreactivity of Methylene Chloride, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, and Other Chlorinated Hydrocarbons in the Presence of Other Organic Materials and Reactive Species

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Abstract

In order to estimate the reactivities of chlorinated solvents in the atmosphere, laboratory studies were carried out on the photolysis rates of mixtures of four common chlorinated solvents, three other chlorinated olefins, and 14 other organic compounds under simulated atmospheric conditions. The reactivity of trichloroethylene was determined, and various competitive reactions were carried out, in the presence of nitric oxide, nitrogen dioxide, ozone, chloral, acetaldehyde, bromotrichloromethane, chlorine, phosgene, and dichloroacetyl chloride. The presence of other organic materials and nitric

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[1]Part XIII: (Dilling et al., 1974).

[2]Part II: (Dilling et al., 1974).

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oxide reduced the photochemical reactivity of trichloroethylene. Vinyl chloride, vinylidene chloride and cis-dichloroethylene behaved like 1-chloroethylene whereas the presence of other organic materials did not significantly affect the photochemical reactivities of methylene chloride and 1,1,1-trichloroethane. Trichloroethylene increased the photochemical reactivity of tetrachloroethylene. The reactivities of most hydrocarbons increased in the presence of trichloroethylene. Chlorine atoms (or other similar species) are suggested as possible reactive intermediates.

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The fate and effect of chlorinated solvents which may be discharged to the environment (Murray and Riley, 1973; Farber, 1973) are important from an ecological standpoint. In the preceding paper (Dilling et al., 1974) the reactivities (rates of disappearance) of four of the more important chlorinated solvents in use today, methylene chloride (CH_2Cl_2), 1,1,1-trichloroethane (CH_3CCl_3), trichloroethylene ($CHCl{=}CCl_2$), and tetrachloroethylene ($CCl_2{=}CCl_2$), and several related compounds were determined under simulated atmospheric conditions.

Changes in the reactivity of one organic compound in the presence of another have been reported (Altshuller et al., 1966; Bufalini and Altshuller, 1967; Wilson, 1969; Wilson et al., 1969). For example, Bufalini and Altshuller (1967) reported that the photochemical reactivity of 1-butene ($EtCH{=}CH_2$) increased in the presence of trans-2-butene (trans-$MeCH{=}CHMe$) and nitrogen dioxide (NO_2) compared with the reactivity of $EtCH{=}CH_2$ in the absence of trans-$MeCH{=}CHMe$. On the other hand the reactivity of $EtCH{=}CH_2$ decreased in the presence of mesitylene ($C_6H_3Me_3$). Since the order of increasing photochemical reactivities of these materials by themselves with NO_2 was $C_6H_3Me_3 < EtCH{=}CH_2 <$ trans-$MeCH{=}CHMe$, these authors concluded that leveling effects should exist when combinations of materials were present, e.g., organic contaminants in the atmosphere. That is, the presence of slow-reacting hydrocarbons would decrease the reactivity of the faster reacting hydrocarbons and conversely, the presence of fast-reacting hydrocarbons would increase the reactivity of slower-reacting hydrocarbons. Photolysis of propylene ($MeCH{=}CH_2$)-nitric oxide (NO) mixtures in the presence of $CHCl{=}CCl_2$ produced slight accelerations of both the formation and decay of oxidant as compared with the photolysis of $MeCH{=}CH_2$-NO alone; there was no significant difference in the level of eye irritants (Wilson, 1969; Wilson et al., 1969).

Therefore it was of interest to determine how the presence of other organic materials affected the photochemical reactivities of the chlorinated solvents. These types of data are 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.

Experimental

General. The reactor design and operation, sampling techniques and analyses, materials, and an example of a typical photolysis have been described in the preceding paper under the heading, Short Term (< 24 Hr) Sea Level Simulated Photolyses (Dilling et al., 1974). In most cases the ratio of organic materials to NO was kept near 2:1 because the highest reactivity generally occurs at this ratio (Tuesday, 1963). All experiments were carried out at 26-28°, 35% relative humidity, and with the same light intensity as previously described (Dilling et al., 1974) unless otherwise specified.

Preparation of Ozone-Air Mixtures. A stream of air was passed into a flask in which was suspended a screen electrode. The electrode was charged by a small Telsa coil. The exit gas which contained a mixture of ozone (O_3) and air was collected in a 20 liter Saran® plastic bag which was then used to fill the previously evacuated photochemical reactor. According to a published procedure (Byers and Saltzman, 1959), the concentration of O_3 in the air stream was determined by bubbling the O_3-air stream at a constant rate for a specified length of time through two gas scrubbing towers. The towers contained a solution of 20 g of potassium iodide in 70 ml of 0.002 N sodium thiosulfate, 50 ml of 0.025 M potassium dihydrogen phosphate, and 50 ml of 0.025 M disodium hydrogen phosphate. The above solution was then titrated with a standard 0.002 N iodine solution which contained 0.04% potassium iodide; a 1% starch solution was used

as an indicator. The air flow through the O_3 generator was adjusted so that the concentration of O_3 used in the photochemical reactions was 10-20 ppm.

Results and Discussion

Since various formulations of $CHCl=CCl_2$ (Table I) are marketed, it was of interest to determine if these grades showed different reactivities under simulated atmospheric conditions. The reactivity differences were small; the formulations with lower stabilizer concentrations were more reactive. Table II shows that there is essentially no difference between the reactivities of uninhibited CH_3CCl_3 and the most highly stabilized CHLOROTHENE® VG. All formulations showed very little or no reaction (Dilling et al., 1974).

The photochemical reactivities of a number of chlorinated compounds, standard hydrocarbons (Dilling et al., 1974), and other organic compounds were investigated in combination with other organic materials which had a wide range of reactivities, as shown in Table III.

$CHCl=CCl_2$ was studied more extensively than the other compounds. In all combinations, except those which involved $CHCl=CCl_2$ with $CCl_2=CCl_2$ and with CH_3CCl_3, the photochemical reactivity of $CHCl=CCl_2$ decreased compared with the reactivity of $CHCl=CCl_2$ in the absence of other organic compounds. In the extreme case with $MeOCH_2CHOHMe$ the reactivity of $CHCl=CCl_2$ was reduced about three-fold. In a mixture of $CHCl=CCl_2$ and g-C_6H_{12}, the reactivity of $CHCl=CCl_2$ was about one-half that of g-C_6H_{12} in the mixture, and was somewhat less than that of g-C_6H_{12} in the absence of $CHCl=CCl_2$. These results are qualitatively consistent with the generation of chlorine atoms as reactive intermediates. g-C_6H_{12} is about five times more reactive toward chlorine atoms than is $CHCl=CCl_2$ (Poutsma and Hinman, 1964).

Table I. Photochemical Reactivity of Various Formulations of $CEl=CCl_2$

Stabilizers	Formulation (concn of stabilizers in ppm) MEK-TRI®	Baker Analyzed	Millinkrodt	HI-TRI®
1-Butene oxide (c-$CHEtCH_2O$)	2,000	1,000	—	—
Epichlorohydrin [c-$CH(CH_2Cl)CH_2O$]	1,100	600	—	—
Ethyl acetate (EtOAc)	500	250	—	—
Diisobutylene (t-$BuCH_2CMe=CH_2$)	250	125	—	—
N-Methyl pyrrole (c-C_4H_4NMe)	125	75	—	—
Diisopropylamine (i-Pr_2NH)	20	30	45	20
Total	3,995	2,075	45	20
Time (hr) for 50% disappearance of $CEl=CCl_2$[a]	4.4	4.1	3.5	3.5

[a]Initial concentrations: 10 ppm $CEl=CCl_2$, 5 ppm NO, 35% relative humidity.

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Table II. Photochemical Reactivity of Various Formulations of CH_3CCl_3

Stabilizers	Formulation (concn of stabilizers in per cent)		
	CHLOROTHENE® VG	Technical	Unbibited
Dioxane [c-O(C_2H_4)$_2$O]	4.0	—	—
Nitromethane ($MeNO_2$)	0.5	—	—
c-$C_5H_8Cl_2$	0.5	—	—
c-$C_4H_4Me_2$	0.0025	—	—
s-Butyl alcohol (s-BuOH)	—	0.25	—
Total	5.0025	0.25	0.0
Time (hr) for indicated % disappearance of CH_3CCl_3	20.9 (< 5%)	16.7 (< 5%)	23.5 (< 5%)

[a]Initial concentrations: 10 ppm CH_3CCl_3, 5 ppm NO, 35% relative humidity.

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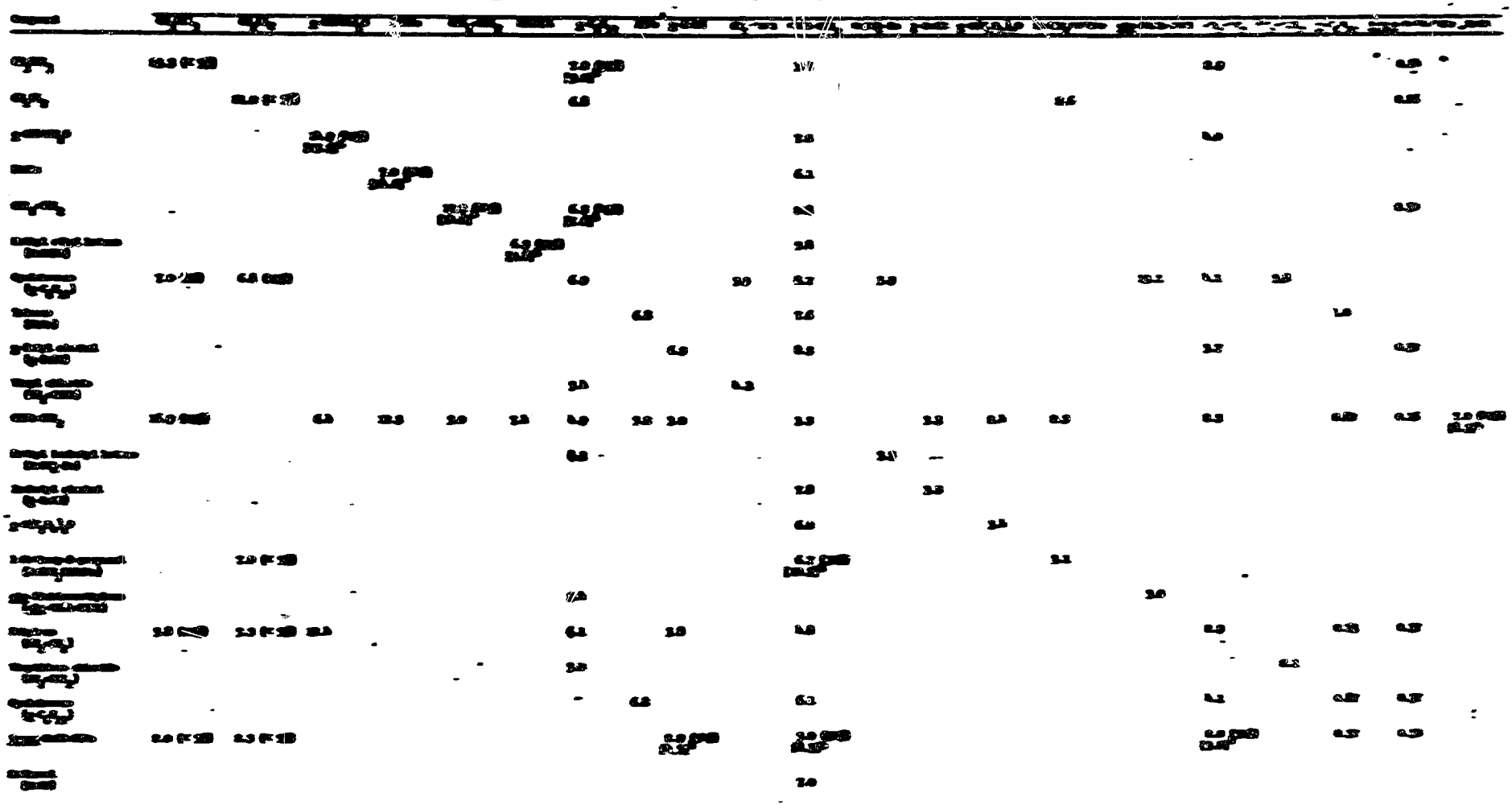

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It should also be noted that in nearly all cases, the photochemical reactivity of the other organic material was substantially increased over that of the reactivity in the absence of $CHCl=CCl_2$. In no case was there a reduction in reactivity. The reactivities of c-CH_2CH_2O, $CCl_2=CCl_2$, n-BuOH, and trans-MeCH=CHMe changed the most dramatically, while the reactivities of EtOAc, i-BuOH, $CH_2=CH_2$, and c-C_6H_{10} changed only slightly. Thus, in an atmosphere which contains a wide variety of reactive materials, it cannot be determined which organic material(s) are the most reactive simply by investigating only single component systems, particularly where $CHCl=CCl_2$ is involved.

In general, the reactivities of CH_3CCl_3, CH_2Cl_2, and $CCl_2=CCl_2$, in combination with materials other than $CHCl=CCl_2$, were not appreciably affected. In only two cases, the combinations of CH_2Cl_2 with c-C_6H_{12} and CH_3CCl_3 with $CH_2=CH_2$, was there the possibility of some interaction. However, in both cases the reactivity of only one of the compounds was affected whereas the other remained nearly unchanged. However the reactivities of the chlorinated solvents are still very low compared with the reactivity of a "slow reactor" such as c-C_6H_{12} alone. Although $CCl_2=CCl_2$ was not affected appreciably by c-C_6H_{12}, $CH_2=CH_2$, or trans-MeCH=CHMe, it was considerably more reactive when $CHCl=CCl_2$ was present.

Since the photochemical reactivity of $CHCl=CCl_2$ was significantly reduced by the presence of a wide variety of organic materials, it was of interest to determine if other olefinic materials would behave similarly. $CH_2=CH_2$, which is normally moderately reactive, shows a slightly reduced photochemical reactivity in combination with a wide variety of materials (Table III). The reactivity of the other organic materials in conjunction with $CH_2=CH_2$ generally increased, particularly in the case of n-BuOH. The photochemical reactivity of trans-MeCH=CHMe, which is very reactive by itself, was only slightly changed by the presence of other organic materials, with the exception of $CHCl=CCl_2$.

The reactivity of some competitive materials did change, e.g., n-BuOH and c-C_6H_{10} increased in reactivity, while $CHCl{=}CCl_2$ decreased markedly in reactivity.

When the chlorinated ethylenes were involved in competition experiments with c-C_6H_{12}, some significant changes were observed. In all cases excepting $CCl_2{=}CCl_2$, the photochemical reactivity of c-C_6H_{12} increased while that of the chlorinated ethylenes decreased. cis-CHCl=CHCl was more than four times less reactive than it was in the absence of c-C_6H_{12}.

A "real" atmospheric environment was simulated by adding $CHCl{=}CCl_2$ to the reactor which contained several other materials which had varying reactivities (Table IV). It was again observed that many of the reactivities changed in the presence of other organic compounds. $CHCl{=}CCl_2$, in particular, was reduced in reactivity by more than a factor of three. It is also interesting to note that the reactivities of c-C_6H_{12} and trans-MeCH=CHMe were nearly identical to those found in the experiment where these two materials were the only organic compounds present (Table III).

The ratio of $CHCl{=}CCl_2$ to other organic materials reasonably can be expected, under real atmospheric situations, to range from very high (at a $CHCl{=}CCl_2$ emission point) to near zero (some distance downwind from the emission point). In order to demonstrate what might happen to the photochemical reactivity of $CHCl{=}CCl_2$ under these conditions, c-C_6H_{12} (an unreactive compound) was chosen as a model organic contaminant in an atmosphere which contained $CHCl{=}CCl_2$. By holding the NO concentration constant, it was found that the half-life of $CHCl{=}CCl_2$ increased from 3.5 hours (only $CHCl{=}CCl_2$ present) to more than 20 hours [ratio of $CHCl{=}CCl_2$ to c-C_6H_{12} 0.2 (Table V)]. A half-life of 20 hours or more indicates that $CHCl{=}CCl_2$ would be much less reactive than c-C_6H_{12} ($t_{1/2}$ = 6.9 hours). In contrast, the photochemical reactivity of c-C_6H_{12} increased markedly, from a half-life of 6.9 hours to < 1 hour, as the concentration of c-C_6H_{12} decreased.

Table IV. Photochemical Reactivity of $CHCl=CCl_2$ in Combination with Several Organic Materials

Compound[b]	Time (hr) for 50% disappearance of compound[a]	
	In mixture	In absence of other organic material[c]
c-$CH_2(CH_2)_4O$	12.9	19.0
$CHCl=CCl_2$	11.1	3.5
$CH_2=CH_2$	3.5	2.9
c-C_6H_{10}	0.35	0.87
trans-MeCH=CHMe	0.35	0.30

[a]All reactions carried out with 35% relative humidity. [b]Each compound initially present at 10 ppm (total 50 ppm organic compounds); NO initial concentration 25 ppm. [c]Initial concentrations: 10 ppm organic compound, 5 ppm NO.

Table V. Effect of Organic Compound Ratio of Photochemical Reactivities of $CHCl=CCl_2$ and $c\text{-}C_6H_{12}$

Initial concentration, ppm[a]		Ratio	Initial NO	Time (hr) for 50% disappearance	
$CHCl=CCl_2$	$c\text{-}C_6H_{12}$	$\frac{[CHCl=CCl_2]}{[c\text{-}C_6H_{12}]}$	concentration, ppm	$CHCl=CCl_2$	$c\text{-}C_6H_{12}$
10	0	∞	5	3.5	--
10	0.01	1000	5	3.3	--
10	0.1	100	5	2.7	~0.9
10	0.2	50	5	3.5	1.3
10	2	5	5	5.6	2.2
10	10	1	5	>16	5.5
2	10	0.2	5	>20	6.7
0	10	0	5	--	6.9
10	2	5	10	6.6	2.8
10	10	1	10	8.7	4.9

[a]All reactions carried out with 35% relative humidity.

The photochemical reactivity of each component in the $CHCl=CCl_2$ - o-C_6H_{12} system also was dependent on the NO concentration, but the effect was dependent on the relative amounts of the two organic components. In the 10:2 ratio ($CHCl=CCl_2$:o-C_6H_{12}) experiments, the reactivity of each organic component decreased as the NO concentration was changed from 5 ppm to 10 ppm. The opposite effect was observed when the initial ratio of organic compounds was 10:10. In the latter case, at the 5 ppm NO level, $CHCl=CCl_2$ appeared to cease reacting, which suggested that all the NO or NO_2 had been consumed.

In a series of experiments similar to those described in the preceding paragraph, o-$CHEtCH_2O$, which has a very low reactivity in the absence of other organic compounds, was used as a competitive material with $CHCl=CCl_2$ at various ratios. Similar to the results described above, a trend was observed where the photochemical reactivity of $CHCl=CCl_2$ decreased as its concentration decreased, and the photochemical reactivity of o-$CHEtCH_2O$ increased as its concentration decreased (Table VI).

The conclusions from these competitive experiments can be summarized as follows. In the presence of other organic materials, the photochemical reactivity of $CHCl=CCl_2$ changed, as did the reactivities of the other organic materials when they were combined with $CHCl=CCl_2$. The photochemical reactivity of $CHCl=CCl_2$ decreased by as much as three times when mixed with $MeOCH_2CHOHMe$, while the photochemical reactivity of the competitive reactant generally increased, in some cases by as much as a factor of two (e.g., with o-$CHEtCH_2O$, trans-MeCH=CHMe, and n-BuOH). If the ratio of $CHCl=CCl_2$ to the organic materials (e.g., o-C_6H_{12}) was < 1, $CHCl=CCl_2$ became quite unreactive and the reactivity of the organic materials was not significantly affected. It follows that in a real atmosphere where the ratio of organic contaminants to $CHCl=CCl_2$ is quite large,

Table VI. Effect of Organic Compound Ratio on Photochemical Reactivities of $CHCl{=}CCl_2$ and o-$CH_3CH_2CH_2O$

Initial concentration, ppm[a]		$\frac{[CHCl{=}CCl_2]}{[o\text{-}CH_3CH_2CH_2O]}$	Initial NO concentration, ppm	Time (hr) for 50% disappearance	
$CHCl{=}CCl_2$	o-$CH_3CH_2CH_2O$			$CHCl{=}CCl_2$	o-$CH_3CH_2CH_2O$
10	0	∞	5	3.5	--
10	2	5	5	4.2	4.1
10	10	1	10	7.0	6.4
0	10	0	5	--	14.9[b] (47%) [15.[c]]

[a]All reactions carried out with 35% relative humidity. [b]Time required for 47% disappearance of o-$CH_3CH_2CH_2O$. [c]Estimated value for 50% disappearance of compound.

the photochemical smog symptoms resulting from $CHCl=CCl_2$ probably would be quite small. If the ratio of $CHCl=CCl_2$ to the other organic materials (e.g., o-C_6H_{12}) was ≥ 1, $CHCl=CCl_2$ appeared to behave as a catalyst and increased the rate of decomposition of the organic materials. $CH_2=CHCl$, $CH_2=CCl_2$, and cis-CHCl=CHCl behaved like $CHCl=CCl_2$. Of the compounds studied only $CHCl=CCl_2$ increased the photochemical reactivity of $CCl_2=CCl_2$. The other organic materials examined did not alter its reactivity significantly. The photochemical reactivities of CH_2Cl_2 and CH_3CCl_3 were not affected significantly by the presence of any other organic materials. The photochemical reactivities of $CH_2=CH_2$, trans-MeCH=CHMe, o-C_6H_{10}, and o-C_6H_{12} generally were less affected by other organic materials, excluding the chlorinated ethylenes, than was the reactivity of $CHCl=CCl_2$. There was some indication of leveling effects (Bufalini and Altshuller, 1967) when only hydrocarbons were involved. When $CHCl=CCl_2$ was one of the components in the combination experiments, the leveling effect was not important, whereas other catalytic effects may be.

The above results which showed that, for example, o-C_6H_{12} was less reactive than $CHCl=CCl_2$ in individual experiments, whereas in experiments with both $CHCl=CCl_2$ and o-C_6H_{12} present, the reactivities were reversed, with o-C_6H_{12} being more reactive. This change in reactivity indicated that a reactive species which was formed from $CHCl=CCl_2$, reacted more rapidly with o-C_6H_{12} than with $CHCl=CCl_2$. An attempt was made to identify this reactive species. This involved the use of NO, NO_2, O_3, and other potentially reactive species with $CHCl=CCl_2$ and other organic materials in individual and competition experiments.

Previous work (Dilling et al., 1974) showed that with slow reacting materials, both NO and NO_2 gave similar results, while with very reactive materials, such as trans-MeCH=CHMe and o-C_6H_{10}, NO_2 produced an increased reactivity over that observed when NO was used (Table VII). Other data, shown in Table VII, indicate that O_3 is much more reactive than is NO or NO_2.

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Table VII. Photochemical Reactivity of Organic Materials with NO, NO_2, and O_3

Compound[a]	Time (hr) for 50% disappearance[b]			Relative reactivity		
	NO[c,d]	NO_2[d,e]	O_3[f]	NO	NO_2	O_3
c-CHEtCH$_2$O	14.9[g] [13.9][h] (47%)	15.2	0.83	0.23	0.19	0.51
c-C_6H_{12}	6.9	7.5	1.0	0.51	0.38	0.42
CHCl=CCl_2	3.5	2.9[i]	0.42	1.0	1.0	1.0
CH_2=CH_2	2.9	2.3[i]	--	1.2	1.2	--
c-C_6H_{10}	0.87	0.19	--	4.0	15.1	--
trans-MeCH=CHMe	0.30	0.17	--	11.7	16.9	--

[a]All compounds at 10 ppm initially. [b]All reactions carried out with 35% relative humidity. [c]All initial concentrations 5 ppm. [d]Data from Dilling et. al. (1974). [e]All initial concentrations 5 ppm except as noted. [f]Initial concentration 15 ppm. [g]Time required for 47% disappearance of c-CHEtCH$_2$O. [h]Estimated value for 50% disappearance of compound. [i]Initial NO_2 concentration 16.8 ppm.

Table VII shows that the relative reactivity of g-$CHEtCH_2O$ is greater with O_3 than with NO or NO_2. The differences in the relative reactivities can be partially explained on the basis that all of the reactions do not involve the same type and quantity of reactive intermediates. For example, some organic materials can cause a faster oxidation of NO to NO_2 than do others, and some organic materials produce O_3 at a faster rate than do others. Consequently if O_3 is one of the key reactive intermediates, then its concentration will be important in determining how reactive a material is in the reactor as well as in the real atmosphere.

The simultaneous use of slow and fast reacting organic materials in competitive photochemical reactions should eliminate some of the differences in relative reactivity caused by different "initiators" such as NO, NO_2 or O_3. Also, this type of system would approximate a real atmosphere more closely. In these competitive experiments the ratios of the photochemical reactivities of the compound pairs were very similar even though the absolute reactivities varied as the "initiator" was varied from NO to NO_2 to O_3 (Table VIII). O_3 appeared to have a slightly greater leveling effect than did NO or NO_2. Since these ratios in the competitive experiments are more nearly alike than those in the individual experiments, this method is suggested as a better way to evaluate how a certain organic material would behave in the real atmosphere. Also, NO, NO_2 and O_3 could be used interchangeably or in combination.

Comparison of the data in Tables VII and VIII further suggests that $CHCl=CCl_2$ caused the formation of some reactive species other than O_3 in the photochemical reactions. Whether NO, NO_2, or O_3 was used as the "initiator", $CHCl=CCl_2$ was more reactive than g-$CHEtCH_2O$ and g-C_6H_{12}, whereas in competitive experiments, $CHCl=CCl_2$ was less reactive. If O_3 were the only important reactive species, one would expect similar ratios of reactivities from individual and combination experiments. The unknown reactive species appeared to be highly reactive toward $CHCl=CCl_2$ in individua

Table VIII. Photochemical Reactivity of Organic Materials in Competition Experiments which Involve NO, NO_2, and O_3

Compound pair[a]	Time (hr) for 50% disappearance of compound in combination[b]			Reactivity Ratio		
	NO[c]	NO_2[c]	O_3[c]	NO	NO_2	O_3
c-C_6H_{12}	4.9	5.0	0.73	1.8	2.1	1.2
CHCl=CHCl	8.7	10.5	0.87			
c-CH_2CH_2O	6.4	8.0	0.5	1.1	1.3	1.0
$CHCl=CCl_2$	7.0	10.5	0.5			
trans-MeCH=CHMe	0.37	0.13	--	1.0	1.1	--
c-C_6H_{10}	0.37	0.15	--			

[a]Initial concentration 10 ppm each. [b]All reactions carried out with 35% relative humidity. [c]Initial concentration 10 ppm.

experiments, but more reactive towards c-C_6H_{12} when the latter was present. This led us to consider possible products from the photochemical oxidation of $CHCl=CCl_2$ such as phosgene ($COCl_2$), chlorine (Cl_2), chloral (CCl_3CHO) (Jeffrey, 1967), dichloroacetyl chloride ($CHCl_2COCl$), etc. (Dilling and Tefertiller, 1974). These materials could lead to free radicals such as chlorine atoms, trichloromethyl radicals ($\cdot CCl_3$), and acyl radicals ($R\dot{C}=O$), etc., under the reaction conditions. Table IX shows the photochemical reactivity of $CHCl=CCl_2$ with a number of these possible products and also with acetaldehyde (MeCHO) and bromotrichloromethane ($CBrCl_3$).

CCl_3CHO increased significantly the reactivity of $CHCl=CCl_2$ in comparison with the effect of NO or NO_2. Chloral would be expected to lead to two types of radicals, trichloroacetyl ($CCl_3\dot{C}=O$) and $\cdot CCl_3$. MeCHO was used to determine if the acetyl radical ($Me\dot{C}=O$) (Altshuller et al., 1967) would lead to similar results. MeCHO decreased the reactivity of $CHCl=CCl_2$ with NO. $CBrCl_3$ which produces $\cdot CCl_3$ radicals, did not enhance the reactivity of $CHCl=CCl_2$ whereas $CBrCl_3$ in combination with NO, did appear to increase the reactivity of $CHCl=CCl_2$. From these experiments, it is not clear why CCl_3CHO was so active.

Chlorine atoms also are potential intermediates in the photochemical oxidation of $CHCl=CCl_2$. Indeed, $CHCl=CCl_2$ was found to be extremely reactive in a photochemical reaction with Cl_2, even more reactive than with O_3. NO in combination with Cl_2 also increased the reactivity of $CHCl=CCl_2$.

Although $COCl_2$ was a product from the photochemical oxidation of $CHCl=CCl_2$ (Dilling and Tefertiller, 1974), and the photolysis of $COCl_2$ would be expected to give chlorine atoms, $COCl_2$ by itself did not increase the reactivity of $CHCl=CCl_2$. A combination of $COCl_2$ and NO did increase the reactivity of $CHCl=CCl_2$ significantly. Formation of chlorine atoms more slowly from $COCl_2$ than from Cl_2 could explain this difference in reactivity.

Table IX. Photochemical Reactivity of $CHCl=CCl_2$ with Possible Photoxidation Products and Other Co-reactants

Co-reactants (concentration in ppm)[a]	Time (hr) for 50% disappearance of $CHCl=CCl_2$ unless otherwise indicated
NO (5)	3.5
NO_2 (16.8)	2.9
CCl_3CHO (2)	3.2
CCl_3CHO (5)	0.93
CCl_3CHO (10)	0.49
MeCHO (10)	4.7 (15%) [15.7][b]
MeCHO (10) + NO (5)	6.4 (30%) [10.7][b]
$CBrCl_3$ (10)	3.0
$CBrCl_3$ (10) + NO (10)	1.3
Cl_2 (1)	1.8 (28%) [3.1][b]
Cl_2 (1) + NO (5)	0.43
Cl_2 (2)	0.18
Cl_2 (5)	<0.08 (85%) [<0.09][b]
Cl_2 (10)	<0.11 (100%) [<0.09][b]
$COCl_2$ (5)	3.8
$COCl_2$ (5) + NO (5)	2.3

[a]Initial concentration of $CHCl=CCl_2$ was 10 ppm in each case. All reactions carried out with 35% relative humidity. [b]Estimated value for 50% disappearance of $CHCl=CCl_2$.

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Using a quite unreactive organic material such as g-CH_2CH_2O, CCl_3CHO by itself or in combination with NO or NO_2 significantly increased the reactivity of g-CH_2CH_2O (Table X). To a lesser extent, both $CHCl_2CCl$ with NO and $CHCl=CCl_2$ with NO increased the reactivity of g-CH_2CH_2O.

Table XI shows data for the reactivity of various organic materials with NO, MeCHO, MeCHO with NO, and CCl_3CHO. MeCHO was chosen since it is a very common product in an oxidizing atmosphere. MeCHO was not as effective an "initiator" as was NO in the photochemical reactions. In the presence of MeCHO and NO, highly reactive compounds, such as g-C_6H_{10} and trans-MeCH=CHMe, became more reactive than in the presence of NO alone. Slower reacting materials, such as g-C_6H_{12} and $CHCl=CCl_2$, became less reactive. MeCHO seems to be an inhibitor in these latter cases. Table XI also shows the reactivity of organic compounds with CCl_3CHO. In combination with compounds which react slowly in the presence of NO, CCl_3CHO increased the compound's reactivity while with materials which react rapidly in the presence of NO, CCl_3CHO caused a rate retardation.

Since Cl_2 greatly increased the reactivity of $CHCl=CCl_2$ (Table IX), it was of interest to determine how Cl_2 would behave in the presence of more than one compound. Table XII shows three combinations with all compounds showing very high reactivities. Especially noteworthy is the combination of $CHCl=CCl_2$ and $CCl_2=CCl_2$ with Cl_2. Based on the known reactivities of $CH_2=CH_2$, $CHCl=CCl_2$, and $CCl_2=CCl_2$ with Cl_2 and with chlorine atoms (Chiltz et al., 1963) it is obvious that there are reactive species other than chlorine atoms involved in the destruction of these olefins. Interestingly the reactivity of $CCl_2=CCl_2$ increased markedly in the presence of $CHCl=CCl_2$, over that observed in the presence of $CH_2=CH_2$, just as the reactivity of $CCl_2=CCl_2$ increased in the presence of $CHCl=CCl_2$ and NO compared with the reactivity observed in the presence of NO alone (Table XI).

Table XI. Photochemical Reactivity of Organic Materials with NO, MeCHO, and CCl_3CHO

Compound[a]	Time (hr) for 50% disappearance of compound unless otherwise indicated[b]			
	NO[c]	MeCHO[d]	MeCHO[e] + NO[e]	CCl_3CHO[d]
o-CHEtCH$_2$O	14.9 (47%) [15.9][f]	--	--	3.3
o-C_6H_{12}	6.9	14.0 (21%) [33.3][f]	13.2 (35%) [21.7][f]	3.4
$CHCl=CCl_2$	3.5	4.7 (15%) [15.6][f]	6.1 (20%) [15.3][f]	0.45
			6.4 (31%)[g] [10.3][f]	
$CH_2=CH_2$	2.9	--	7.6 (36%) [10.6][f]	3.1
o-C_6H_{10}	0.87	4.4 (18%) [12.2][f]	0.30 0.67[g]	1.8
trans-MeCH=CHMe	0.30	2.4 (19%) [6.3][f]	0.22	2.3

[a]All compounds at 10 ppm initially. [b]All reactions run with 35% relative humidity. [c]All initial concentrations 5 ppm. [d]All initial concentrations 10 ppm. [e]All initial concentrations 10 ppm except as noted. [f]Estimated value for 50% disappearance of compound. [g]Initial concentration of MeCHO 5 ppm.

Table XII. Photochemical Reactivity of Organic Materials in Competition Experiments Which Involve Cl_2

Compound pair[a]	Time (hr) for 50% disappearance (unless otherwise indicated) of compound in combination[b]
$CHCl=CCl_2$	<0.12 (100%)
$CCl_2=CCl_2$	<0.12 (100%)
$CH_2=CH_2$	0.37
$CCl_2=CCl_2$	1.5 (46%) [1.7][c]
$CH_2=CH_2$	0.57
$CHCl=CCl_2$	0.57 (49%) [0.58][c]

[a]Initial concentration 10 ppm each. [b]All reactions carried out with 35% relative humidity. Initial Cl_2 concentration 5 ppm. [c]Estimated value for 50% disappearance of compound.

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Other possible reactive species in these photochemical reactions are singlet oxygen (Pitts et al., 1969; Kummler et al., 1969; Coomber et al., 1970; Hamming, 1970; Kummler et al., 1970; Altshuller and Bufalini, 1971), oxychlorine radicals such as ClO· and ClOO· (Kiryushin and Poluektov, 1968; Johnston et al., 1969; Pierrard, 1969; Altshuller and Bufalini, 1971), and hydroxyl radicals (Stedman et al., 1970).

In conclusion, we have found that the relative photochemical reactivities of slow reacting organic materials are similar whether NO or NO_2 is added to the reactor in individual experiments. Replacing NO with NO_2 significantly affected the reactivities of fast reacting compounds. Use of O_3 as the "initiator" also leads to relative reactivities which are different from those observed with NO or NO_2. If more than one organic material is investigated in competitive experiments, the relative photochemical reactivities of these materials with NO, NO_2, or O_3 are quite similar. Since a real atmosphere generally contains a mixture of organic pollutants, these results suggest that competitive experiments which involve more than one organic material (perferably several materials) are necessary in order to more accurately assess the relative photochemical reactivities of various organic materials. Our results also suggest that the key reactive intermediate(s) in these photochemical reactions is not NO, NO_2, or O_3, but rather some other species which is formed during these reactions. The competitive experiments especially indicate that a common reactive intermediate , oxygen atoms possibly (Altshuller and Bufalini, 1965), is formed irregardless of whether NO, NO_2 or O_3 is used as the "initiator". Chlorine atoms may be reactive intermediates when $CHCl=CCl_2$ is photolyzed in the presence of NO, NO_2 or O_3. Cl_2 is more reactive than O_3 with $CHCl=CCl_2$ in photochemical reactions. Photolysis of $CHCl=CCl_2$ and $CCl_2=CCl_2$ together in the presence of Cl_2, leads to very rapid destruction of both olefins.

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Organic Photochemistry. XV.[1] Environmental Fate and Effect of Chlorinated Solvents. IV.[2] Product Studies on the Simulated Atmospheric Photolyses of Methylene Chloride, 1,1,1-Trichloroethane, Trichloroethylene, and Tetrachloroethylene

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Abstract

In order to determine the products formed from four important chlorinated solvents in the atmosphere, laboratory studies were carried out under simulated atmospheric conditions in a flow reactor. Under sea level conditions, which included nitrogen dioxide and water vapor in air with ultraviolet radiation (λ>295 nm) followed by trapping the products with the water, trichloroethylene gave dichloroacetic acid (40%) and hydrogen chloride (52%); tetrachloroethylene gave trichloroacetic acid (36%), hydrogen chloride (16%), and chlorine (33%). Phosgene was detected as an intermediate from

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[1]Part XIV: (Dilling and Bredeweg, 1974).

[2]Part III: (Dilling and Bredeweg, 1974).

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both chlorinated olefins while trichloroacetyl chloride, chlorine, and carbon dioxide were also detected from tetrachloroethylene. Under high altitude conditions, which involved dry air and short wavelength light (λ254 nm principally), methylene chloride gave hydrogen chloride (9%), chlorine (16%), chloric acid (8%), and perchloric acid ($\geq$30%); 1,1,1-trichloroethane gave hydrogen chloride (20%), chlorine (39%), chloric acid (3%), perchloric acid ($\geq$24%), acetic acid (10%), and a trace of chloroacetic acid.

The fate and effect of chlorinated solvents which may be discharged to the environment (Murray and Riley, 1973a; Murray and Riley, 1973b; Farber, 1973) are important from an ecological standpoint. In preceding papers of this series (Dilling et al., 1974; Dilling and Bredeweg, 1974) the reactivities (rates of disappearance) of the four most widely used chlorinated solvents, methylene chloride (CH_2Cl_2), 1,1,1-trichloroethane (CH_3CCl_3), trichloroethylene ($CHCl=CCl_2$), and tetrachloroethylene ($Cl_2C=CCl_2$), were determined under simulated atmospheric conditions. The two olefinic solvents, $CHCl=CCl_2$ and $Cl_2C=CCl_2$, underwent reaction under simulated sea level atmospheric conditions which included ultraviolet radiation of wavelength greater than 290 nm, nitric acid (NO) or nitrogen dioxide (NO_2), and water vapor in air (Dilling et al., 1974). CH_2Cl_2 and CH_3CCl_3 were unreactive under these simulated sea level conditions, but did react rapidly under simulated high altitude atmospheric conditions which included short wavelength ultraviolet radiation (primarily 254 nm) in air (Dilling et al., 1974). Having determined that these four compounds do decompose under certain simulated atmospheric conditions, another important consideration is, into what products are these materials transformed under these conditions. The experiments reported in this paper were carried out under conditions as near as possible to those found in the environment. However, higher concentrations of the compounds in air than would normally be found in the environment were required, in order to collect enough material in a reasonable length of time for reliable product identification.

No data have been reported on the products from either CH_2Cl_2 or CH_3CCl_3 under simulated atmospheric conditions. However, one can postulate in some cases what the products might be, based on other chemical studies of these materials. Liquid CH_2Cl_2 was unreactive toward oxygen (O_2) even at 290° (Carlisle and Levine, 1932a; Hardie, 1964a), and produced only trace amounts of hydrogen chloride (HCl) (Archer, 1970). Photolysis of liquid CH_3CCl_3 in the presence of air gave chloroacetyl chloride ($CH_2ClCOCl$), chloroacetic acid (CH_2ClCO_2H)(Archer and Harter, 1964), and HCl (Scott, 1961). $COCl_2$ was reported as a product from CH_3CCl_3 when oxidized by the atmosphere at an elevated temperature (Hardie, 1964b).

The products formed from $CHCl=CCl_2$ under simulated atmospheric conditions were reported to be phosgene ($COCl_2$), carbon monoxide (CO), and dichloroacetyl chloride ($CHCl_2COCl$)(Wilson et al., 1968). It has been suggested that formyl chloride (HCOCl) is probably a product (Altshuller and Bufalini, 1971). No formaldehyde (HCHO) was formed (Wilson et al., 1969; Altshuller and Bufalini, 1971), and the amount of total aldehydes formed probably was no more than the background (i.e. with no $CHCl=CCl_2$ present)(Hamming, 1967). Vapor phase photooxidation of $CHCl=CCl_2$ with short wavelength light gave $CHCl_2COCl$ and $COCl_2$ (Dahlberg, 1969). Liquid phase photooxidation gave $CHCl_2COCl$, trichloroethylene oxide (o-$CHClCCl_2O$), $COCl_2$, CO, HCl, chlorine (Cl_2), formic acid (HCO_2H), and glyoxylic acid ($HCOCO_2H$) in varying amounts under different conditions (Carlisle and Levine, 1932b; Müller and Ehrmann, 1936; Kirkbride, 1942; Gilbert, 1960; Shepherd, 1962; Yasnitskii et al., 1965; Howard and Montgomery, 1967; Yasnitskii et al., 1967). Photooxidation

of $CHCl_2COCl$ gave CO and $COCl_2$ via radical intermediates which initiated the oxidation of chloroethylenes (Yasnitskii et al., 1966). Vapor phase oxidation of $CHCl=CCl_2$ gave carbon dioxide (CO_2) and HCl (Hardie, 1964c). The oxidation of liquid $CHCl=CCl_2$ with air or O_2 gave $CHCl_2COCl$, o-$CHClCOCl_2O$, $COCl_2$, CO, HCl, chloral (CCl_3CHO), and various peroxides (Erdmann, 1912; Mugdan and Wimmer, 1929; Carlisle and Levine, 1932b; Mugdan and Wimmer, 1934; McKinney et al., 1955; Shepherd, 1962; Hardie, 1964a; Shell International, 1966; Howard and Montgomery, 1967; Jeffrey, 1967; Mayo and Honda, 1968; Archer, 1970). $CHCl=CCl_2$ reacted with ozone (O_3) to give HCl, $COCl_2$, CO, $CH_2ClCOCl$, and an "oxide of chlorine" (Erdmann, 1912).

The only product data reported for $CCl_2=CCl_2$ under simulated atmospheric conditions was that there was no significant increase in the total amount of aldehydes formed compared with a blank run (Hamming, 1967). The vapor phase photooxidation of $CCl_2=CCl_2$ with O_2 or air gave trichloroacetyl chloride (CCl_3COCl) and $COCl_2$ (Calfee and Wallace, 1956; Bertrand et al., 1971; Dahlberg et al., 1972). The mechanism probably involved the intermediacy of the chlorooxy radical (ClO.)(Kiryushin and Poluektov, 1968). The liquid phase photooxidation of $CCl_2=CCl_2$ with O_2 gave CCl_3COCl, $COCl_2$, HCl, and various peroxides (Besson, 1895; Kirkbride, 1941; Rushmer and Smith, 1947; Yasnitskii et al., 1963a; Hardie, 1964d). Photolysis of CCl_3COCl with O_2 gave $COCl_2$ and CO via free radicals which were able to initiate the oxidation of chloroethylenes

(Yasnitskii et al., 1966). Photooxidation of $CCl_2=CCl_2$ in the presence of water gave trichloroacetic acid (CCl_3CO_2H) (Bailey and Hickson, 1941; Yasnitskii et al., 1963b; Yasnitskii et al., 1970). Oxidation of liquid $CCl_2=CCl_2$ with O_2 gave CCl_3COCl, tetrachloroethylene oxide (o-CCl_2CCl_2O), $COCl_2$, and HCl (Müller and Ehrmann, 1936; Shepherd, 1962; Jeffrey, 1967; Archer, 1970), although it has been reported that $CCl_2=CCl_2$ was stable toward O_2 in the dark (Hardie, 1964d). O_2 reacted slowly with liquid $CCl_2=CCl_2$ to give CCl_3COCl and $COCl_2$ (Besson, 1894; Swarts, 1898; Hardie, 1964d).

Experimental

Flow Reactor. The reaction vessel and associated gas handling equipment are shown in Figure 1.

For the experiments with $CHCl=CCl_2$ and $CCl_2=CCl_2$ dry cylinder air and a dry air-NO_2 mixture from Mathason Gas Products were passed through 1/4 inch Nupro In-line check valves, 1/4 inch Nupro needle valves and Brooks 1A-15-1 rotameters. Two of the air streams were passed through liquid bubbling tubes with sintered glass gas dispersion tubes. One tube contained water at -25°, and the other contained the chlorinated solvent at a lower temperature (CH_2Cl_2, -78°; CH_3CCl_3, -25°; $CHCl=CCl_2$, -32°; $CCl_2=CCl_2$, -15°). The low temperature was necessary in order to obtain the desired concentration of the chlorinated compound in the air stream.

The low temperature was maintained by circulating CH_2Cl_2 from a refrigeration unit through a jacketed vessel which surrounded the lower part of the bubbling tube except for CH_2Cl_2 where dry ice was used. The concentrations of chlorinated sol-

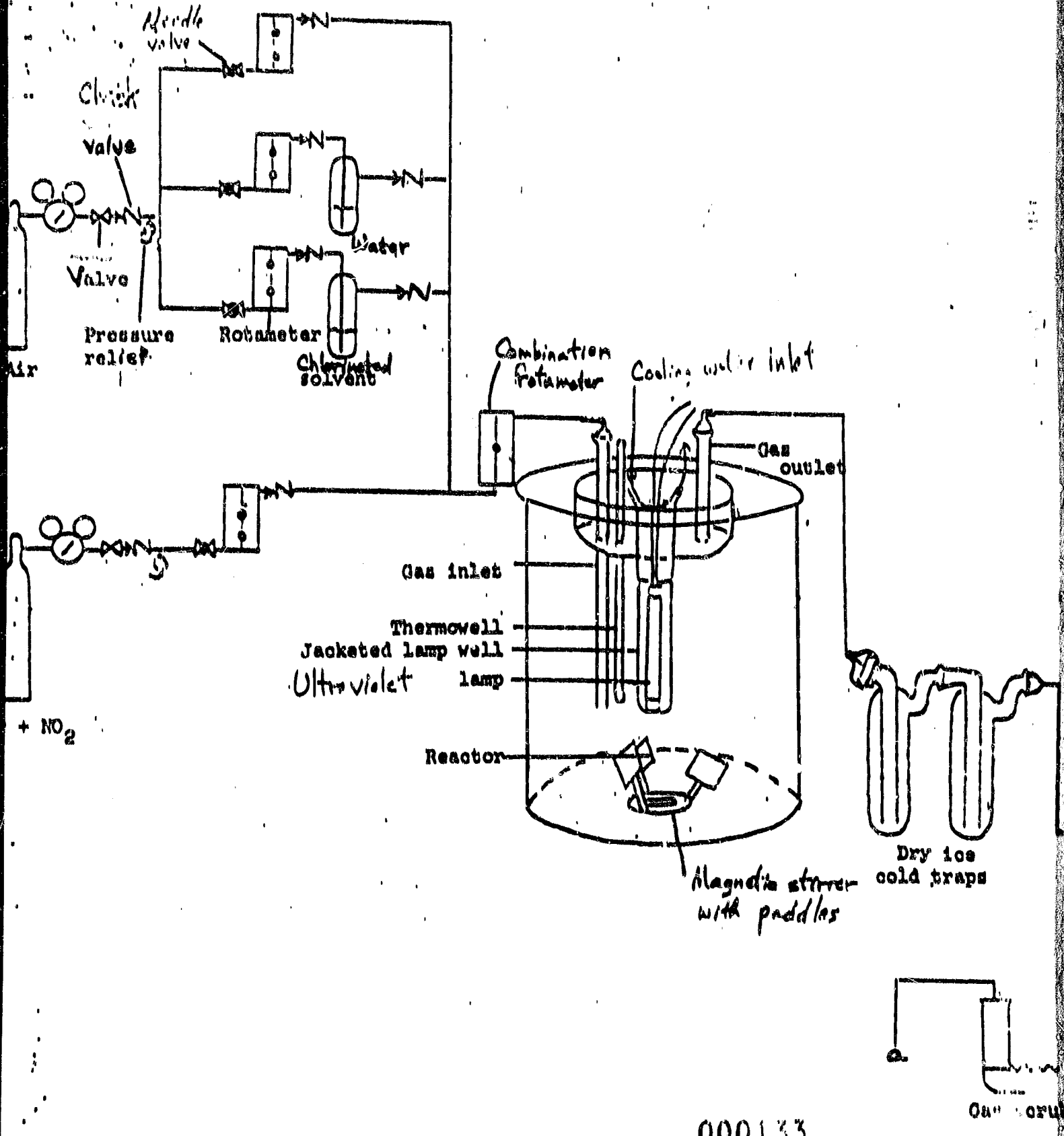

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Figure 1. Photochemical flow reactor (in some cases an additional scrubber and (or) another dimensional was added at reactor)

vents in the air streams were determined by weight loss of the bubbling tube and weight gain of the dry ice trap in blank runs. The air stream, which was passed through the water, was essentially saturated with water as determined by a control experiment. The four air streams which contained NO_2, the chlorinated solvent, water, and air alone in the desired proportions were each passed through check valves and combined. For the reactions with CH_2Cl_2 and CH_2CCl_2, the NO_2 and water were omitted. The combined gas streams were passed through a Brooks 2-15-3 rotameter and into the reactor. The flow meters were calibrated with the actual gas mixtures used in the reactions.

The gas mixture was introduced near the bottom of the reactor and dispersed by means of a magnetically driven large Teflon paddle stirrer. The reactor was a large flat bottomed cylindrical vessel with a volume of ~26 liters. The top edge of the vessel was ground flat. A flat glass plate was mounted on the top, sealed with Silicone or Apiezon grease, and secured with spring clips. The reactor temperature was maintained at 25±1° by wrapping the sides of the aluminum foil covered vessel with flat sided rubber tubing through which water from a refrigerated bath was circulated.

The radiation source for the reactions of $CHCl=CCl_2$ and $CCl_2=CCl_2$ was a Hanovia 450 watt medium pressure mercury arc lamp. The lamp was placed in a water cooled Pyrex well which transmitted only wavelengths greater than 295 nm as determined by measuring the

emission spectrum with a Perkin-Elmer 202 ultraviolet-visible spectrophotometer. The lamp intensity is estimated to be ·50 times that of sunlight in the 295-400 nm region, based on the manufacturer's specifications. The radiation source for the CH_2Cl_2 and CH_3CCl_3 reactions was a 10 watt Hanovia low pressure mercury arc lamp which has been described previously (Dilling et al., 1974).

For the reactions of $CHCl=CCl_2$ and $CCl_2=CCl_2$ the exit gas mixture from the reactor was passed through two dry ice traps, the first of which was of about one liter capacity. The second trap had a capacity of ~350 ml. Most of the condensable products and water were collected in the first trap. The noncondensable gases were then passed through a low back pressure gas scrubber which contained standard sodium hydroxide solution. For the reactions of CH_2Cl_2 and CH_3CCl_3 the exit gas stream was passed through two 350 ml dry ice traps, then through the gas scrubber, and finally through a one liter dry ice trap. In some cases a second caustic gas scrubber was also used.

All connections between the various parts of the system were constructed from 1/4 inch stainless steel or Teflon tubing and Swagelok fittings.

Simulated Sea Level Atmospheric Photolysis of $CHCl=CCl_2$. Water (75 ml) and $CHCl=CCl_2$ (50 ml) were placed in their respective bubbling tubes (Figure 1). The air flow rates were as fol-

lows: 25 cc/minute through the $CHCl=CCl_2$ which was 175 ppm $CHCl=CCl_2$ (molar or volume basis) after mixing with the other gas streams, 140 cc/minute through the water which was 35% relative humidity after mixing with the other gas streams, and 235 cc/minute of the air-NO_2 mixture [90 ppm NO_2 as determined by a modified Saltzman procedure (Dilling et al., 1974)] which was 50 ppm NO_2 after mixing with the other gas streams. The total flow rate was 400 cc/minute which corresponded to a 65 minutes residence time in the reactor. Into the gas scrubber was placed 100 ml of 1.000 N NaOH and 115 ml of water. Dry ice-ethanol was used to cool the cold traps.

The reaction was run for 166 hr during which time 3,980 liters (calculated from flow rates) of air (slightly above atmospheric pressure), 29.53 g of water, and 3.67 g (27.9 mmoles) of $CHCl=CCl_2$ had passed through the reactor. No deposit was detected on the lamp well, the reactor surface, or the gas lines. Nearly all of the condensable material was found in the first cold trap. A total of 35.75 g of material (one phase) was obtained.

After they melted, the aqueous solutions from the cold traps were transferred to a 50 ml volumetric flask with rinsing and were made up to volume with water. A 5.0 ml aliquot was removed, acidified with nitric acid (HNO_3) and titrated potentiometrically for chloride ion (Cl^-) with 0.100 N $AgNO_3$ on a Precision-Dow Recordomatic Titrator. The 20.67 ml of titrant required corresponded to 20.7 mmoles of Cl^- in the original 50 ml of solution.

Another 5.0 ml aliquot of the aqueous reaction solution was removed and titrated for acidity with 1.000 N NaOH on a Sargent-Welch Recording Titrator, Model DG. The 4.09 ml of titrant required to reach pH 7 corresponded to 40.9 mmoles of strong acid (H^+) in the original 50 ml of solution.

After another 2 ml of the aqueous reaction mixture was removed (for gc analysis, etc.), the remaining 38 ml of solution was extracted continuously with 200 ml of ether for 77 hr. The ether extract was evaporated under vacuum to ~5 ml. After a small amount of water was separated, 4.842 g of the ether solution remained. G c analysis (2 ft x 1/4 inch column packed with 1.5% LAC 446 and 2% H_3PO_4 on 60-80 mesh Chromosorb WAW, column temp 127°, injection port temp 140°, detector block temp 300°, He flow rate 50 cc/minute) of this ether solution showed a single product, t_R 4.3 minutes, which had the same retention time as an authentic sample of dichloroacetic acid ($CHCl_2CO_2H$). To a 1.640 g portion of the ether solution was added 85.0 mg of 1-chloronaphthalene (1-$C_{10}H_7Cl$) as an internal g c standard, t_R 1.2 minutes. G c analyses of this solution showed the presence of 0.559 g of $CHCl_2CO_2H$ which corresponded to 2.17 g (61% yield based on carbon) of $CHCl_2CO_2H$ in the original 50 ml of aqueous solution. The 33.8 mg atoms of chlorine which are contained in this amount of $CHCl_2CO_2H$ represents 40% of the chlorine present in the $CHCl=CCl_2$ used. A portion of the ether extract was evaporated. The mass spectrum of the residue indicated the sample to be almost entirely $CHCl_2CO_2H$ by

comparison with the spectrum of an authentic sample. Minor amounts of other compound(s) may have been present.

G c analysis of the original aqueous solution showed only $CHCl_2CO_2H$. G c analyses of the ether extract on longer columns (from which $CHCl_2CO_2H$ was not eluted) showed traces of two short retention time components which were not identified.

The sodium hydroxide solution in the scrubber was transferred to a 250 ml volumetric flask with rinsing and made up to volume with water. A 10.0 ml aliquot was acidified with nitric acid (HNO_3) and titrated for Cl^-; 9.11 ml of 0.100 N $AgNO_3$ was required. This corresponded to 22.8 mmoles of Cl^- in the total scrubber solution. The excess base in a 25.0 ml aliquot of the scrubber solution was titrated with 1.000 N hydrochloric acid. Three breaking points were observed at 5.61 ml (pH = 10.4), 5.95 ml (pH = 8.2), and 6.88 ml (pH = 4.8). The 6.88 ml of titrant corresponded to the absorption of 31.2 mmoles of H^+ which had been picked up by the caustic scrubbing solution. Titration of sodium carbonate and sodium bicarbonate solutions indicated that the extra breaking points noted above were not due to the presence of carbon dioxide.

The total Cl^- in the traps and scrubber was 43.5 mmoles which corresponded to 52% of the theoretical amount of chlorine in the $CHCl{=}CCl_2$ used. The total H^+ in the traps and scrubber was 72.1 mmoles.

Simulated Sea Level Atmospheric Photolysis of $CCl_2=CCl_2$.

Water (75 ml) and $CCl_2=CCl_2$ (50 ml) were placed in their respective bubbling tubes. The gas flow rates were set at 25 cc/minute through the $CCl_2=CCl_2$, 140 cc/minute through the water, 28 cc/minute of an air-NO_2 mixture (1000 ppm NO_2), and 207 cc/minute of diluent air. The total gas flow rate was 400 cc/minute and consisted of 142 ppm $CCl_2=CCl_2$, 35% relative humidity, and 70 ppm NO_2 in air. The residence time in the reactor was 65 minutes. Into the gas scrubber were placed 150 ml of 1.000 N NaOH and 100 ml of water.

The reaction was run for 167 hr. During this time, 4.21 g of $CCl_2=CCl_2$ (25.4 mmoles $CCl_2=CCl_2$, 101.6 mg atoms chlorine) and 27.73 g of water had passed through the reactor. The NO_2 concentration in the gas stream was determined after the termination of the photoreaction by filling a large gas bulb with the gas mixture which was used throughout the reaction and analyzing the contents by the modified Saltzman technique.

The reactor was rinsed with water until the rinses were neutral. This solution contained 5.3 mmoles of H^+ and no Cl^-. C o analysis showed the presence of 5.66 mmoles of CCl_2CO_2H, which corresponds to 17.0 mg atoms of chlorine.

A total of 28.87 g of acidic liquid was collected in the two cold traps. Contained in this liquid were 5.63 mmoles of Cl^- and 13.00 mmoles of H^+. A continuous ether extraction was run for approximately 105 hr on a portion of the cold-trap solution. C o analysis showed the presence of 5.43 mmoles of CCl_3CO_2H in the ether extract, which corresponded to 16.3 mg atoms of chlorine. No other products were detected. Infrared analysis confirmed the presence of CCl_3CO_2H in the ether extract, and also indicated another unidentified product. Mass spectroscopic analysis showed only CCl_3CO_2H.

Cl^- titration of the trap solution which remained after ether extraction showed the presence of 5.65 mmoles, which indicated that all of the Cl^- remained in the solution after ether extraction. Base titration showed 5.20 mmoles of H^+ in the solution. Thus, all the acid, with the exception of the HCl, had been extracted from the trap solution.

Analysis of the solution from the gas scrubber showed the presence of 27.50 mmoles of Cl^- and 16.50 mmoles of hypochlorite ion (OCl^-) which was determined by the iodide-thiosulfate method (Furman, 1962). A portion of the scrubber solution was acidified to pH 1 and continuously extracted with ether for approximately 70 hr. C o analysis of the ether extract showed 1.25 mmoles of CCl_3CO_2H present in the total scrubber solution, which corresponded to 3.75 mg atoms of chlorine. Titration of the scrubber

solution with 1.000 N HCl indicated that 65.50 mmoles of base had been neutralized by acidic products. This was an excess of 20.25 mmoles of H^+ over that which could be accounted for by the chlorinated products.

The overall recovery of products was 33.1 mmoles of Cl^-, 16.5 mmoles of $COCl^-$, 12.3 mmoles of $COCl_2CO_2H$ which corresponds to 37.0 mg atoms of chlorine, and 83.8 mmoles of H^+. Assuming the $COCl^-$ arises from Cl_2, the yields (based on total chlorine present in the $COCl_2$-$COCl_2$) of products were 33% Cl_2, 16% HCl, and 36% $COCl_2CO_2H$.

Simulated High Altitude Atmospheric Photolysis of CH_2Cl_2. CH_2Cl_2 (50 ml) was placed in the bubbling tube, and 290 cc/minute of dry air was passed through. The resulting stream was diluted with more dry air to give a total gas flow of 1200 cc/minute. This gave a concentration of 155 ppm CH_2Cl_2 in air and a residence time of 22 minutes in the reactor. Two gas scrubbers were used in the photoreaction. Into each was placed 250 ml of 1.000 N NaOH.

The reaction was run for 165 hr. At the completion of the reaction, 6.51 g of CH_2Cl_2 (76.7 mmoles, 153.4 mg atoms of chlorine) had passed through the reactor. A small amount of white solid, identified silicon dioxide (SiO_2) by infrared analysis, was deposited throughout the reactor. Infrared analysis also

indicated that there was a small amount of unidentified organic material associated with the SiO_2. A portion of the Apiezon grease which had been used to seal the system and which had turned black during the photoreaction was collected and analysed for total chlorine. While the analyses were poorly reproducible, they averaged around 4 wt %.

A total of 3.13 g of liquid had collected in the two dry ice cold traps. It was possible to recover a very small amount of yellow liquid from the second cold trap. Mass spectroscopic analysis of this liquid indicated the presence of water, HCl, and a large amount of CO_2. There were several minor unidentified ion-peaks. The two cold traps were rinsed with water until the rinses were neutral. The rinses were made up to volume with water in a 500 ml volumetric flask. Analyses indicated a total of 1 mmole of chlorate (ClO_3^-), which was determined by the ferrous sulfate method (Kolthoff et al., 1961), and 21 mmoles of perchlorate (ClO_4^-), which was determined by the ferrous 1,10-phenanthroline method (Fritz et al., 1964). There was no Cl^- or OCl^- present. Titration with 1.000 N NaOH showed 23.0 mmoles of H^+ in the total cold trap solution.

The reactor and connecting lines were rinsed with water and made up to 1000 ml. Present in the solution were 2 mmoles of ClO_3^- and 22 mmoles of ClO_4^-. No Cl^- or OCl^- was found. Base titration showed 22.6 mmoles of H^+ in the total solution.

The solution from the first gas scrubber contained 18 mmoles of Cl^-, 12 mmoles of OCl^-, and 7.25 mmoles of ClO_2^-. It was not possible to analyze for ClO_4^- in the presence of the ClO_2^-. An attempted acid titration of the solution was not successful. The second gas scrubber contained 7.75 mmoles of Cl^-, 1.25 mmoles of ClO_2^-, and 0.50 mmole of ClO_4^-. There was no OCl^-. Acid titration of the solution, however, indicated that a total of 36.75 mmoles of H^+ was present; only 9.50 mmoles of H^+ could be accounted for by HCl, chloric acid ($HClO_3$), and perchloric acid ($HClO_4$). O c and nmr analyses for HCO_2H were negative. The unidentified acid does not contain chlorine, as neutron activation analysis showed only 10.1 mg atoms of chlorine in the solution.

The solution in the last cold trap was made up to 250 ml. It contained 1.75 mmoles of ClO_4^-, 0.5 mmole of ClO_2^-, and 2.4 mmoles of H^+.

The overall recovery of products was 25.8 mmoles of Cl^-, 12.0 mmoles of OCl^-, 12.0 mmoles of ClO_2^-, ≥45.2 mmoles of ClO_4^-, and ≥84.8 mmoles of H^+. Assuming the OCl^- arises from Cl_2, the yields (based on total chlorine present in the CH_2Cl_2) of products were 16% Cl_2, 9% HCl, 8% $HClO_2$, and ≥30% $HClO_4$.

Simulated High Altitude Atmospheric Photolysis of CH_2Cl_2.

CH_2Cl_2 (50 ml) was placed in the bubbling tube. Dry air (18 cc/minute) was passed through the CH_2Cl_2 and the resulting CH_2Cl_2 air stream was diluted with more dry air to give a total gas flow

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of 1250 cc/minute. This gave a concentration of 169 ppm CH_3CCl_3 in the air and a residence time of 21 minutes in the reactor. In the single gas scrubber was placed 250 ml of 1.000 N NaOH.

The reaction was run for 167 hr. During this time, 12,525 liters of air and 11.51 g of CH_3CCl_3 (86.3 mmoles, 258.9 mg atoms of chlorine) had passed through the reactor. A small amount (≥30 mg) of SiO_2 was collected.

A total of 3.53 g of acidic liquid was found in the first two dry ice cold traps. The reactor and the connecting lines in the system also contained acidic liquid. The two cold traps, reactor, and connecting lines were rinsed with water until the rinses were neutral. The rinses and the 3.53 g of acidic liquid were combined in a 1000 ml volumetric flask and made up to volume with water. An aliquot was titrated with $AgNO_3$; these were 5.4 mmoles of Cl^- in the total solution. A 50 ml aliquot was removed and titrated for H^+ with 1.000 N NaOH. Two breaks were observed, the first at pH 3.95 after the addition of 3.23 ml of titrant, and the second at pH 8.50 after an additional 0.44 ml of titrant had been added. This corresponds to 64.6 meq of strong acid and 8.8 meq of weak acid.

A continuous ether extraction was run on 500 ml of the rinse solution for approximately 150 hr. Subsequent titration of the extracted water indicated that all of the weak acid, but only

12% of the strong acid, had been extracted into the ether. The ether was evaporated to a small volume. Mass spectroscopic analysis confirmed acetic acid (HOAc) as the primary product, with a small amount of CH_2ClCO_2H and another minor unidentified component.

The pH of 200 ml of the extracted water solution was raised to 7.5 with NaOH, and the solution was evaporated to dryness. The resultant white solid was identified by infrared analysis as primarily a hydrate of sodium perchlorate ($NaClO_4$) by comparison with authentic spectra; a minor amount of a carboxylic acid salt and a nitrate (NO_3^-) or a carbonate (CO_3^{-2}) were also indicated to be present. Powder x-ray diffraction analysis confirmed $NaClO_4$ as the major constituent. Mass spectroscopic analysis showed only CO_2 at 460°. Analysis of the extracted water solution showed the presence of 61.6 mmoles of ClO_4^- in the entire extracted solution.

The NaOH solution from the gas scrubber was made up to volume with water in a 250 ml volumetric flask. Analyses indicated 95.0 mmoles of Cl^-, 50.0 mmoles of OCl^-, and 6.75 mmoles of ClO_3^- in the total scrubber solution; ClO_4^- could not be determined.

The water which had collected in the last cold trap downstream from the gas scrubber was made up to volume with water in a 250 ml volumetric flask. Titration indicated little or no Cl^-. A 50 ml aliquot was titrated with 1.000 N NaOH. The titration

started at pH 7.2; a breaking point was observed at pH 9.7 after the addition of 0.53 ml NaOH. This corresponds to 2.65 mmoles of H^+ in the total solution. No ClO_2^- was found, but 2.0 mmoles of ClO_4^- in the total solution was found.

The overall recovery of products was 100.4 mmoles of Cl^-, 50.0 mmoles of $COCl^-$, 6.8 mmoles of ClO_2^-, $\geq$61.9 mmoles of ClO_4^-, and $\geq$76.1 mmoles of H^+. Assuming the $COCl^-$ arises from Cl_2, the yields (based on total chlorine present in the CH_2CCl_2) of products were 39% Cl_2, 20% HCl, 3% $HClO_2$, and $\geq$24% $HClO_4$.

Static Photolysis of CCl_2=CCl_2. The photoreactor, described previously for short-term (<24 hr) sea level simulated photolyses (Dilling et al., 1974), was charged with 100 ppm of CCl_2=CCl_2, 50 ppm of NO, and enough water for 35% relative humidity. The reaction mixture was irradiated as described previously (Dilling et al., 1974) for 7 hr at which time 60% of the CCl_2=CCl_2 had reacted; 50% of the CCl_2=CCl_2 had reacted in 4.8 hr. The contents of the reactor were drawn through a trap which was cooled in liquid nitrogen. Mass spectroscopic analysis of the contents of the trap indicated the presence of $Cl_2CHCOCl$ (and/or c-CCl_2CCl_2CO), m/e 145 (Cl_2CHCO^+), 117 (CCl_2H^+), 110 (Cl_2CO^+), 82 (CCl_2^+); $COCl_2$, m/e 98 (M^+), 63 ($COCl^+$); Cl_2, m/e 70 (M^+), 35 (Cl^+); and CO_2, m/e 44 (M^+), in addition to CCl_2=CCl_2. The chlorine isotopic abundance ratios were consistent with the assigned formulas.

Repetition of this reaction without the water vapor gave essentially the same results.

The preceding reaction was repeated except that the water vapor was omitted. G c analysis (6 ft x 3 mm ID glass column packed with 3.8% UC-W98 on 80-100 mesh Diatoport S, column temperature 60°, electron capture detector) of the gaseous reaction mixture confirmed the presence of $COCl_2$ by comparison of its retention time with that of an authentic sample.

Static Photolysis of $CHCl=CCl_2$. As in the preceding experiment, 100 ppm $CHCl=CCl_2$ and 50 ppm of NO in air were irradiated. G c analysis confirmed the presence of $COCl_2$.

Results

Vapor phase irradiation of $CHCl=CCl_2$ (175 ppm), in a flow reactor, in air which contained NO_2 (50 ppm) and water vapor (35% relative humidity) at 25° with a medium pressure mercury arc lamp, ($\lambda > 295$ nm) and with a residence time of 65 minutes in the radiation zone gave essentially complete conversion of the $CHCl=CCl_2$ to $CHCl_2CO_2H$ and HCl (Equation 1).

$$CHCl=CCl_2 + air + NO_2 + H_2O \xrightarrow{h\nu} \underset{(40\%)}{CHCl_2CO_2H} + \underset{(52\%)}{HCl} \qquad (1)$$

The conditions of the product collection were such that any hydrolyzable materials would have been isolated as the hydrolysis products. The yields throughout this paper (except for HOAc) are based only on the number of chlorine atoms present in the compounds. The yield of $CHCl_2CO_2H$ was 61% based on the carbon content.

$COCl_2$ was detected in a gas sample which resulted from the photolysis of $CHCl=CCl_2$ (100 ppm) and NO (50 ppm) in air in a static reactor.

Irradiation ($\lambda>295$ nm) of $CCl_2=CCl_2$ (142 ppm) in air which contained NO_2 (70 ppm) and water vapor (35% relative humidity) in the flow reactor (65 minutes residence time) gave CCl_2CO_2H, HCl, Cl_2 (or some other product which analyzed as OCl^- and Cl^- in aqueous solution) and unidentified organic products (Equation 2).

$$CCl_2=CCl_2 + air + NO_2 + H_2O \xrightarrow{h\nu} \underset{(36\%)}{CCl_2CO_2H} + \underset{(16\%)}{HCl} + \underset{(33\%)}{Cl_2} \qquad (2)$$

The conversion of the $CCl_2=CCl_2$ was essentially complete. The reaction mixture from $CHCl=CCl_2$ was not analyzed for OCl^-, but there could not have been more than 8% since 92% of the chlorine was accounted for in the two products shown in Equation 1. There was another strong acid(s), which did not contain chlorine, formed in the photolysis of both $CHCl=CCl_2$ and $CCl_2=CCl_2$.

$COCl_2$ was detected in the vapor phase from a static photolysis of $CCl_2=CCl_2$ (100 ppm), NO (50 ppm), and water vapor (35% relative humidity) in air. Mass spectroscopic analysis of the condensable products from this reaction mixture indicated the presence of CCl_2COCl, $COCl_2$, Cl_2, and CO_2.

Photolysis of CH_2Cl_2 (155 ppm) in dry air under simulated high altitude conditions (λ primarily 254 nm) in the flow reactor at ~25° with a residence time of 22 minutes in the radiation zone gave HCl, Cl_2, $HClO_2$, $HClO_4$, and traces of unidentified organic products (Equation 3).

$$CH_2Cl_2 + \text{air} \xrightarrow{h\nu} \underset{(9\%)}{HCl} + \underset{(16\%)}{Cl_2} + \underset{(8\%)}{HClO_2} + \underset{(\geq 30\%)}{HClO_4} \qquad (3)$$

Irradiation of CH_2ClCl_2 (169 ppm) under simulated high altitude conditions similar to those used for CH_2Cl_2 gave the same four inorganic chlorine products in addition to HOAc and a trace of CH_2ClOCO_2H (Equation 4).

$$CH_2ClCl_2 + \text{air} \xrightarrow{h\nu} \underset{(20\%)}{HCl} + \underset{(39\%)}{Cl_2} + \underset{(3\%)}{HClO_2} + \underset{(\geq 24\%)}{HClO_4} + \underset{(10\%)}{HOAc} + CH_2ClOCO_2H \qquad (4)$$

The HOAc yield is based on the carbon content. The CH_2Cl_2 and CH_2ClCl_2 were completely consumed in the latter two reactions.

Discussion

The chloroacetic acids produced from $CHCl=CCl_2$ and $CCl_2=CCl_2$ almost certainly arose from hydrolysis of the chloroacetyl chlorides, and the HCl from $COCl_2$ and possibly HCOCl. These hydrolyses likely occurred in the cold traps where the water and organic products both condensed, and thus were not photoreactions. The detection of CCl_3COCl and $COCl_2$ from the photolysis of $CCl_2=CCl_2$ and the reported formation of $CHCl_2COCl$ and $COCl_2$ from $CHCl=CCl_2$ (Wilson et al., 1968) support this conclusion. $COCl_2$ is known to hydrolyse very rapidly in the liquid phase ($t_{1/2} \approx 0.1$ sec at 25°)(Manogue, 1958; Ugi and Beck, 1961). $CHCl_2COCl$ and CCl_3COCl hydrolyse even faster (Ugi and Beck, 1961). HCOCl is unknown, and if formed as a transient intermediate would likely break down to HCl and CO. Thus if these acid chlorides were formed at low concentrations in the atmosphere from $CHCl=CCl_2$ and $CCl_2=CCl_2$, the acid chlorides would be destroyed rapidly on contacting rain or other liquid water bodies. The vapor phase hydrolysis of $COCl_2$ in the dark was quite slow as shown by injecting $COCl_2$ into air which contained water vapor and following the $COCl_2$ rate of disappearance by g c.

Since one mole of HCl is generated for each mole of $CHCl_2COCl$ formed under the simulated atmospheric conditions of this study, $CHCl=CCl_2$ breaks down to about two parts of $CHCl_2COCl$ and one part of other HCl generators (presumably $COCl_2$ and HCOCl)(Scheme I).

Scheme I

$$CHCl{=}CCl_2 + O_2 \xrightarrow[NO_2]{h\nu} \underset{(60\%\ of\ Cl)}{CHCl_2COCl} + \underset{(32\%\ of\ Cl)}{(COCl_2 + HCOCl)}$$

$$\downarrow H_2O \qquad\qquad \downarrow H_2O$$

$$\underset{(40\%\ of\ Cl)}{CHCl_2CO_2H} + \underset{(20\%\ of\ Cl)}{HCl} \qquad \underset{(32\%\ of\ Cl)}{HCl}$$

The major difference between the photolyses of $CHCl{=}CCl_2$ and $CCl_2{=}CCl_2$ under similar conditions was the formation of an appreciable amount of Cl_2 from the latter (Scheme II).

Scheme II

$$CCl_2{=}CCl_2 + O_2 \xrightarrow[NO_2]{h\nu} \underset{(48\%\ of\ Cl)}{CCl_3COCl} + \underset{(4\%\ of\ Cl)}{COCl_2} + \underset{(32\%\ of\ Cl)}{Cl_2}$$

$$\downarrow H_2O \qquad \downarrow H_2O \qquad \downarrow H_2O,\ OH^-$$

$$\underset{(36\%\ of\ Cl)}{CCl_3CO_2H} + \underset{(12\%\ of\ Cl)}{HCl} \qquad \underset{(4\%\ of\ Cl)}{HCl} \qquad \underset{(16\%\ of\ Cl)}{OCl^-} + \underset{(16\%\ of\ Cl)}{Cl^-}$$

Cl_2 was detected both directly, by mass spectroscopy, and indirectly, by thiosulfate titration of the iodine liberated from

the reaction of potassium iodide with OCl^- which resulted from the reaction of the Cl_2 with aqueous base.

The excess acid ($[H^+] > [Cl^-]$ + $[CHCl_2CO_2H$ or $CCl_3CO_2H]$) produced in the photolysis of $CHCl=CCl_2$ and $CCl_2=CCl_2$ is likely HNO_3, which arose from the reaction of NO_2, a portion of which probably passed through the photoreactor unchanged, with water in the traps and scrubber (Cotton and Wilkinson, 1966).

The formation of $HClO_3$ and $HClO_4$ from CH_2Cl_2 and CH_2CCl_2 in air with short wavelength ultraviolet radiation is indicative of highly oxidizing conditions. Ozone is known to be generated rapidly under these conditions (Dilling et al., 1974).

Some of the products identified from the photoreactions under these simulated high altitude conditions were probably formed through the intermediacy of various chlorine oxides (Sneed et al., 1954; Remy, 1956), which in the presence of water or alkali, gave the oxyacids of chlorine or the salts. The presence of $HClO_3$ and $HClO_4$ in the water which accumulated in an empty cold trap downstream from two NaOH scrubbers during the photolysis of CH_2Cl_2 indicates that at least part of the acid is not formed directly during the photolysis. Cl_2 could have formed directly from CH_2Cl_2 or CH_2CCl_2, from oxidation of HCl, or via the decomposition of a chlorine oxide intermediate. Alternatively, a chlorine oxide itself could have provided the oxidizing power observed in the caustic scrubbers (e.g. $Cl_2O + H_2O \longrightarrow 2\ HOCl$).

All of the products observed in this study were those which probably resulted from reactions which are unimolecular with respect to the chlorinated solvents, i.e. there were no products which require the interaction of two or more organic molecules. Thus, even though the concentrations of the chlorinated solvents may have been considerably higher than those found under many environmentally significant conditions, the products would very likely be the same at lower concentrations.

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Organic Photochemistry. XVI.[1] Environmental Fate and Effect of Chlorinated Solvents. V.[2] Total Oxidant Formation from Simulated Atmospheric Photolyses of Methylene Chloride, 1,1,1-Trichloroethane, Trichloroethylene, Tetrachloroethylene, and Vinyl Chloride

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Abstract

In order to estimate the amount of oxidant (ozone, etc.) generated in the presence of several chlorinated solvents in the atmosphere, laboratory studies were carried out under simulated atmospheric conditions which included ultraviolet radiation (λ >290 nm), nitrogen dioxide, and water vapor in air in a small

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[1]Part XV: (Dilling and Tefertiller, 1974).

[2]Part IV: (Dilling and Tefertiller, 1974).

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reactor (~10 liters). The oxidant was measured by titration of the iodine liberated on passing the reaction mixture through a neutral buffered potassium iodide solution. Trichloroethylene and vinyl chloride caused the formation of significant amounts of oxidant. Tetrachloroethylene caused only a small amount of oxidant to be formed at short irradiation times and little or no oxidant at longer times. Methylene chloride and 1,1,1-trichloroethane contributed little or nothing to the formation of oxidant.

The fate and effect of chlorinated solvents which may be discharged to the environment (Murray and Riley, 1973a; Murray and Riley, 1973b; Farber, 1973) are important from an ecological standpoint. In preceding papers of this series (Dilling et al., 1974; Dilling and Bredeweg, 1974; Dilling and Tefertiller, 1974) the reactivities of, and products from, the four most widely used chlorinated solvents, methylene chloride (CH_2Cl_2), 1,1,1-trichloroethane (CH_3CCl_3), trichloroethylene ($CHCl=CCl_2$), and tetrachloroethylene ($CCl_2=CCl_2$), were determined under simulated atmospheric conditions. $CHCl=CCl_2$ was moderately reactive, and $CCl_2=CCl_2$ was slightly reactive under simulated sea level atmospheric conditions which included ultraviolet radiation of wavelength greater than 290 nm, nitric oxide (NO) or nitrogen dioxide (NO_2), and water vapor in air. CH_2Cl_2 and CH_3CCl_3 were unreactive under these conditions. Vinyl chloride ($CH_2=CHCl$), a large volume monomer, was nearly as reactive as $CHCl=CCl_2$ (Dilling et al., 1974).

An important criterion of the adverse effect of compounds on the environment is the amount of oxidants, principally ozone (O_3), peroxyacetyl nitrate ($MeCO_3NO_2$), and NO_2 (Pitts, 1969), which are generated due to the presence of the compound in a photolyzing air mass (Leighton, 1961; Altshuller and Bufalini, 1965; Altshuller and Bufalini, 1971). The objectives of the

present work were to determine the amounts of total oxidant generated due to the presence of the four chlorinated solvents and CH_2=CHCl, and also to develop a simple method, which utilized a small (~10 liter) photoreactor, by which total oxidant formation could be conveniently determined.

As far as we are aware, no data have been reported on oxidant formation due to the presence of CH_2Cl_2 or CH_2=CHCl other than a preliminary report of our data for CH_2Cl_2 (Farber, 1973). CH_3CCl_3 was found to contribute, in a minor way, to oxidant formation (0.11 ppm O_3 under conditions where CHCl=CCl_2 produced 0.45 ppm O_3) (Fuller, 1968; Farber, 1973).

In general, most workers have found that CHCl=CCl_2, under simulated atmospheric conditions, had a medium capacity for producing oxidant as compared with other common solvents or atmospheric pollutants (Harton and Bolse, 1958; Brunelle, et al., 1966; Hamming, 1967; Fuller, 1968; Wilson, 1969; Wilson et al., 1969b; Altshuller and Bufalini, 1971; Farber, 1973). The agreement of results between different workers was not very good. Some claimed that CHCl=CCl_2 produced oxidant more slowly than did ethylene (CH_2=CH_2) (Wilson, 1969; Wilson et al., 1969a) while others claimed a rate comparable to that of CH_2=CH_2 but less than that of propylene ($MeCH$=CH_2) (Altshuller and Bufalini, 1971). Still another group claimed a stable ozone concentration

of zero (Schuck and Doyle, 1959). The maximum concentration of oxidant formed with $CHCl=CCl_2$ was higher than that with $CH_2=CH_2$ (Wilson, 1969; Altshuller and Bufalini, 1971), and about the same as that with $MeCH=CH_2$ (Altshuller and Bufalini, 1971). The oxidant dosage with $CHCl=CCl_2$ was about the same as with $CH_2=CH_2$, but less than with $MeCH=CH_2$ (Altshuller and Bufalini, 1971). Another report indicated that the amount of O_3 formed in the presence of $CHCl=CCl_2$ was at least as great as that produced by automobile exhaust (Hamming, 1967). The amount of oxidant formed decreased sharply as the $CHCl=CCl_2$:NO ratio fell (Wilson, 1969; Wilson et al., 1969b). Addition of $CHCl=CCl_2$ to $MeCH=CH_2$ caused a slight increase in the amount of oxidant formed compared to that formed with $MeCH=CH_2$ alone (Wilson, 1969; Wilson et al., 1969b). Injection of a high concentration of $CHCl=CCl_2$ into a reactive photolyzing air mass showed no increase in oxidant level over that without added $CHCl=CCl_2$ (Wilson, 1969; Wilson et al., 1969b).

According to most workers, oxidant or O_3 was either not generated (Brunelle et al., 1966; Hamming, 1967) or only a very small amount was generated (Schuck and Doyle, 1959; Fuller, 1968; Farber, 1973) in the presence of $CCl_2=CCl_2$ under simulated atmospheric conditions. One report (Horton and Bolze, 1958) indicated that more oxidant was produced in the presence of $CCl_2=CCl_2$ than in the presence of $CH_2=CH_2$, $MeCH=CH_2$, or toluene (PhMe).

Experimental

Reactor. The photoreactor (9.58 liters capacity), described previously for short term (<24 hr) sea level simulated photolyses (Dilling et al., 1974), was used after slight modification. An exit port was added at the end opposite the sample port so that the contents of the reactor could be swept out with a gas stream. The temperature of the reactor was maintained at 24-25° by means of a Haake constant temperature bath.

Materials. High purity dry air and prepurified nitrogen from Matheson were used as received.

Cylinder NO_2 from Matheson was purified by condensing gas from the cylinder in a wet ice cooled trap, freezing the liquid NO_2 (N_2O_4) in dry ice and degassing to 0.01 mm Hg. The sample was thawed by warming to 0°, refrozen and again degassed. This procedure was repeated two more times to insure that all air and other noncondensable impurities had been removed. The purified NO_2 was transferred to a 1 liter sample bulb equipped with a stopcock on one end and a piece of gage-glass tubing on the other end to which was attached a rubber septum secured with Swagelok fittings. This flask was evacuated, loaded with a known pressure of purified NO_2 and brought to atmospheric pressure with air. Samples were withdrawn by syringe from this

sample bulb and injected into the photoreactor. The NO_2 was not purified for the reactions with PhMe, CH_3CCl_3, and $CHCl=CCl_2$. This difference may account for the apparent oxidant abscissa displacements in Figures 3 and 4 as compared with Figures 2, 5 and 6.

CH_2Cl_2, "Distilled in Glass", from Burdick and Jackson Laboratories was used as received. Uninhibited CH_3CCl_3 and $CHCl=CCl_2$ were redistilled. $CCl_2=CCl_2$ (Dow Per®) was washed with aqueous HCl and distilled, bp 121°, prior to use. Cylinder $CH_2=CHCl$ from Matheson was placed in a black Teflon gas bag from which the $CH_2=CHCl$ was withdrawn by a syringe for injection into the photoreactor. Mass spectroscopic analysis of the $CH_2=CHCl$ showed a purity of 99.2%. PhMe was redistilled. All of the organic materials were subjected to careful analysis by gas chromatography (gc), mass spectrometry and ultraviolet analyses. No impurities were detected except as noted.

Neutral buffered potassium iodide (KI) solution (Saltzman, 1965) was prepared by dissolving successively 13.61 g of potassium dihydrogen phosphate, 14.20 g of anhydrous disodium hydrogen phosphate, and 10.00 g of KI in 500 ml of water. This mixture was diluted to a volume of 1.00 liter to give a 1% KI solution that was 0.1 M in each of the phosphate salts. This solution was transferred to a brown bottle, stoppered, and stored in the dark for 24 hr before use.

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Sodium thiosulfate ($Na_2S_2O_3$, 10.0 ml of 0.1000 N solution) from Curtin Scientific Co. was standardized against potassium iodate in the conventional manner and diluted to 1.00 liter with distilled water to give a solution which was 0.00100 N.

Analyses. The disappearance of the organic compounds was monitored by periodically withdrawing samples by syringe from the reactor for gc analyses. CH_2Cl_2 and $CCl_2=CCl_2$ were determined with a thermal conductivity gc equipped with a 10 ft x 1/4 inch column packed with 20% Apiezon L using a helium flow rate of 40 cc/minute at 75° and 150° respectively. CH_3CCl_3, $CHCl=CCl_2$ and PhMe were determined with a flame ionization gc equipped with a 3 ft x 1/4 inch column packed with 20% Porapak Q at 175°. $CH_2=CHCl$ was determined with an electron capture gc equipped with a 10 ft x 1/8 inch column packed with 20% N,N-bis-2-cyanoethyl-formamide on 80-100 mesh Chromosorb W at 80°.

The total oxidant was determined by flushing the entire reactor contents through a gas-absorbing tower (Smith et al., 1964) which contained 25 ml of the 1% KI solution. The reactor was flushed with nitrogen at a rate of 1 liter/minute for 30-45 minutes. This time period should remove 95.7-99.0% of the reactor contents according to the equation $C = C_o e^{-(f/v)t}$, where C is the concentration at time t, C_o is the initial concentration, f is the flow rate, and v is the reactor volume.

Attempts to flush the reactor contents with smaller quantities of KI and (or) higher flow rates gave consistently low oxidant values, as determined by titration with $Na_2S_2O_3$. The validity of the method was tested by generating small quantities of O_3 with an electric discharge in a flask, and removing two aliquots by syringe. One was injected into the photoreactor and flushed through a KI solution as described; the other was slowly bubbled directly through an equivalent volume of KI solution. Titration of each solution (several runs) indicated that over 95% of the O_3 in the reactor was accounted for. After the reactor contents had been flushed into the KI solution, the gas tower was rinsed with 15-20 ml of water, 0.75 ml of soluble starch solution was added, and the resulting solution was titrated with 0.001 N $Na_2S_2O_3$. The concentration of oxidant was calculated from the equation, ppm oxidant (as O_3) = 1.276 x ml of 0.001 N $Na_2S_2O_3$. It was necessary to flush the entire reactor in order to have sufficient quantities of O_3 for analysis.

Example of a Typical Photolysis. To the evacuated chamber was added 1.8 µl of CH_3CCl_3, 1.0 cc of a NO_2 (120 mm Hg pressure) -air (640 mm) mixture, and 95 µl of water to give concentrations of 50 ppm (molar or volume basis) CH_3CCl_3 and 12 ppm NO_2 at 43% relative humidity, after the reactor was filled to atmospheric pressure with air. The contents of the reactor were irradiated

for 15 hr. The results of CH_3CCl_3 and oxidant analyses are shown in Figure 3. The reaction was repeated several times for different lengths of time to obtain the other oxidant values shown in Figure 3.

Results and Discussion

Many workers have reported that O_3 did not appear in simulated atmospheric photolyses until the NO had been converted to NO_2 (Leighton, 1961; Altshuller and Bufalini, 1965; Pitts, 1969). It is also clear that nearly the same maximum O_3 concentration is attained irregardless of whether NO or NO_2 is part of the starting mixture. In order to avoid this "induction period" and to thereby shorten the analysis time, NO_2 was used in all of our experiments. The concentration of the organic compound used was usually 50 ppm; this concentration was necessary in order to facilitate gc monitoring of the chlorinated organic compound in some cases. Some experiments were also carried out with ~12 ppm of the chlorinated compounds. A ratio of 4:1 for organic compound:NO_2 was used for all experiments. The range for most of the earlier work was from 2:1 to 8:1.

The precision with which the oxidant could be measured in these experiments suffered from several factors. The

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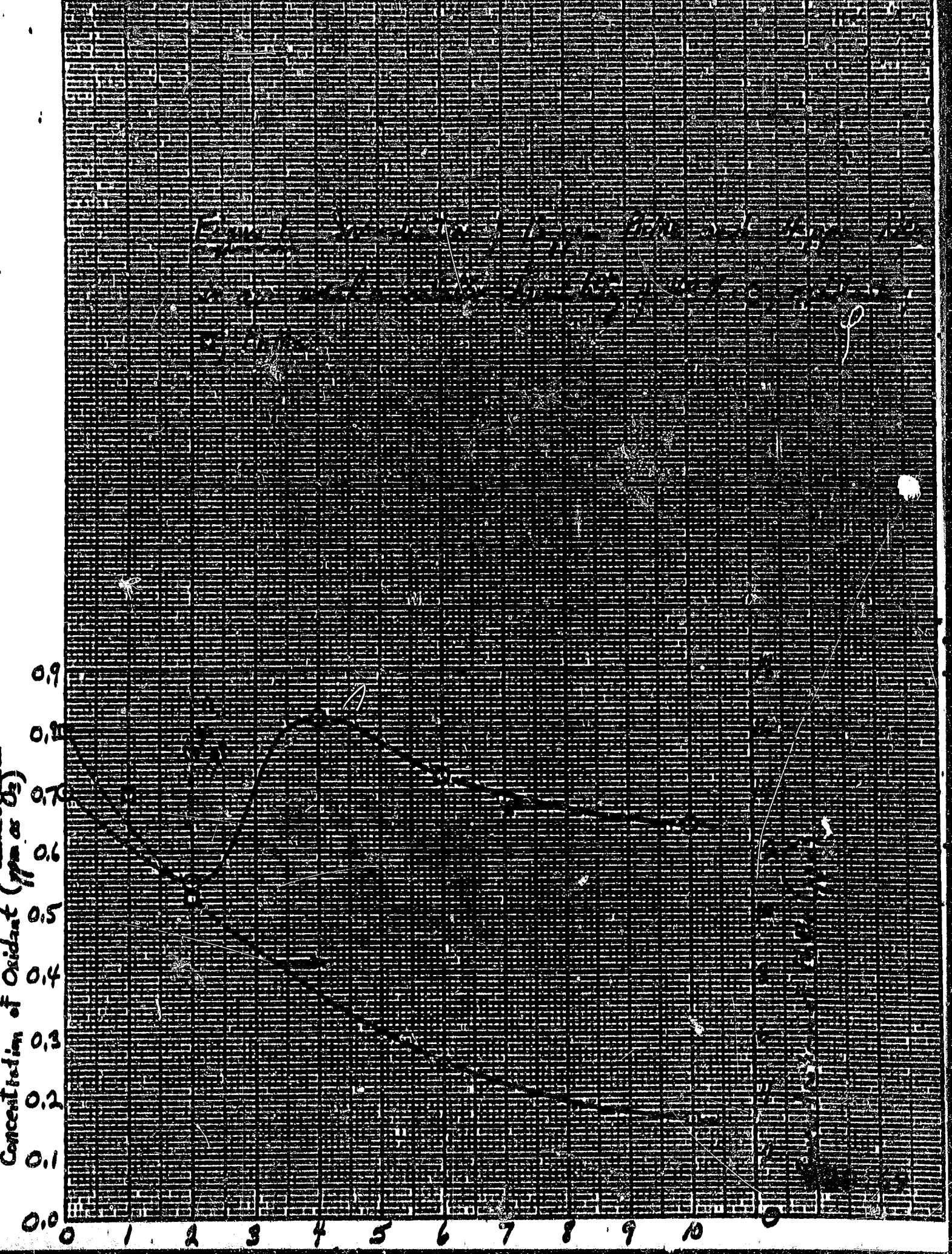
major one was the fact that each datum point came from a separate experiment. Other factors were the difficulty of introducing NO_2 reproducibly and the large volume of air which was flushed through the absorbing reagent. An estimate of the precision in the oxidant measurement is about ± 10-15%. While this precision is not as good as one might wish, the data obtained still give adequate representation of oxidant formation, particularly when one is concerned with relative values of various compounds.

In order to validate the method for total oxidant determination, PhMe was subjected to the procedure. This compound had been studied earlier (Levy and Miller, 1970) and was found to lead to moderate quantities of O_3 at a relatively rapid rate. The photolysis of 16 ppm PhMe was carried out in the presence of 4 ppm NO_2. Figure 1 shows the results of this irradiation. The maximum oxidant concentration (0.8 ppm) was reached after 4 hours irradiation. Levy and Miller (1970) reported a maximum O_3 concentration of 0.44 ppm after 3.3 hours whenthe initial concentrations were 4 ppm PhMe and 2 ppm NO. In a previous paper of this series (Dilling et al., 1974) a half-life of 6.8 hours was reported for PhMe (10 ppm) when irradiated in the presence of NO (5 ppm); the data in Figure 1 indicate a half life of ~3.5 hours. Bearing in mind


Concentration of Oxidant (ppm as O3)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0 1 2 3 4 5 6 7 8 9 10

the differences in starting concentrations, light intensities, and differences in reaction times for NO versus NO_2, the agreement in the data seems satisfactory.

Data for the photolysis of CH_2Cl_2 are shown in Figure 2. There was no disappearance of CH_2Cl_2 within experimental error, in agreement with our previous report (Dilling et al., 1974). The oxidant levels were essentially identical with a blank run in which the CH_2Cl_2 was omitted. NO_2 at an initial concentration of 13 ppm in the blank, gave an oxidant level of 3.5 ppm as O_3 (Saltzman, 1965); this level fell off rapidly to ~0.8 ppm in ~3 hours. Under the conditions of these experiments this level (0.8 ppm) remained relatively constant for at least 15 hours of continued irradiation. Photolysis of an NO_2 blank which started at 2.7 ppm resulted in a total oxidant decrease from 0.7 ppm to 0.3 ppm in 4 hours.

The value for total oxidant obviously includes both O_3 and NO_2 as well as any other oxidizing species which might be produced by photolysis during the course of the experiment. In the early stages of irradiation the oxidant is almost entirely NO_2. However in the later stages of the reaction, where O_3 may be the predominant oxidant, the O_3 concentration cannot be obtained by subtracting the NO_2 (blank) curve from the total oxidant curve because the addition of an organic material can

drastically alter the ($NO_2 + O_2 \xrightleftharpoons{h\nu} NO + O_3$) equilibrium (Altshuller and Bufalini, 1965).

The photolysis of CH_3CCl_3 was studied at two concentrations, 50 ppm and 10 ppm. The results are illustrated in Figure 3. There was relatively little disappearance of the CH_3CCl_3. The data at 10 ppm indicate that the oxidant level decreased rapidly until it approached zero. The lowest oxidant level that could be measured was about 0.05 ppm. The curve for the 50 ppm reaction again rapidly decreased, but asymptotically approached ~0.1 ppm, which is very low, considering the high initial concentration of CH_3CCl_3.

$CHCl=CCl_2$ produced much more oxidant (Figure 4) than did CH_2Cl_2 and CH_3CCl_3 in the early stages of the photolysis. The formation of a significant amount of oxidant from $CHCl=CCl_2$ is in agreement with most of the references cited above. The rapidity with which oxidant forms is perhaps surprising. That it is real, however, is borne out by the fact that several of the points in Figure 4 were obtained in duplicate. Again, however, some of the oxidant may be due to a slower decrease in the NO_2 concentration.

$CCl_2=CCl_2$ was found to be rather reactive from the standpoint of disappearance of organic; the concentration dropped from 50 ppm to <5 ppm in 4 hours. In this respect it is quite

Concentration of Oxidant (ppm as O_3)

Concentration of CH_3CCl_3 (ppm)

similar to $CHCl=CCl_2$. However, $CCl_2=CCl_2$ is rather dramatically different from $CHCl=CCl_2$ with respect to oxidant formation. A comparison of the curves in Figure 5 shows that photolysis of $CCl_2=CCl_2$ led to slight oxidant formation initially but that the final oxidant level reached after 15 hours of irradiation was lower than that for the blank. This same behavior was also observed in experiments at lower concentrations (13 ppm $CCl_2=CCl_2$, 2.8 ppm NO_2). The total oxidant in the presence of $CCl_2=CCl_2$ decreased from 0.7 ppm to 0.2 ppm in 4.4 hours; the final oxidant concentration in the blank was 0.3 ppm.

Oxidant formation in the presence of $CH_2=CHCl$ (Figure 6) was comparable to that in the presence of $CHCl=CCl_2$. The rate of $CH_2=CHCl$ disappearance was similar to that of $CCl_2=CCl_2$ in the initial stages, but became slower as time increased.

In summary, the relative oxidant forming potentials of the five chlorinated compounds studied are as follows:

$CHCl=CCl_2 \approx CH_2=CHCl > CCl_2=CCl_2 > CH_2Cl_2 \approx CH_3CCl_3$.

Concentration of $CH_2=CHCl$ (ppm)

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Chemical Name (300 per name)	CAS No. (10)
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Nancy P. Tefertiller and Wendell L. Dilling

The Simulated Atmospheric Photoreactivity of Methyl Chloroform, Methylene Chloride, and Carbon Tetrachloride*

The ultimate fate of chlorinated solvents discharged to the environment is of increasing concern, as ecological considerations are leading to tighter governmental regulation of these materials. Methyl chloroform and methylene chloride have been reported to be stable to photochemical degradation for short periods of time when irradiated with sea level type irradiation ($\lambda > 295$ mμ). If these materials are persistent, the question can be raised as to whether, with continued release to the atmosphere, they could accumulate at high altitude and be a cause of future environmental problems.

This study was undertaken to determine over what periods of time methyl chloroform and methylene chloride were stable under environmentally simulated conditions at sea level, then how rapidly they, along with CCl_4, would disappear when exposed to simulated high altitude type irradiation ($\lambda > 254$ mμ).

Methyl chloroform (50 ppm) did not react when irradiated with sunlamps ($\lambda > 295$ mμ) for 4 weeks in the presence of NO_x (~ 10 ppm) and moisture (35% RH). Methylene chloride was found to be stable under the same conditions for approximately 6 days, then it rapidly disappeared. All 3 solvents were at least 90% reacted within 10 min, however, when irradiated with light typical of that found at high altitude. Therefore a buildup of these materials in the upper atmosphere does not appear likely.

* Organic Photochemistry, XX.

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I. INTRODUCTION[1]

It had been reported by Corwin Fredewey[2] that methyl chloroform (10 ppm) in air containing 35% relative humidity and 5 ppm NO reacted less than 5% in 23.5 hr when irradiated with sunlamps ($\lambda > 295$ mμ) emitting sea level type irradiation. Similarly, in the presence of 16.8 ppm NO_2 it reacted less than 5% in 8 hours. Methylene chloride under the same conditions showed less than 5% reactivity either in 21 hours in the presence of 5 ppm NO or in 7.5 hours in the presence of 16.8 ppm NO_2. Further work by Arrington and Hines[3,4] confirmed that methyl chloroform (10 ppm and 40 ppm) and methylene chloride (10.5 ppm and 50 ppm) were unreactive photochemically when irradiated 15 and 14 hours respectively in the presence of NO_x and moisture and that neither exhibited oxidant forming potential.

The present study was undertaken to determine whether methyl chloroform and methylene chloride would be stable under prolonged irradiation by sunlamps under simulated sea-level atmospheric conditions and to determine whether there was any possibility of their buildup at higher altitudes should they prove to be persistent at sea level. Carbon tetrachloride was included in the study using simulated high altitude irradiation (λ 254 mμ), as it has been observed by scientists in remote areas of the world, leading to questions of its persistence in the environment.

II. RESULTS AND CONCLUSIONS

Methyl chloroform (50 ppm) in air containing 35% relative humidity and 10 ppm NO_2 at ~ 27°C was stable for 4 weeks when irradiated with GE sunlamps emitting low altitude light (> 295 mμ) (Figures 1 and 2). When irradiated at 33°C with a Hanovia low pressure mercury arc lamp (λ = 254 mμ) simulating high altitude irradiation, the methyl chloroform was 94% gone in 10 minutes (Figure 3). The presence of NO_2 and moisture in the air were not necessary to the reactivity of the methyl chloroform (Figures 4 and 5), and the solvent was stable in the presence of ozone in the dark. There appeared to be no adsorption of methyl chloroform onto the reactor walls.

FIGURE 1

UPA LEVEL IRRADIATION
OF METHYL CHLOROFORM

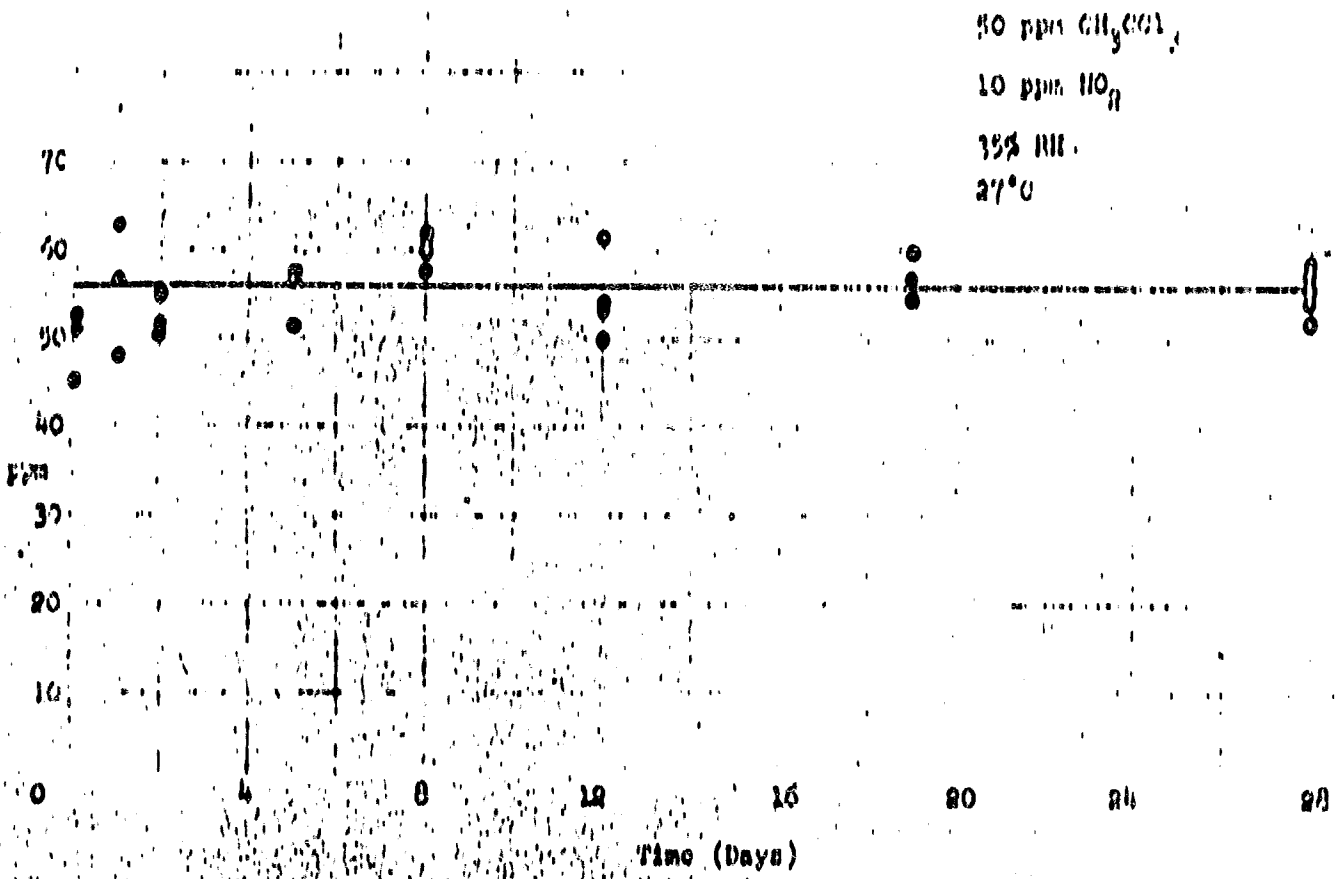

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FIGURE 2

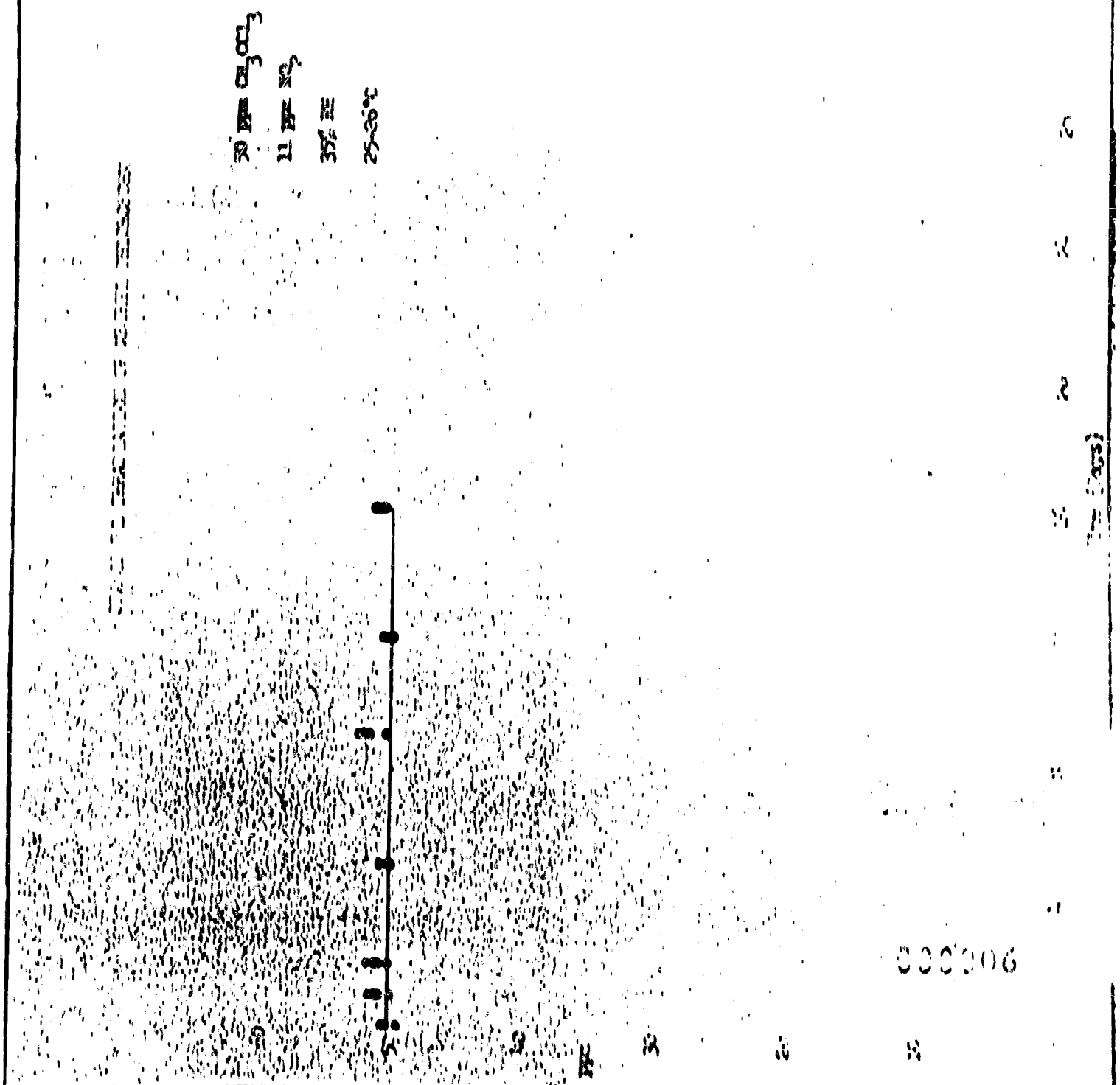
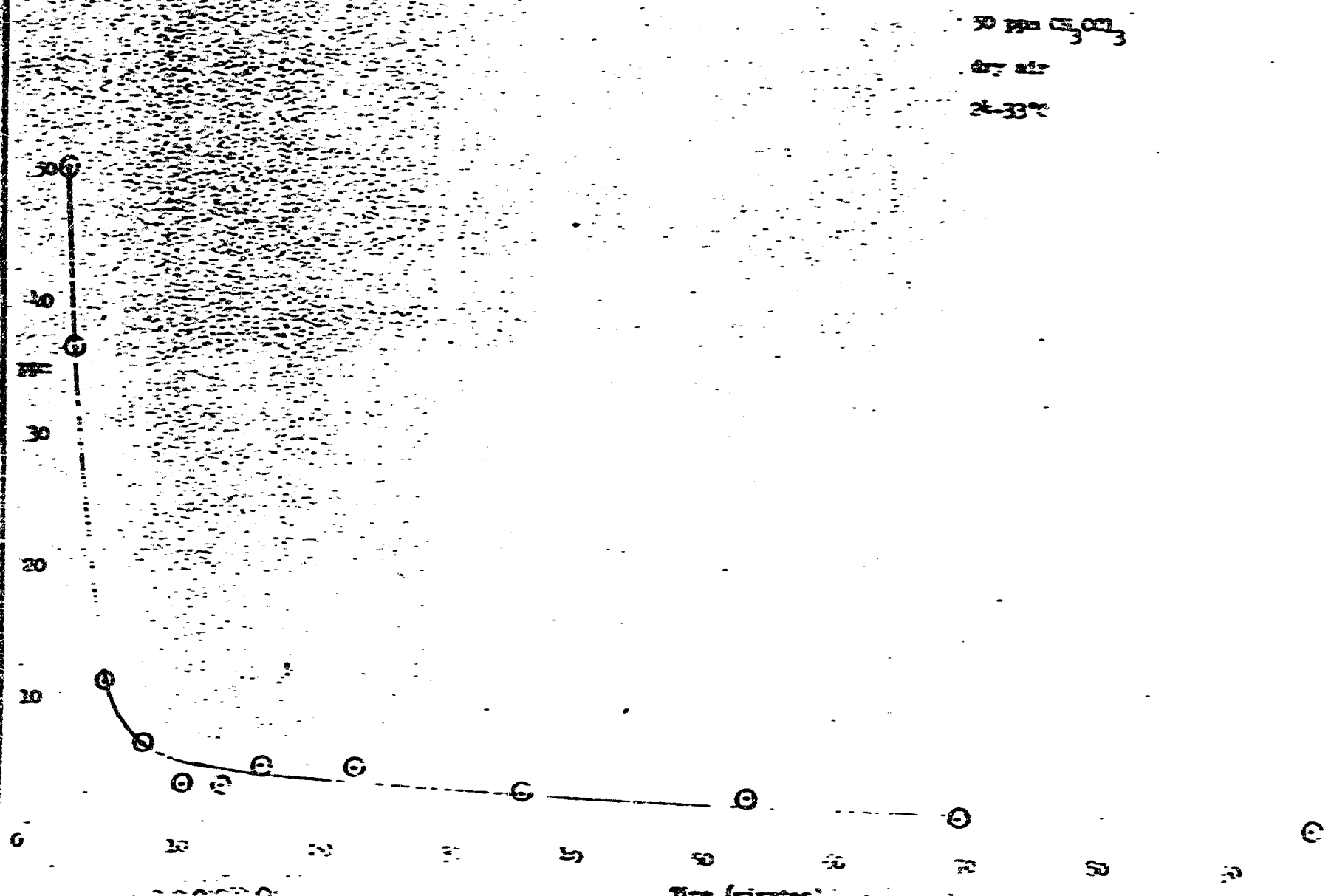


FIGURE 4

[illegible] OF METHYL CHLOROFORM, DRY AIR



Methylene chloride (50 ppm) in air at 20-30°C containing 3% relative humidity and 10.5 ppm NO_2 was stable for approximately 6 days under sea-level irradiation, then rapidly disappeared (Figures 6 and 7). It was possible to account for 77% of the chlorine from the CH_2Cl_2 as inorganic Cl^- after rinsing the reactor with dilute NaOH solution. With high altitude irradiation, it too, was 99% reacted in 10 minutes in dry air with no NO_2 present (Figure 8).

Carbon tetrachloride was not studied under low altitude conditions, but was found to be 96% photodegraded in 10 minutes at a level of 50 ppm in dry air with high altitude irradiation (Figure 9).

Some unidentified photoproducts were observed in the high altitude irradiation of all three solvents. These were generally of a low concentration with the exception of one product from CCl_4 which reached a concentration of about a third (as area %) of the starting CCl_4, and then disappeared with further irradiation.

III. DISCUSSION

Both methyl chloroform and methylene chloride appear to be stable in air when irradiated with sunlamps which simulate sunlight at sea level. Methyl chloroform, in particular, showed no activity in four weeks. The sharp disappearance of the methylene chloride after several days irradiation is unexplained. It can be theorized that an impurity in the CH_2Cl_2, as yet unidentified, inhibited the reaction until the impurity itself had been photoreacted; that a product was formed at an extremely low level which catalyzed the reaction; or that there was an agent on the surface of the glass which effected a delayed reaction. The fact that at the completion of the reaction 77% of the Cl_2 from the CH_2Cl_2 could be accounted for by $AgNO_3$ titration as Cl^-, however, indicates that reaction did occur, rather than an accident to the system (such as a leak) which might have caused the CH_2Cl_2 to be lost.

FIGURE 6

SEA LEVEL PHOTOREACTION OF METHYLENE CHLORIDE

50 ppm CH_2Cl_2

10.5 ppm NO_2

35% RH

25-26°C

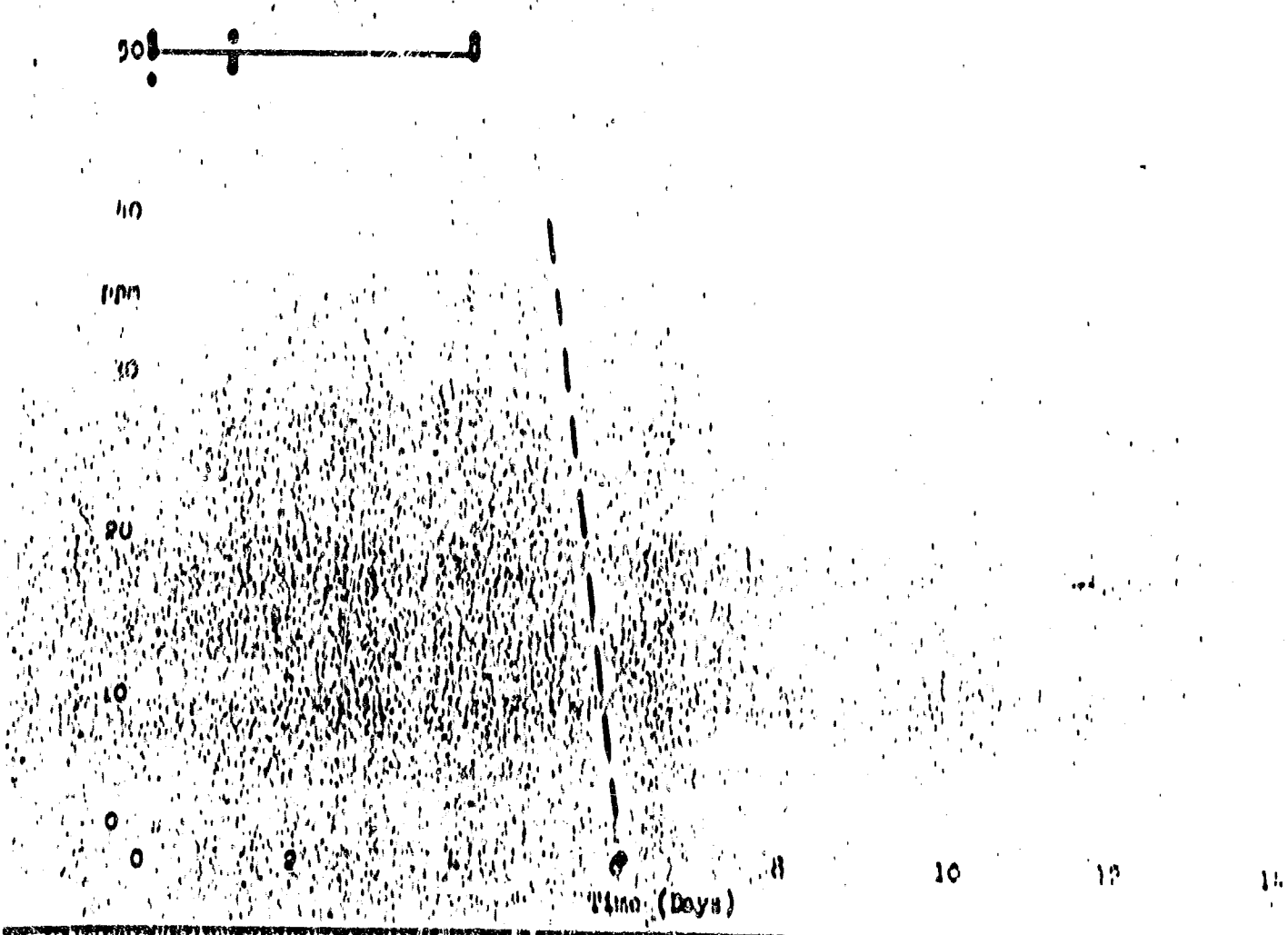


FIGURE 7

SEA LEVEL PHOTOREACTION OF METHYLENE CHLORIDE

50 ppm CH_2Cl_2

10 ppm NO_x

65% RH

25-30°C

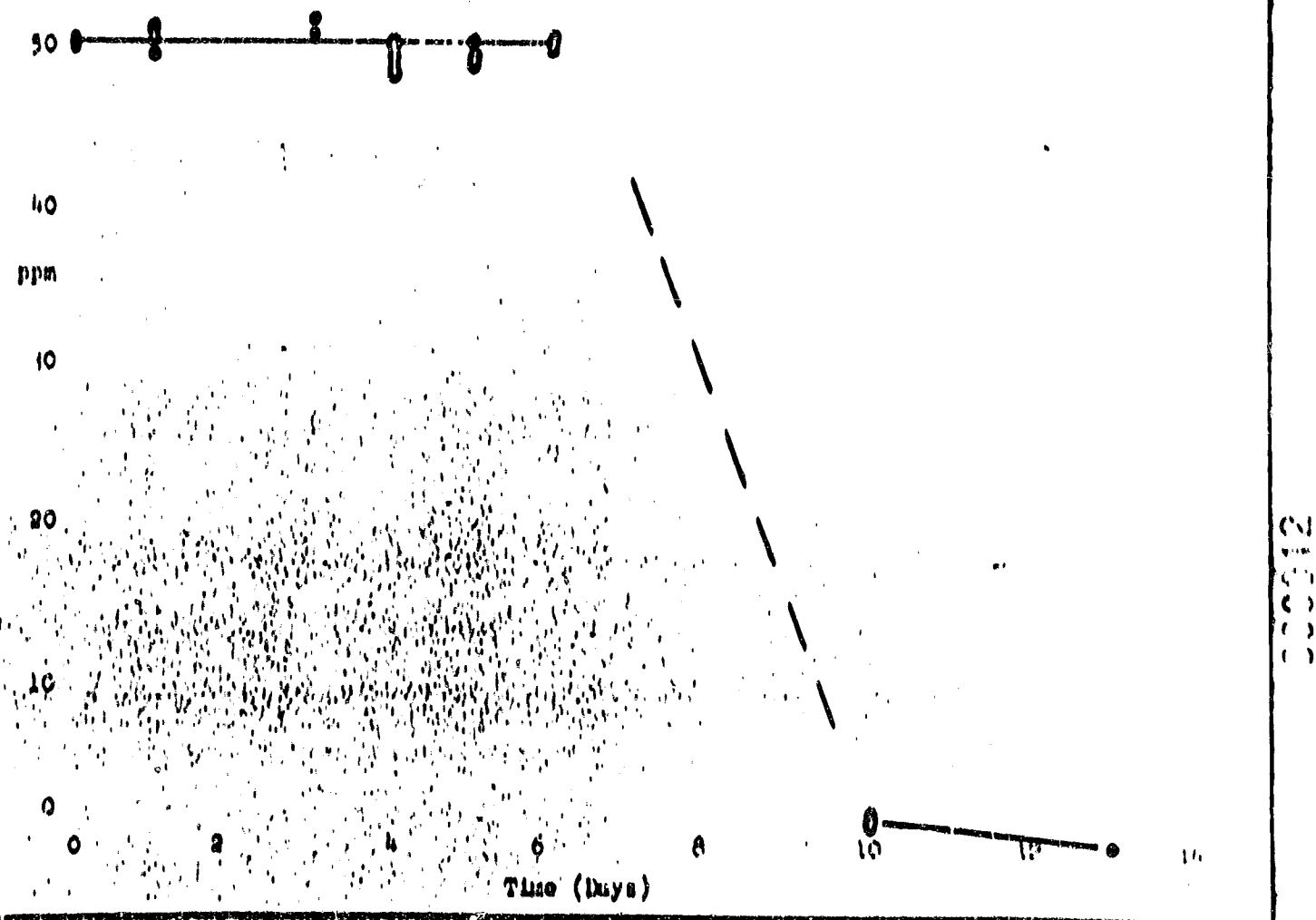


FIGURE 1

HIGH ALTITUDE PHOTOREACTION OF METHYLENE CHLORIDE, DRY AIR

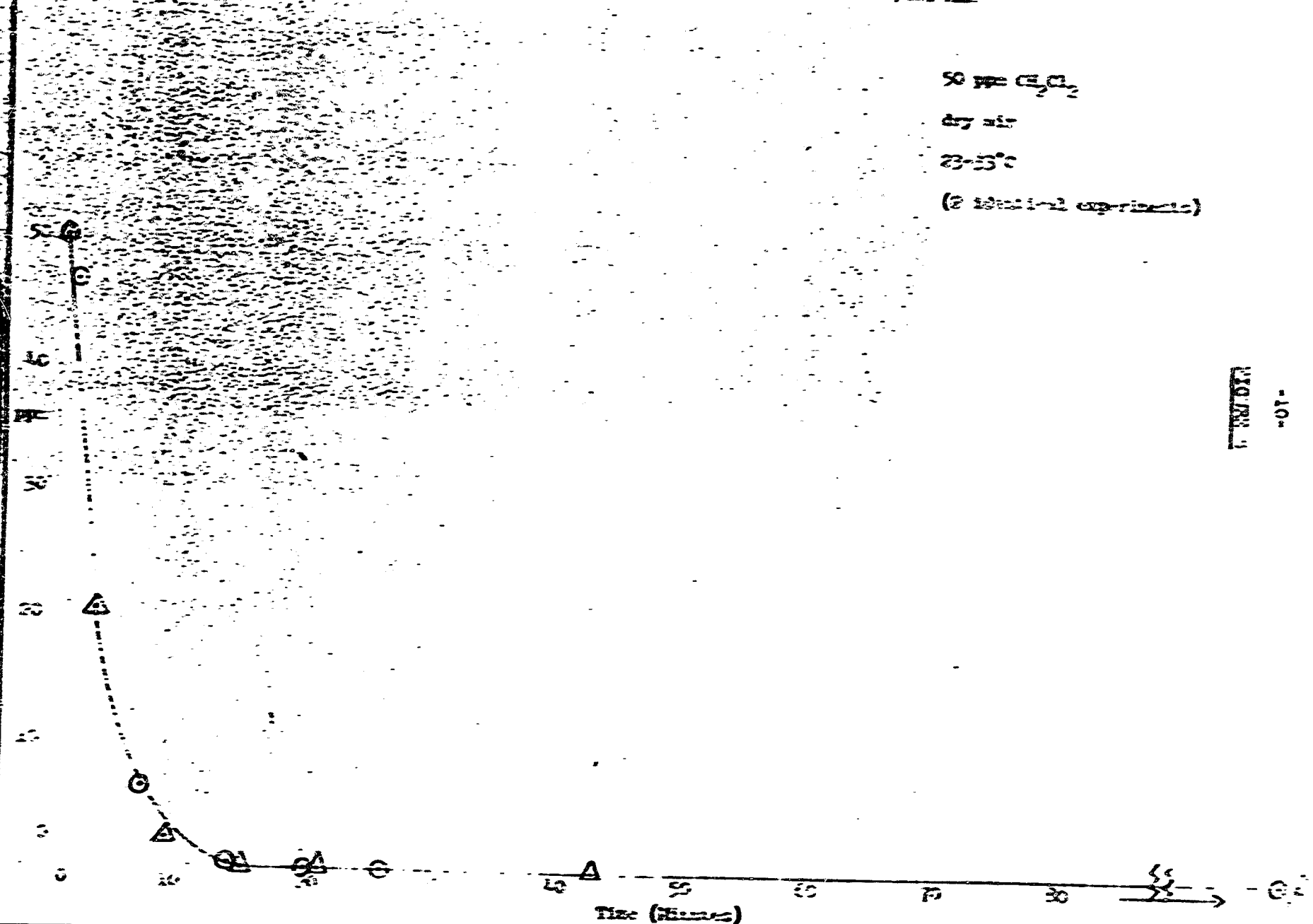


FIGURE 1

Sunlight which reaches the surface of the earth has been filtered through the earth's ozone layer (5-20 mi in depth) where the short wavelength ultraviolet radiation is absorbed.[4] Consequently, it is of longer wavelength (> 290 nm) and lower energy than that radiation in the upper regions of the atmosphere (~ 25 mi) which still contains the high energy, short wavelength UV light. Even though the spectral distribution from sunlight is a continuum while the emission from a medium pressure mercury arc lamp such as the sunlamps used in this work is a series of lines, the overall spectral output from the two sources parallel each other rather closely in this region of the spectrum (> 290 nm).[6] Therefore the sunlamp equipped with a Pyrex filter should be a reasonably good simulator of sunlight in this spectral region. It is not surprising that methyl chloroform and methylene chloride were not reactive when irradiated with sunlamps as their ultraviolet spectra show no absorption in that spectral region. Apparently these solvents are unreactive toward the radicals derived from the photolysis of nitrogen dioxide.

The high energy radiation observed at higher altitudes would be expected to cause a more efficient photoreaction of the chlorinated solvents which have been studied in this work. The Hanovia low pressure mercury arc lamp used to simulate high altitude radiation has 90% of its emitted light at 254 nm where the solvents still have tailing absorption.[7] The lamp's intensity (see Appendix) as measured by Roger Bredeweg of the Analytical Lab., is 30-120 times that of sunlight just inside the earth's atmosphere at an altitude of ~ 25 miles (see Appendix). While more intense than naturally occurring sunlight, the rapidity with which the chlorinated solvents were photolyzed allows one to draw a crude extrapolation as to how long these solvents would exist should they manage to reach the upper regions of the earth's atmosphere. Based only on lamp intensity, one would expect at least a 90% photoreaction of the solvents with 5-20 hours irradiation. There appears to be little danger of their accumulation under these conditions.

The exact temperature of the atmosphere at the altitude which the lamp is simulating is unknown; at 11 kilometers (~ 7 mi) the temperature is -50 to -60°C and rises to about 0°C at 50 km (~ 30 mi).[8] The photoreactions described in the work were run close to room temperature; temperature often has little effect on the rate of photoreactions. The photoreactions were run at 740-750 mm. pressure; the pressure at high altitudes is lower. Pressure probably has an effect on the rate of these photoreactions. The rate could be either increased or decreased with a decrease in pressure depending on the mechanism of the photodecompositions.

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There is probably not much water in the stratosphere at higher altitudes, most being found in the troposphere (< 11 km).[9]

IV. EXPERIMENTAL

A. General Techniques

a) Sea level irradiation - The photochamber used in this work was designed to minimize the possibility of leakage from the chamber during extended photoreactions. Consequently, there were only two openings, a 4 mm teflon stopcock and a capillary tubing sample port which was sealed with a silicone rubber septum. The chamber was a flat-ended pyrex cylinder of 11.6 liters capacity. At each end was positioned a sunlamp. Between each lamp and the end of the reactor was a 3/8" thick quartz plate to reduce warming of the photoreactor by the sunlamps. The ends of the reactor were further cooled by forced air which had been passed over a cold water cooling coil. The entire system was placed in a hood to maintain adequate air flow to dissipate generated heat. The temperature was measured by a thermocouple taped to the outside of the reactor near the center of the cylinder. (Figure 10)

Irradiation was provided by 2 GE RS-275W sunlamps with an intensity of 1 to 2 times that of sunlight[2] and wavelengths greater than 295 mμ.

The hood in which the apparatus was placed was covered by black cloth to eliminate the possibility of eye injury from the ultraviolet irradiation.

b) High altitude irradiation - The photochamber for these experiments was a Pyrex sphere of 12.1 liters capacity having a 4 mm teflon stopcock and a capillary tubing sample port closed by a silicone rubber septum. The photoreactor had a ground glass joint into which was fitted the ultraviolet lamp. A silicone grease seal was used to eliminate the possibility of leakage from the reactor. The system was proven capable of holding a vacuum for several days. Blowers were directed at the top and bottom of the photoreactor to dissipate the small amount of heat generated by the lamp. The temperature of the system was measured by a thermocouple taped to the outside of the flask near the middle between the blowers.

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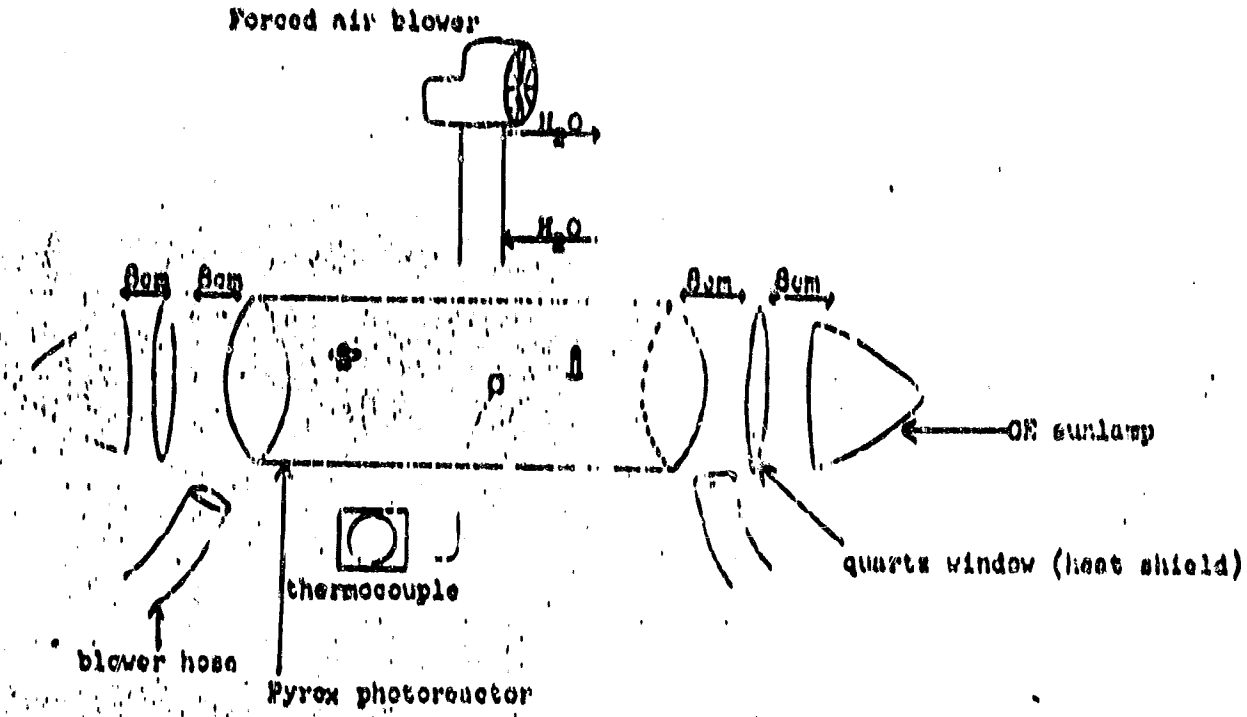
FIGURE 10

SEA LEVEL IRRADIATION APPARATUS



An a high voltage existed across the leads of the lamp the entire apparatus was enclosed in a wire mesh, grounded cage with safety switches which would turn off the lamp if the doors were opened accidentally while the lamp was on. The entire cage was covered by black cloth to prevent escape of short wavelength UV radiation which is potentially damaging to the eyes. The cage was ventilated into a nearby hood by an auxiliary blower, as the lamp generated ozone during its operation. (Figure 11)

Irradiation was provided by a Rayonet low pressure mercury arc lamp (see Appendix). Approximately 90% of its emitted wavelength was at 2537 Å.[7] There may also be emitted a shorter wavelength line at 1849 Å which would cause the generation of the ozone observed each time the lamp was used. The lamp can be said to approximate the sun's emission. It is 10-40 times as intense as the sun's radiation would be just outside the earth's atmosphere. Just within the earth's atmosphere (at ~ 25 miles) where the sun's intensity is cut to ~ 1/3 of its original intensity in the 2000-3000 Å range by absorption of its short wavelength radiation by ozone, the lamp would be 30-120 times as intense as sunlight at that altitude.

a) Gas mixture - The simulated atmosphere used in the photoreactions was obtained by premixing to the desired proportions a dry air stream with an air stream saturated with water and one containing a known amount of NO_2. The gas streams were monitored by flowmeters and the photochamber was flushed with the gas mixture for a period of time calculated to replace 99.9% of the ambient air in the photochamber.[3] A sample of the gas mixture was then removed and analyzed for ppm NO_2 using a modified Saltzman procedure and a calibration curve prepared by M. J. Mintz.[9] Finally a quantity of chlorinated solvent calculated to give 50 ppm (on a molar basis) in air was injected through the septum into the reactor. The system was allowed to equilibrate overnight, blanks (0 time) were analyzed, and the photoreactions were run for varying lengths of time with the two types of irradiation. Gas samples (2 or 10 ml) were removed at specific intervals by gas-tight syringe and analyzed by gas-liquid chromatography.

b) Analysis - Two gas chromatography systems were used to analyze for chlorinated solvent concentration. The first was by thermal conductivity GC analysis using a F & M 500 instrument equipped with a 10' x 1/4" 20% apiezon L column

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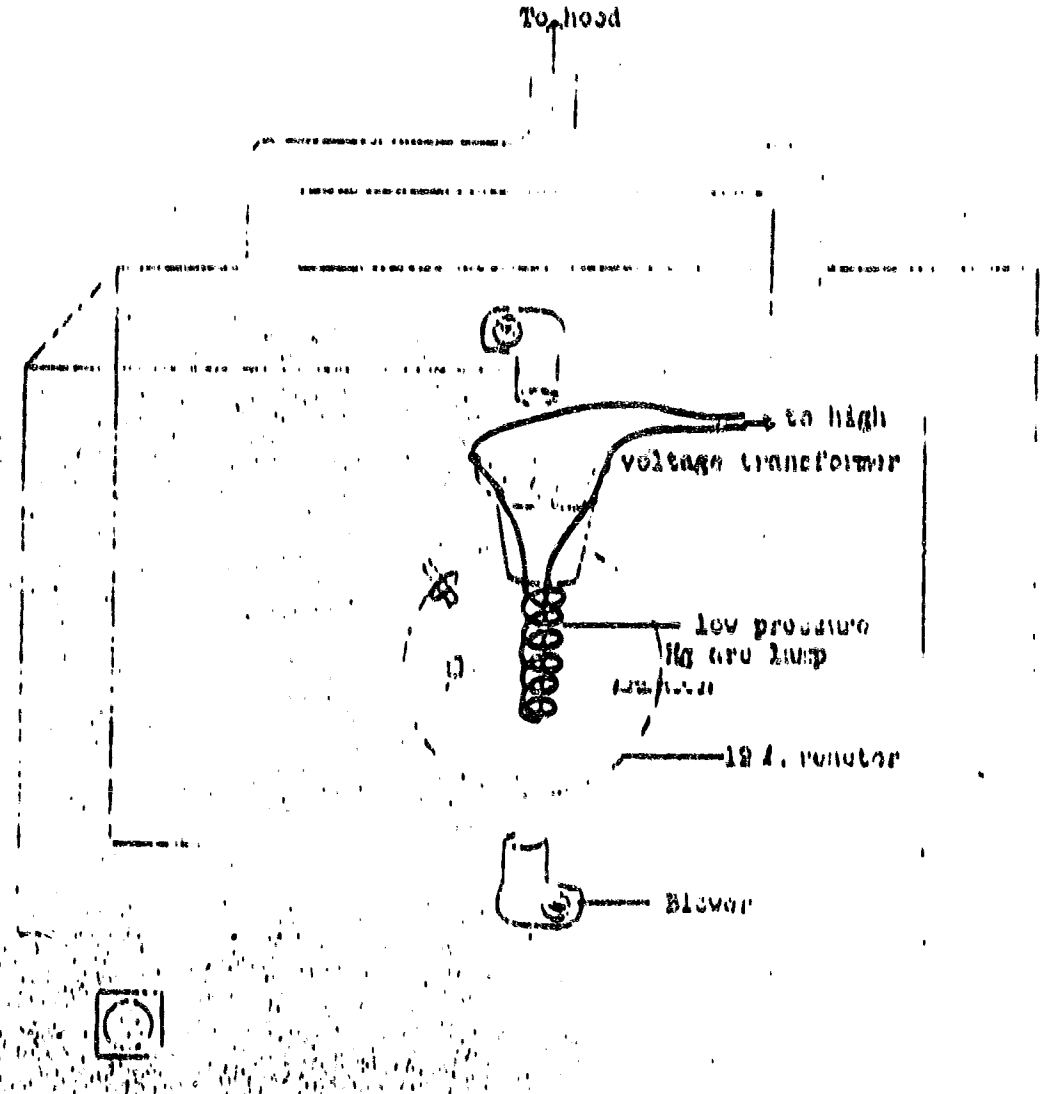
FIGURE

HIGH ALTITUDE IRRADIATION APPARATUS



on chromosorb W/AW, and a column temperature of 150°. The detector temperature was 300°, the injector was 140° and the helium flow rate through the column was 45 cc/min. A 10 cc gas sample was used, and while it was possible to increase the sensitivity of the filaments by increasing the filament current to 175 milliamps (vs. 150 under typical operating conditions), the resultant peak size was quite small. The peak area of all gas samples was measured by planimeter. There was observed considerable scatter in the data and the best that was hoped for was 10% reproducibility. A liquid standard consisting of methyl chloroform in trichloroethylene was used to calibrate the GC sensitivity with each gas run.

During the course of this work a hydrogen flame ionization gas chromatography system became available which allowed much greater sample reproducibility, usually within 4%. It consisted of an Aerograph Hy-Fi Model 550-B with a Bendix electrometer equipped with a 10' x 1/8" N,N-bis(2-cyanoethyl)formamide column 20% on chromosorb W. The column temperature was generally 87° and the carrier gas was nitrogen. It was possible to use a 2 cc gas sample size with this system.

A gas standard bulb was used to calibrate the H_2 flame GC. It consisted of 50 ppm chlorinated solvent in a 12.6 liter glass sphere having only two openings, a 4 mm stopcock and a capillary sampling port closed by a silicone rubber septum. Samples from this bulb were reproducible over at least 6 weeks, indicating no leakage from the bulb.

D. Reagents

Carbon tetrachloride and methylene chloride, Burdick & Jackson, distilled in glass; methyl chloroform, Dow-distilled, center cut BP 74° at 760 mm; air, dry cylinder air from Matheson Gas Products, NO_2 in air, made to specification by Matheson Gas Products, analyzed to be 95 ppm in air by the modified Saltzman procedure.

E. The Photoreaction of Methyl Chloroform

a) Simulated sea level environment - Methyl chloroform (50 ppm) in the presence of 10 ppm NO_2 and 35% relative humidity at 87° was irradiated for 28 days using the GE sunlamps (Figure 1). The methyl chloroform was analyzed by thermal conductivity GC and found to be stable under these conditions.

The above experiment was repeated under the same conditions using the flame-ionization GC method of analysis in order to reduce the scatter in the data and was found to be stable when irradiated for 16 days (Figure 2).

b) Simulated high altitude irradiation - Methyl chloroform (90 ppm) in the presence of 9 ppm NO_2 and 35% RH at 25-33°C was 90% photoreacted within 10 minutes when irradiated with the low pressure Hg arc lamp (Figure 3). Analysis was by thermal conductivity and, while the CH_3CCl_3 was difficult to distinguish from background noise after 5 minutes, it appears that there is a residual amount (~ 3 ppm) which remains in the system. This is probably an artifact of the system (i.e. CH_3CCl_3 may have dissolved into the rubber septum and is slowly being released back into the reactor), and later experiments with CCl_4 using the more sensitive flame-ionization GC indicate that the small residuals observed in several reactions will eventually disappear with further irradiation.

Effect of NO_2 - The above reaction was repeated with no NO_2 present. The CH_3CCl_3 appeared to be completely gone within 6 min. (Figure 4). The apparent complete reaction of the CH_3CCl_3, compared to that run in the presence of NO_2, is probably not significant because of the analytical uncertainty.

Effect of moisture - CH_3CCl_3 (90 ppm) in dry air (obtained by running Matheson dry cylinder air through a drying tower filled with Drierite interspersed with P_2O_5) was irradiated for 96 min. at 24-33°C. The CH_3CCl_3 was approximately 99% reacted in 10 min. (Figure 5). Flame-ionization GC was used and confirmed a low residual of CH_3CCl_3 in the reaction. It also indicated the appearance, then subsequent disappearance of a possible very low level unidentified photoproduct.

Stability of methyl chloroform in the presence of ozone - CH_3CCl_3 (90 ppm) was added to an ozone-filled photoreactor and held in the dark for 4 days. The CH_3CCl_3 was analyzed after 1 day and 4 days. There was no decrease in the concentration of CH_3CCl_3 as compared to pre-ozone samples with no ozone present.

Adsorption of methyl chloroform on the reactor walls - CH_3CCl_3 (90 ppm) was allowed to equilibrate overnight in the photoreactor and its GC peak area measured. A second addition of 90 ppm CH_3CCl_3 was made to the photoreactor and this was also allowed to equilibrate overnight. The subsequent peak area was about double that of the first CH_3CCl_3 injection (within experimental error) indicating no significant adsorption of the first addition of CH_3CCl_3 onto the reactor walls.

D. The Photoreaction of Methylene Chloride

Simulated sea level environment

Methylene chloride (50 ppm) in the presence of 10.5 ppm NO_2 and 35% RH was irradiated at 25-26° for 6 days. The CH_2Cl_2 was stable for 4 days, then disappeared completely by the sixth day (Figure 6).

The above reaction was repeated. The CH_2Cl_2 was stable for 6 days, almost completely gone by 10 days and totally reacted in 13 days (Figure 7). The photoreactor was rinsed with 20 ml of 0.01N NaOH and 40 ml of DI water, the rinses were evaporated to a small volume, acidified and titrated with N/10 $AgNO_3$. It took 0.36 ml N/10 $AgNO_3$ to reach an end point, a recovery of 76.6% of the chlorine from the initial CH_2Cl_2 (1.91 µl or 0.0170 mM Cl^-) as Cl^-.

Simulated high altitude irradiation

Methylene chloride (50 ppm) in dry air was irradiated for approximately 2.5 hours with the low pressure Hg arc lamp. The CH_2Cl_2 was 25% reacted within 10 minutes and completely reacted within 40 minutes (Figure 8). The experiment was repeated in order to observe the behavior of an impurity which appeared in the equilibrated gas mixture only after the CH_2Cl_2 had been injected into the reactor (perhaps a residue coming off the reactor walls from previous photoreactions) and which disappeared rapidly upon irradiation. A small photoproduct then formed having the same retention time, but it, too, disappeared with further irradiation. A second, even smaller unidentified photoproduct may have been observed.

A GC peak identified as water was confirmed in these experiments. The source of the moisture is unknown, but the air in these reactions is dry cylinder air further treated in a drying tower as described.

E. The Photoreaction of Carbon Tetrachloride

Simulated high altitude irradiation

CCl_4 (50 ppm) in dry air was irradiated at 23-31°C for 320 minutes. The CCl_4 was 90% reacted within 10 minutes, leaving a low level of CCl_4 (~ 5 ppm)

which gradually disappeared over the next [illegible] (Figure 5). A photoproduct having the same retention time as that seen in the CH_2Cl_2 photoreaction was observed. At its greatest concentration (at ~13 min. [illegible]) it represented [illegible] % of starting CCl_4. The product is unidentified. It is not phosgene, formic acid, formaldehyde, hexachloroethane or tetrachloroethylene. It gradually disappeared with continued irradiation, with most of it being photoreacted within one hour. There may have been one other, very small photoproduct observed in the reaction.

V. ACKNOWLEDGEMENTS

The authors wish to thank Michael J. Mintz of Organic Chemicals Production Research for the design of the photoreactors and many helpful discussions.

VI. TOXICITY AND HANDLING PRECAUTIONS

The chlorinated solvents used in this work did not present a health hazard at the levels used. Routine precautions were taken in their handling. Care was taken to avoid accidental release of potentially eye damaging ultraviolet radiation by covering both apparatus with black cloth. The ozone generated by the low pressure mercury arc lamp was monitored by Richard Olson of Chemical Biology Research (Industrial Hygiene Section)[10] and the level found to be far less than the Threshold Limit Value (0.1 ppm) outside the enclosed work cage. The ozone inside the work cage (≤ 0.8 ppm) was forced into a nearby hood by an auxiliary blower, having an alarm to indicate blower failure.

VII. ECOLOGY

The data in this report was gathered in order to learn more about the interaction of the above solvents with the environment and to prevent the development of future problems. While CH_3CCl_3 and CH_2Cl_2 appear to be stable at low altitudes, they previously have been shown not to lead to oxidant formation and therefore not to contribute to photochemical smog.[3,4] At higher altitudes, as indicated, the solvents would not be persistent.

[illegible]

1. [illegible] XX. [illegible] XIX. W. L. [illegible], Dow Chemical Company report [illegible] ([illegible]).

2. [illegible] J. [illegible]

3. J. F. Arrington and M. J. Mintz, Dow Chemical Company Report

4. M. J. Mintz, Dow Chemical Company

5. Lewis Keller, "Ultraviolet Radiation," Second Edition, John Wiley and Sons, Inc., New York, 1965, pp. 118-120.

6. H. B. [illegible] and W. L. Dilling, Dow Chemical Company

7. J. G. Calvert and J. N. Pitts, Jr., "Photochemistry," John Wiley and Sons, Inc., New York, 1966, pp. [illegible], 689.

8. Chem. Eng. News, Special Report, "Chemistry and the Atmosphere", March 28, 196[illegible], Vol. 44, pp. 1A-54A.

9. M. J. Mintz, Dow Chemical Company

10. R. Olson, Dow Chemical Company

Appendix

Relative Energy of Rocket's Low Pressure Mercury Arc Lamp Emitted and Solar Insolation Outside Earth's Atmosphere

Rocket's Low Pressure Mercury Arc Lamp

Wavelength (Å)	Intensity (in watts/cm^2) at 2.5 meters from Lamp
8537	3.5 (±7%)
2654	0.016
2967	0.038
3109	0.11
3341	0.006
3657	0.093
4068	0.055
4358	0.063
5461	0.036

Total energy at 8537 Å of 2.5 watter lamp assumed to be a cylinder (10 cm. long x 2.8 cm. diameter) (actually a coil); light intensity (power) = 11.7 milliwatts/cm^2 at surface of lamp.

Solar Radiation Outside Earth's Atmosphere
(Calculated from data in ref. 5, p. [illegible])

Wavelength range (mμ)	Power in wavelength range (milliwatts/cm²)	
140-145	0.00001	
145-155	0.00007	
155-165	0.00018	
165-175	0.00041	
175-185	0.00091	
185-195	0.0017	
195-202.5	0.00225	
202.5-207.5	0.0025	
207.5-212.5	0.0050	
212.5-217.5	0.0090	
217.5-222.5	0.015	
222.5-227.5	0.021	
227.5-232.5	0.026	
232.5-237.5	0.027	
237.5-242.5	0.029	
242.5-247.5	0.032	
247.5-252.5	0.032	
252.5-257.5	0.050	
257.5-260	0.0325	Total, 140-260 mμ = 0.287
260-262.5	0.0325	
262.5-267.5	0.10	
267.5-272.5	0.125	
272.5-277.5	0.11	
277.5-[illegible]	0.060	Total, 140-[illegible] mμ = 0.714
280-2[illegible]	0.060	
282.5-287.5	0.1[illegible]	
287.5-292.5	0.26	
292.5-297.5	0.315	
297.5-300	0.1525	Total, 140-300 mμ = 1.672

Solar Radiation Outside Earth's Atmosphere
(Calculated from data in ref. 5, p. [illegible])

Wavelength range (mμ)	Power in wavelength range (milliwatts/cm²)	
140-145	0.00001	
145-155	0.00007	
155-165	0.00018	
165-175	0.00041	
175-185	0.00091	
185-195	0.0017	
195-202.5	0.00225	
202.5-207.5	0.0025	
207.5-212.5	0.0050	
212.5-217.5	0.0090	
217.5-222.5	0.015	
222.5-227.5	0.021	
227.5-232.5	0.026	
232.5-237.5	0.027	
237.5-242.5	0.029	
242.5-247.5	0.032	
247.5-252.5	0.032	
252.5-257.5	0.050	
257.5-260	0.0325	Total, 140-260 mμ = 0.287
260-262.5	0.0325	
262.5-267.5	0.10	
267.5-272.5	0.125	
272.5-277.5	0.11	
277.5-280	0.060	Total, 140-[illegible] mμ = 0.714
280-282.5	0.060	
282.5-287.5	0.17	
287.5-292.5	0.26	
292.5-297.5	0.315	
297.5-300	0.1525	Total, 140-300 mμ = 1.672

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ECOLOGY OF CHLORINATED SOLVENTS - DETECTION AND DETERMINATION IN WATER

L K

Chemical Name (300 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75-Ø9-2

1A

D-605

878211816

THE DOW CHEMICAL COMPANY

February 9, 1971

TO: D. A. Rausch, 2020 Building

FROM: M. J. Mintz and N. B. Tefertiller

SUBJECT: Ecology of Chlorinated Solvents--Detection and Determination in Water

During the past month we have made a preliminary evaluation of three techniques for the identification and quantification of chlorinated solvents in water. In addition we evaluated some "real" samples to test the techniques with materials obtained in the field. Two sets of samples were taken from the Midland Waste Water Plant, bracketing the chlorinator to test for the production of chlorinated hydrocarbons in that unit, and one set from the Tittabawassee River on either side of the Dow plant. Since a report on this work is currently being written the results will only be briefly discussed.

I. Hollow Fiber-Mass Spec. This technique, developed by Westover (Chem. Physics Res.), utilizes a bundle of 10-15 silicone rubber fibers connected to a mass spec as a probe for water analysis. The limits of detection for this technique appear to be ~100 ppb. Analyses plagued by loss of solvents from prepared 100 and 1000 ppb water standards:

$CHCl_3$	No Loss
perc	Rapid Loss
trichlor	Rapid Loss
EDC	Moderate Loss

Analysis of field samples:

Midland Waste Water Plant (250# Cl_2/4,900,000 gals)

Before Chlorinator	No Chlorinated Solvents
After Chlorinator	No Chlorinated Solvents

Tittabawassee River

Mapleton	CH_2Cl_2 (~100-200 ppb) EDC (tentative)
Above Dow	No Chlorinated Solvents

II. Carbon Adsorption-DTA/Mass Spec. This technique was developed by Westover and Hart and applied to our samples by George Kallos. The carbon

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samples were prepared in our laboratory by passing 17 liters of water through 3 gms (0.5cc) of activated charcoal during 2.5-3.5 hours. The limits of detection for this technique (analysing the wet carbon sample) are on the order of 500-1000 ppb.

Analysis of field samples:

Midland Waste Water Plant

Before Chlorinator	Toluene Benzene No Chlorinated Solvents
After Chlorinator	Acetone Benzene No Chlorinated Solvents

Tittabawassee River

Mapleton	(water)	Methylene Chloride EDC Chlorinated Aromatics
	(particulate)	Methylene Chloride Dichloroethylene Toluene Chlorinated Highers
Above Dow		Benzene

III. Ether Extraction-Electron Capture GC. This technique developed by Jank Hall of the Analytical Labs was applied to our samples by Mr. Hall. The limits of detection for this technique are about 10 ppb, by far the lowest of the three techniques evaluated. As was the case with the hollow fiber-mass spec technique these analyses were also plagued by loss of solvents from prepared 100 and 1000 ppb water standards:

$CHCl_3$	No Loss
perc	Severe Loss
trichlor	Severe Loss
EDC	Moderate Loss

Analysis of field samples:

Midland Waste Water Plant

Before and After Chlorinator	(<10 ppb)	
	EDC Trichlor perc	one or both

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Tittabawassee River

Mapleton	$CHCl_3$	13 ppb
	C_2Cl_4	14 ppb
	1,1,1-Trichlor	<10 ppb
	Didn't look for CH_2Cl_2	
Above Dow	No Chlorinated Solvents	

While this latter technique is certainly the most sensitive, the ease of operation and essentially instant readout of the hollow fiber-mass spec method make it the method of choice, if its limits of sensitivity are acceptable. The carbon adsorption technique is slow, cumbersome and relatively inaccurate and should probably be excluded as a method of analysis for grab samples.

Considering the results bracketing the chlorinator in the Midland Waste Water Plant it does not seem likely that production of chlorinated solvents takes place under these conditions (by reaction of chlorine with dissolved organics). However, this does not rule out the possible production of chlorinated solvents in the chlorination of industrial effluents where the dissolved and suspended organics are quite different from those found in municiple waste streams.

M. J. Mintz
Britton Research
498 Building

Nancy Tefertiller

N. B. Tefertiller
Britton Research

cc

Research Manager

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AN EVALUATION OF THE

ABILITY OF MALE SPRAGUE-DAWLEY

RATS TO TOLERATE SEVERAL DAILY

6-HR EXPOSURE TO METHYLENE CHLORIDE

VAPOR FOLLOWING PARTIAL HEPATECTOMY

Chemical Name (200 per name)	25	CAS No. (10)	24
METHYLENE CHLORIDE		75-09-2	

1A

D-592

8782/1815

SUMMARY REPORT

Methylene Chloride: An Evaluation of the Ability of Male Sprague-Dawley Rats to Tolerate Several Daily 6-Hr Exposures to Methylene Chloride Vapor Following Partial Hepatectomy.

The purpose of the present study was to determine whether partially hepatectomized male Sprague-Dawley rats could tolerate several daily 6 hour inhalation exposures to methylene chloride. Five rats per group were partially hepatectomized as described by Higgins and Anderson (1931). An additional five rats per group were sham hepatectomized. The day following surgery, the rats were exposed to 0, 1500 or 3500 ppm of methylene chloride vapor, 6 hours/day, for 3 days. On the fourth day the rats were necropsied and sections of liver taken for microscopic examination.

At sacrifice, no statistically significant differences were observed in the body weights or in the absolute and relative liver weights of the treated versus control animals within the partially hepatectomized or sham operated groups. Similarly, the partially hepatectomized and sham operated rats exposed to 1500 or 3500 ppm of methylene chloride did not show any recognizable treatment-related hepatic histopathologic effects different from that which was seen in the respective control groups.

Based on these observations, it would be anticipated that longer-term studies with either sham or partially hepatectomized rats, exposed to these levels of methylene chloride, would be compatible with a normal life expectancy in view of the absence of significant toxicity in this short-term probe.

Higgins, G. M. and Anderson, R. M. (1931). Experimental pathology of the liver. I. Restoration of the liver of the white rat following partial surgical removal. Arch. Pathol., 12, 186-202.

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Doc Title: ACUTE TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARD OF METHYLENE CHLORIDE - URETHANE GRADE

LK 5 3 83

Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-Ø9-2

1A

D-609

8182117

BIOMEDICAL RESEARCH
Division Toxicology

DOW CHEMICAL U.S.A

SUBMITTED BY	CHARGE	DATE	PAGE
L. C. Renfro		January 27, 1975	NUMBER

ACUTE TOXICOLOGICAL PROPERTIES AND INDUSTRIAL HANDLING HAZARDS OF METHYLENE CHLORIDE - URETHANE GRADE

REPORTED BY T. G. Pullin, R. J. Schnobel CHECKED BY R. V. Johnston, D.V.M.

INFORMATIVE SUMMARY WITH CONCLUSIONS BASED ON THE SAMPLE RECEIVED. ADDITIONAL INFORMATION INCLUDING THE EFFECTS OF REPEATED EXPOSURE MAY BE REQUIRED AS SPECIFIC USES AND FORMULATIONS ARE DEVELOPED OR IF PROCESS CHANGES OCCUR.

A sample of methylene chloride-urethane grade was submitted to the Comparative Toxicology Research Laboratory for evaluation of acute oral lethality, eye and skin irritation properties, acute inhalation toxicity, and for definition of industrial handling hazards involving acute exposures.

A member of the Industrial Health Team (Toxicologist, Industrial Hygienist, and/or Industrial Physician) should be consulted for recommendation of additional safety evaluations or handling procedures which may be needed to support manufacture and/or use of this test material.

The acute oral lethality of the test material is low. The acute oral LD_{50} determined in twelve female albino rats is greater than 2.0 g/kg body weight. There is little likelihood that internal injury would result from acute ingestion of amounts of the material one might encounter incidental to industrial handling.

Eye contact with the test material resulted in moderate pain, moderate conjunctival inflammation to the male albino rabbit. If contact occurs, contaminated eyes should be flushed with copious amounts of flowing tap water for at least 15 minutes. Safety glasses are recommended whenever the likelihood of eye contact exists.

Prolonged contact (24 hours) with the skin of the male albino rabbit open to air resulted in a slight chemical burn. Prolonged and/or repeated skin contact should be avoided. If contact occurs, the skin should be decontaminated with soap and water and contaminated clothing thoroughly cleaned before re-use.

The test material may present a definite problem from a single acute exposure to the vapor or fumes at room temperature. The concentrated vapors of the material are sufficient to cause marked anesthesia. Exposure to the vapors or fumes of the test material should be avoided.

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MAR 24 1975

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BIOMEDICAL RESEARCH
TEXAS DIVISION TOXICOLOGY

DOW CHEMICAL U.S.A.

FREEPORT, TEXAS

DATA SHEET OF PROPERTIES, HEALTH HAZARDS, AND PRECAUTIONS FOR SAFE HANDLING OF MATERIALS

MOLECULAR FORMULA: CH_2Cl_2

NAME: Methylene Chloride, Urethane Grade

MOLECULAR WEIGHT: 84.94

INDUSTRIAL HYGIENE STANDARD: 200ppm *

EVALUATION: Wk. 7 Vol. 5

CITY:

STRUCTURAL FORMULA OR COMPOSITION: Methylene Chloride CH_2Cl_2

BOILING POINT	EXPLOSIVE LIMITS	FLASH POINT	IGNITION TEMP	MELTING POINT	VAPOR PRESS.
40 oc 760 mmHg	None	None oc	oF	-92 oc	410 mmHg 25°

CORROSIVENESS (To Common Metals)	PHYSICAL STATE	COLOR
None	Liquid	Water clear - APHA 0-5

CHEMICAL REACTIVITY	ODOR (Indicate Concentration in Air)
Not very reactive.	Ethereal - sweet

STABILITY (To air, Change, Heat, Light)

Stable.

TYPE OF CONTACT	CLASSIFICATION OF TOXIC PROPERTIES	
EYES	☒ May cause considerable discomfort, chemical conjunctivitis, corneal edema, and/or irritation of the eyelids	☐ May cause some permanent loss of vision (this includes damage to cornea or internal injury which is immediately noted in one week)
	☐ May cause sufficient injury to the eye to result in loss of time from work (this includes damage to the cornea which heals or nearly heals in a week and/or conjunctivitis, conjunctival hemorrhage with edema)	☐ Vapor exposure may cause severe pain, lacrimation or serious injury to the eyes
SKIN	☐ Single prolonged exposure (hours) causes no effect. Several repeated prolonged exposures may or may not cause the development of some slight irritation.	☐ Single short exposure (minutes) may cause considerable irritation and/or single prolonged or frequently repeated short exposures cause a burn and/or may cause systemic injury, even death.
	☒ Single prolonged exposure may cause considerable discomfort, considerable irritation (possibly a mild burn) and/or systemic injury (not fatal)	☐ An extensive rapidly caused severe burns and/or serious systemic injury, even death.
DUST OR MIST ☐	☐ No systemic injury expected. No irritation to nose and throat in dusty or misty atmospheres	☐ Dusty or misty atmospheres painful to nose and throat (irritation) and in most humid and/or exposure may cause serious systemic injury even death.
	☐ Throat and nose irritation in a dusty or misty atmosphere is harmful but not intolerable and/or prolonged or repeated exposures may cause systemic injury.	☐ Short exposure (minutes) may cause death or serious systemic injury.
VAPOR Tested at room temperature unless otherwise indicated.	☐ Exposure may not cause any effects other than some very slight irritation of eyes or throat. Only persons of the most	☒ Exposure may cause extreme discomfort, considerable irritation and/or may cause intolerable irritation to the respiratory passages.
	☐ Single exposures (breathing) of hour or frequently repeated exposures of shorter duration, may cause slight anesthesia and/or a light systemic injury, and/or cause appreciable, but not dangerous, irritation of respiratory passages.	☐ Short exposures may cause unconsciousness, and/or serious systemic injury, including death.
		☐ Even very short exposure will cause serious systemic injury or death.
INGESTION	☒ Amounts which may be swallowed incidental to industrial handling will not cause injury. However, substantial quantities should be considered more or less serious effects may occur.	☐ Amounts which may be swallowed incidental to industrial handling and use may cause serious injury.

COMMENTS: *This applies to the pure material.

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DEGREES OF EXPOSURE RELATED TO TYPES OF OPERATION

		PRECAUTIONS ARE CODED AS FOLLOWS: EYES	SKIN	DUST OR MIST	VAPOR	INGESTION
I	NO CONTACT	Characterized by remote control operation with equipment isolated from the work area. The persons entering isolated area will require the personal protection outlined for IV below.	A	A		A	A
II	MINOR CONTACT	Characterized by closed systems with equipment vented outside the work area, instrument control, mechanical handling of materials in bulk. Examples are continuous reactors, drum and filters, enclosed conveyors, ventilated packaging.	A	A		A	A
III	OCCASIONAL DAILY CONTACT	Characterized by manual handling of materials in containers such as bags, drums and barrels. Contamination may be present on outside parts. Many batch operations fall into this category.	B	B		D	A
IV	UNLESS CONTACT LIKELY	Characterized by hand sampling, entry for the purpose of emergency repair, equipment cleaning, maintenance, taking a drum of waste, packaging contents of dusty materials without ventilation, charging and tray drying.	B	B		D	A

EYE CONTACT

A No eye protection needed.

B Use safety glasses without side shields.

C Use safety glasses with side shields.

D Use chemical workers goggles.

E Use gas tight goggles or a full face gas mask.

SKIN CONTACT

A Plain coveralls and gloves worn during the usual working of most times should be adequate for outdoor operations. Gloves contaminated clothing and shoes must be removed and kept from the skin and must be thoroughly cleaned before reuse.

B Same as A, but with the addition of rubber aprons, rubber sleeves, and rubber gloves when the operation requires.

C Clothing and shoes must be changed and taken home daily upon any detectable contact. Each use will require special consideration to determine suitable protective devices and standards of personal cleanliness.

D Impervious clothing such as rubber boots, rubber aprons, and rubber gloves will be required. Specific items will be dictated as required by circumstance.

E Any exposure to obviously dusty atmospheres will require a dust respirator as per the approval of the U.S. Bureau of Mines for use with toxic dusts.

RESPIRATORY PROTECTION

DUST OR MIST

A No respiratory protection.

B No protection required for exposure of thirty min. Duration or less in obviously dusty atmospheres. Exposure of longer duration will require the use of a dust respirator bearing the approval of the U.S. Bureau of Mines for the use with toxic dusts.

C Any exposure to dusty atmospheres will require the use of an air line respirator, blower mask, or chemical mask.

VAPOR

A No precautions necessary.

B No precautions necessary for single exposures of less than 1 hour. Longer single exposures or frequently repeated exposures will require a gas mask or fresh air hood approved with appropriate canister.

C No precautions necessary for single exposures of no more than ten minutes. Longer exposures or frequent single or repeated, will require gas mask or respirator equipped with appropriate canister.

D Gas mask with appropriate canister required at all times.

E Evacuate area at once and enter only with airline respirator, blower mask, or chemical mask.

INGESTION

A No unusual precautions required.

B Food and tobacco should not be present in the work area. Hands and face should be washed before smoking and eating.

COMMENTS

GOOD PRACTICE REQUIRES THAT GROSS AMOUNTS OF ANY CHEMICAL BE REMOVED FROM THE SKIN AS SOON AS IS PRACTICAL.

SUITABLE GAS MASK CANISTER

* Organic vapor.

1-000X-1

Signed: L. C. Fullin, E. G. Horvath — Checked: R. V. Johnston, R. L. Daniel

Date: February 19, 1975 — Date: February 19, 1975

OFFICE OF TOXIC SUBSTANCES

REV. 7/27/82

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Contractor 21

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• RI • UP • HEHSD 82 SU HS FN

Doc Title 23

ELEVATED CARBON MONOXIDE LEVELS SECONDARY TO ACUTE OCCUPATIONAL EXPOSURE TO DICHLOROMETHANE PREPARED BY NORTHWESTERN UNIV MEDICAL SCHOOL CHICAGO

Chemical Name (300 per name)	25	CAS No. (10)	26
DICHLOROMETHANE		75-09-2	

1A

12-31-81

D-629

87-3211818

ELEVATED CARBON MONOXIDE LEVELS SECONDARY TO ACUTE OCCUPATIONAL EXPOSURE TO DICHLOROMETHANE

Honorio T. Benzon, M.D.,* Louis Claybon, M.D., M.S.,**
and Edward A. Brunner, M.D., Ph.D.***

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** First year post- graduate trainee, Department of Anesthesia, University of Pennsylvania Hospitals

*** Professor and Chairman, Department of Anesthesia, Northwestern University Medical School

RECEIVED

This work was done at Northwestern University Medical School, 303 East Chicago Avenue, Chicago, Illinois 60611

ELEVATED CARBON MONOXIDE LEVELS SECONDARY TO ACUTE OCCUPATIONAL EXPOSURE TO DICHLOROMETHANE

Methylene chloride, CH_2Cl_2 is a volatile solvent used in industry primarily as a degreasing and metal cleaning agent. It is rapidly metabolized to carbon monoxide. Reports of acute exposure to methylene chloride in industrial operations have appeared rarely in the literature. However, the hazards of its use as a paint remover by hobbyists in poorly ventilated areas has recently been reported. [1] With increasing use both in industry and by hobbyists, a greater incidence of methylene chloride toxicity can be expected.

Report of Cases

Case 1. A 40-year-old Mexican male who worked at a degreasing factory was overcome by methylene chloride fumes from an industrial leak. He lost consciousness for an undetermined amount of time, but was awake when examined in an emergency room 1½ hours after exposure. He was oriented, complained of shortness of breath, and had a hacking cough. Airway protective reflexes were intact. Lung examination revealed diffuse rhonchi and mild inspiratory wheezes. Chest X-ray showed increased vascular markings with diffuse, fluffy radiodensities. After drawing an arterial blood gas sample, he was given 500 mg hydrocortisone sodium succinate, intravenously, and 50% oxygen by mask with a high flow system. In the Intensive Care Unit (ICU), an indwelling arterial line was placed in the radial artery. Frequent blood gas analysis was done using 2 IL 513 analyzers (Table 1).

Oxyhemoglobin, reduced hemoglobin, and carboxyhemoglobin were measured on an IL 182 Co-oximeter, and inspired oxygen tension was regulated to keep the calculated oxygen content above 16 volumes per cent. Lactic acid on admission was 33 mg % (normal less than 20 mg %) and P50 was 22 mm Hg (normal 27.5 mm Hg). EKG showed sinus tachycardia. Hydrocortisone sodium succinate, 100 mg, intravenously, was given every six hours for 24 hours. The inspired oxygen concentration was gradually decreased from 50 to 30%. COHb levels slowly decreased from 19% to 4% over 24 hours. The patient was discharged on the third hospital day with a COHb level of 2% (within normal range in Chicago).

Case 2. A 50-year-old Mexican male, working at the same degreasing factory was exposed to methylene chloride fumes concurrently with the patient described above. The patient felt dizzy and nauseated, but did not lose consciousness. He was awake, oriented, and complained of mild shortness of breath on arrival at an emergency room. Physical examination revealed minimal rales, but no rhonchi in the lungs and an early mid-diastolic heart murmur at the lower left sternal border. Chest X-ray showed increased vascular markings in the upper lobes without other changes suggestive of pulmonary edema. LDH, SGOT, and CPK values were elevated. EKG on admission revealed a left anterior fascicular block and sinus bradycardia. Treatment consisted of oxygen therapy and hydrocortisone sodium succinate, 500 mg intravenously.

He was taken to the ICU and frequent arterial blood gas analysis was accomplished, the result of which appears in Table II.

The EKG the next day showed complete right bundle branch block, left anterior fascicular block, and non-specific ST-T wave changes. The possibility of a myocardial infarction was raised, but serial EKG's showed no further changes. Serum CPK isoenzyme analyses on two occasions showed that the enzyme came entirely from skeletal muscle. Chest X-ray (four views of the heart) and fluoroscopy on the fourth hospital day were normal. The patient's past history was suggestive of ischemic heart disease.

Treatment consisted of oxygen therapy and methylprednisolone sodium succinate, 4 mg intravenously, every six hours for 24 hours. The patient responded well and was transferred to the ward on the third day with a COHb level of 3%. He was discharged on the 17th hospital day.

DISCUSSION

Methylene chloride is a halogenated hydrocarbon used in industry as a degreaser, a solvent, an aerosol propellant, and in the preparation of heat sensitive oils and waxes. It has been used as a general anesthetic in obstetrics. [2] Its low toxicity compared to carbon tetrachloride, trichloroethylene, or tetrachloroethylene makes it highly desirable for industrial degreasing operations. It has little hepatic or renal toxicity and exerts no clinically evident central nervous system depression at ambient air concentrations of up to 500 ppm.

Approximately 250,000 tons are produced each year [3] and 70,000 workers are exposed annually. One-sixth of the amount produced is used in retail paint thinners and removers. Toxicity appears to be due more to metabolism to carbon monoxide [4] than to narcotic properties of the chemical. The elevation of carbon monoxide for some hours after the acute exposure is due to the saturation of the tissues with methylene chloride and consequent metabolism to CO. [5-6]

Carbon monoxide is an ubiquitous man-made pollutant in the lower atmosphere. It is an odorless, colorless, and tasteless gas and results from incomplete combustion of carbonaceous materials used in fuels for transportation and heating and is generated in industrial processing and burning. [7] It is also produced endogenously in man as a by-product during catabolism of hemoglobin and other heme compounds. [8] Carbon monoxide combines with hemoglobin to form carboxyhemoglobin (COHb), its affinity for hemoglobin being 240 times greater than oxygen. [9] The dissociation curve of HbO_2 in the presence of COHb is shifted to the left with flattening of the slope. [10-11] The oxygen carrying capacity of the blood is reduced by both of these factors. This results in tissue hypoxia, the signs and symptoms of which correlate closely with the COHb content of the blood. [9] Of special interest with regard to Case 2 are the EKG changes - inversion of the T wave, elevation of the ST segment, atrioventricular dissociation, and heart block - all of which have been described. Previously, these EKG changes were thought to appear at levels of 40% COHb in acute cases,

or at 21% COHb, or an equivalent O_2 deficiency in chronic exposures.[12] Heart block has been noted at levels exceeding 75% COHb for one hour or longer. Recent reports have documented adverse effects in patients with heart disease with COHb levels as low as 5%.[13-15]

Factors that modify the severity of CO poisoning are the concentration of the gas in the inspired air, the duration of exposure, the cardiac output, the oxygen demand of the tissues, and the hemoglobin concentration of the blood. Treatment is directed towards providing adequate tissue oxygenation. Patients should be removed from the site of exposure and given oxygen. The half-life of COHb under ambient conditions is 320 minutes; with 100% oxygen, half-life is reduced to 80 minutes.[16]

In these two cases, both men were exposed to unknown ambient air concentrations of methylene chloride. Patient 1 who was overcome initially by a large dose of methylene chloride suffered loss of consciousness with amnesia. His COHb on admission was only 20%, a level not adequate to cause loss of consciousness in most circumstances. His loss of consciousness is probably primarily due to the direct narcotic effect of methylene chloride plus any additive effects of carbon monoxide. Central nervous system damage in cases of chronic methylene chloride exposures is to be suspected. Neurological, behavioral, and toxicological follow-up is indicated.

We believe carbon monoxide poisoning posed the greatest threat to our patients. The oxyhemoglobin dissociation curve is shifted to the left. The P_{50} value was 22 mmHg in patient 1. Tissue hypoxia and

consequent anaerobic metabolism is to be anticipated. The lactic acid value of 33 mg % illustrates this. Furthermore, CO is formed continuously from the methylene chloride retained in the tissues. Studies in rats have shown that at twenty-four hours after exposure to methylene chloride, 2% of the original dose is still retained in the body.[4] The elevated COHb levels persisted for as long as 26 hours, as shown in Figure 1. The elimination of CO did not follow the pattern of pure CO poisoning. In methylene chloride poisoning, the rate of CO production can be equal to the rate of CO elimination even after the exposure has been terminated. The CO half life is seven hours with a load time of 14½ hours in patient 1 and 10-3/4 hours with the same load time in patient 2. Both patients were given 35-50% O_2 rather than 100% O_2, which is the recommended treatment for CO poisoning, but the blood oxygen content was closely monitored and regulated. It may be more advisable to use 100% inspired oxygen initially. This may be tapered as the COHb levels fall to acceptable levels.

TABLE 1

HRS. Post-Exposure	% Inspired O_2	pH	PCO_2	PO_2	HbO_2	COHb
2 Hrs.	21%	7.28	40	59	75%	19%
4 Hrs.	21%	7.29	45	71	82%	18%
5½ Hrs.	50%	7.38	33	165	94%	16%
7 Hrs.	40%	7.33	32	103	82%	18%
8½ Hrs.	35%	7.36	30	138	79%	19%
9½ Hrs.	35%	7.32	29	146	80%	20%
12½ Hrs.	35%	7.34	30	137	82%	18%
14½ Hrs.	35%	7.35	31	151	81%	20%
17 Hrs.	35%	7.35	33	155	84%	13%
20½ Hrs.	35%	7.35	32	145	88%	11%
24½ Hrs.	35%	7.40	31	143	93%	7%
28 Hrs.	35%	7.42	31	141	95%	4%
52 Hrs.	21%	7.47	35	82	96%	2%

TABLE 2

HRS. Post-Exposure	% Inspired O_2	pH	PCO_2	PO_2	HbO_2	COHb
2 Hrs.	21%	7.39	34	72	87%	11%
6 Hrs.	50%	7.38	36	166	89%	10%
7½ Hrs.	40%	7.39	31	160	91%	9%
9½ Hrs.	40%	7.39	29	152	91%	9%
11½ Hrs.	40%	7.40	30	105	90%	9%
13½ Hrs.	40%	7.39	30	135	90%	10%
16 Hrs.	40%	7.40	32	151	90%	9%
19½ Hrs.	40%	7.38	34	100	97%	8%
23½ Hrs.	40%	7.42	32	110	93%	7%
28½ Hrs.	40%	7.42	30	101	95%	4%
34½ Hrs.	40%	7.41	35	130	97%	3%
45½ Hrs.	28%	7.42	31	90	97%	2%
53½ Hrs.	21%	7.41	30	70	94%	3%
94½ Hrs.	21%	7.51	28	83	94%	4%
77½ Hrs.	21%	7.50	33	60	96%	2%

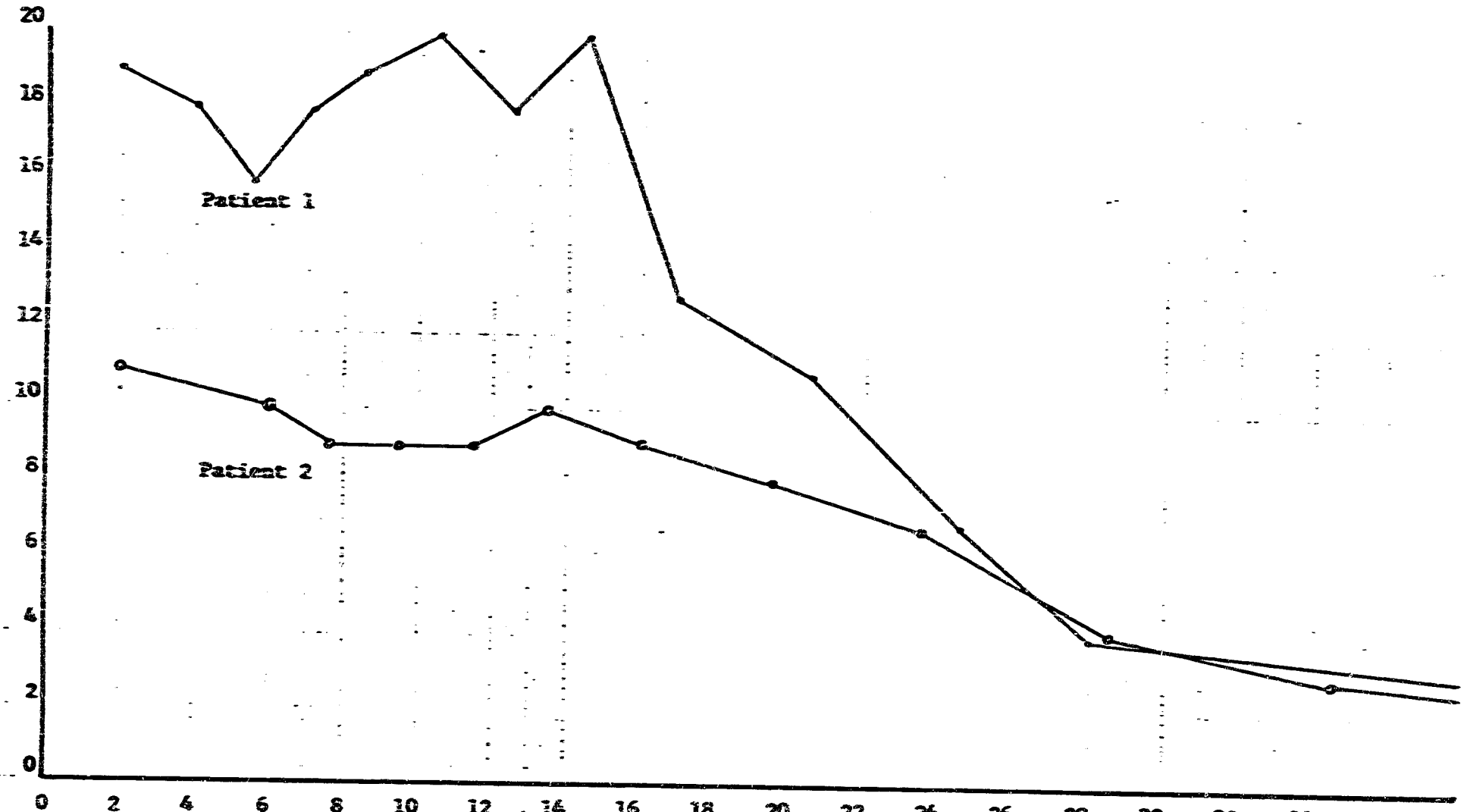

Figure 1 Carboxyhemoglobin levels after exposure to dichloromethane

8782/1818

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HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: METABOLISM DATA AND OXYGEN HALF SATURATION PRESSURES

L.K 6 [illegible]

Chemical Name (300 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75-Ø9-2

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D633

80-821819

July 9, 1982

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: METABOLISM DATA AND OXYGEN HALF SATURATION PRESSURES

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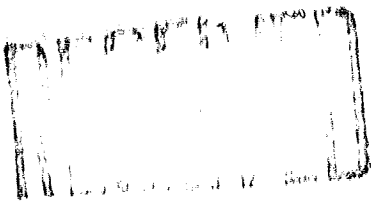

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*Former employee of Dow Chemical, U.S.A.

HEALTH EVALUATION OF EMLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: METABOLISM DATA AND OXYGEN HALF SATURATION PRESSURES

KEYWORDS

Carboxyhemoglobin

Hemoglobin Oxygen Affinity

Methylene Chloride

Occupational Exposure

Oxygen Transport

000003

A B S T R A C T

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: METABOLISM DATA AND OXYGEN HALF SATURATION PRESSURES

(Ott MG, Skory LR, Holder BB, Bronson JM, and Williams PR)

In vivo metabolism of methylene chloride to form carboxyhemoglobin has been demonstrated in a number of experimental studies. The present investigation was undertaken to quantitate the relation-ships between methylene chloride exposure, percent carboxyhemoglobin saturation, alveolar air carbon monoxide and oxygen half-saturation pressures in an occupational setting.

As expected, methylene chloride exposure produced dose-related increases in carboxyhemoglobin and alveolar air carbon monoxide, and a corresponding decrease in oxygen half-saturation pressures. The calculated decrease in oxygen half-saturation pressure was between 2 and 4 mm Hg among persons exposed to 300 + ppm methylene chloride for an 8-hour work day. Additional analyses indicated that this shift in the oxyhemoglobin dissociation curve could be explained on the basis of the known carbon monoxide interactions with hemoglobin.

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: METABOLISM DATA AND OXYGEN HALF SATURATION PRESSURES

The discovery that methylene chloride ($MeCl_2$) is biotransformed in the body to form carboxyhemoglobin (COHb) has raised questions as to what effect $MeCl_2$ exposure may have on tissue oxygenation.[1,16] COHb produced because of the high affinity of carbon monoxide (CO) for hemoglobin, affects oxygen transport in two ways; first, by the partial replacement of oxyhemoglobin with COHb and, secondly, by increasing oxygen affinity for hemoglobin through CO, oxygen, and hemoglobin interactions.[3]

$MeCl_2$ is also capable of directly interacting with hemoglobin as evidenced by its anti-sickling properties.[9,13] It was first suggested that $MeCl_2$ might increase the affinity of CO for hemoglobin, thereby, accounting in part for the increased COHb seen with $MeCl_2$ exposure.[14] However, subsequent in vitro studies using whole blood have shown that low partial pressures of $MeCl_2$ do not influence CO binding to hemoglobin.[4,5] The immediate objective of the present study was to quantitate in an occupational setting, the interrelationships among $MeCl_2$ exposure, oxygen half-saturation pressures, blood COHb, and alveolar CO. The broader objective was to provide an additional perspective to the mortality and blood constituent findings described in earlier reports.[10-12]

MATERIALS AND METHODS

Two hundred sixty eight employees from two fiber production plants voluntarily participated in this investigation. In the study plant, employees were exposed to $MeCl_2$, acetone, and methanol, the methanol being present in a ratio to $MeCl_2$ of about 1 to 10. Employees of the comparison plant were exposed to similar acetone concentrations but not to $MeCl_2$ or methanol. Based on results of an intent-to-participate questionnaire, individuals were entered into the study so as to achieve approximately equal numbers of participants from the two plants among both men and women. The distribution of the 268 employees by sex, race, smoking habits and plant is given in Table 1. There were more nonwhite men and women in the study group reflecting the larger numbers of nonwhites at that plant location. The age range of participants was 19 to 62 in the study plant, and 21 to 63 in the comparison plant; the average age being 40.4 and 39.4 years, respectively. There were geographic differences between the two plants, the study plant being located in flat terrain (elevation of 169 meters above sea level) and the comparison plant being located in a mountainous area (elevation of plant was 483 meters with surrounding elevations of 1280 meters above sea level). The altitude differences correspond to approximately 5 to 14% lower partial oxygen pressures at the comparison plant site.

The design of the study permitted estimation of possible drift in the measurements over time since about one-fourth of the

comparison plant participants were evaluated first, then all study plant participants and finally the remaining comparison plant participants. The following variables were determined twice for each participant, immediately preceding and following the workshift: blood COHb, alveolar air CO, and the partial oxygen pressure required to keep 50% of the blood oxygen-carrying capacity saturated with oxygen at PH 7.4 and 37°C (P_{50}). Personal monitoring of each employee for $MeCl_2$ exposure was carried out during the workshift to coincide with the time period between the two blood samples. The environmental monitoring procedures have been described elsewhere.(10)

Alveolar air CO was determined by collecting end expiratory air in a Saran* bag and analyzing the sample with an Ecolyzer Series 2200 CO Analyzer. COHb was determined by Diagnostic Laboratories, Charlotte, North Carolina using a Co-Oximeter IL 182 three wavelength differential spectrophotometer.

Analytical Procedure For Determination of P_{50}

The measurement of P_{50} requires determination or knowledge of the partial oxygen pressure and the corresponding oxygen saturation percent and pH. The measurement of pH was with a PHM73 pH Meter supplied by the London Company, Cleveland, Ohio. Oxygen saturation was measured with the OSM2 Hemoximeter (manufactured by Radiometer, Copenhagen). A general procedure

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for using the OSM2 Hemoximeter to determine P_{50} was presented by Siggaard-Andersen.(15) While it is possible to measure actual venous pH, partial oxygen pressure and oxygen saturation, an equilibration procedure was selected since (1) the partial oxygen pressure calculated from the oxygen concentration of the equilibrating gas is not subject to the variance attending direct measurements of partial oxygen pressure, and (2) after equilibration, all samples have approximately the same partial oxygen and carbon dioxide pressures so that oxygen saturation readings are within a narrower range. Three tanks of equilibration gas with a gas mixture of 3.7% oxygen, 5.6% carbon dioxide and balance nitrogen were supplied by Matthewson Gas, Chicago, Illinois. Oxygen content of each tank was analytically measured to three significant digits. The partial oxygen pressure of the equilibrating gas was corrected on a twice daily basis for changes in barometric pressure.

About 5 ml of blood was drawn from an antecubital vein, half of that amount being used for determination of total hemoglobin and COHb, the remainder being used for P_{50} determination. Measurement of P_{50} was performed in duplicate using two tonometers (Model IL 237, Instrumentation Laboratories, Lexington, Massachusetts). After a 9 minute equilibration period, blood samples were extracted and directly injected into both the Hemoximeter and the pH electrode, recording, in order, the oxygen saturation and pH twice. The same procedure was then repeated

with a blood sample from the second tonometer. A major concern was the stability of COHb during tonometry for nine minutes with a CO-free gas. However, experiments showed that at the low oxygen concentration in the equilibration gas, the loss of CO was only about 1 to 2% of the amount present in the sample. Thus, the impact of COHb stability on the oxygen half-saturation pressures was considered to be minimal under the conditions of this study.

Computations of P_{50} were based on the equation given by Riggard-Anderson using the single point principle.(15) The extrapolation to 50 percent oxygenation was based on Hill's plot with an assumed slope of 2.7. Extrapolation to a constant pH of 7.4 assumed a Bohr coefficient of -0.4. Oxygen saturation was expressed as a percentage of total available hemoglobin rather than total hemoglobin, a convention followed by Collier.(3) The expression for calculating oxygen saturation percent was: 100 x (Hemoximeter reading % - COHb %)/(100 - COHb %). Although the slope in the Hill equation varies slightly depending on the presence of COHb(3), this variability had little effect on extrapolation to half-saturation pressures as oxygen saturation readings were all between 39% and 70%.

Collier obtained a measure of the shift in the oxyhemoglobin dissociation curve (ODC) in the presence of COHb by first determining half-saturation pressure with or without COHb present and then using an empiric correction to remove COHb effects.(3)

Estimation of the corresponding CO-free P_{50} may be performed using an empiric procedure analogous to that used by Collier or by solving the equations given by Ledwith.(8) Both approaches were used in the present investigation.

RESULTS

The distribution of individual $MeCl_2$ exposure measurements as obtained by personal monitoring on the day of P_{50} determination is given in Figure 1. Monitoring data were available for 135 of 136 employees who participated in this aspect of the health surveillance program. The bimodal distribution of $MeCl_2$ exposure was due to differences in $MeCl_2$ use levels in two separate blocks within the study plant.(10)

The relationships between $MeCl_2$ exposure and percent COHb saturation before and after-shift are depicted in Figure 2. Separate equations were fitted for smokers and nonsmokers; however, the smoking contribution to COHb was nearly constant across $MeCl_2$ exposure concentrations both before and after-shift. The linear association between $MeCl_2$ exposure and before-shift COHb reflects residual CO metabolism associated with previous-day exposure to $MeCl_2$. The significant quadratic fit for post-shift COHb indicates partial saturation of the enzyme system for metabolizing $MeCl_2$. Very similar results were obtained when alveolar CO was related to $MeCl_2$ exposure (see Figure 3). This finding

would be expected in view of the relationship between alveolar CO and COHb. The correlation between the change in COHb during the work shift and the corresponding change in alveolar CO was 0.79.

Several quality assurance steps were taken to evaluate the possibility of drift in P_{50} determinations during the course of the study. As previously mentioned, measurements of P_{50} in the comparison plant were made both before and after completion of the measurements among study plant participants. There was only a 0.2 mm Hg difference in the group mean P_{50} for nonsmoking comparison plant participants in the November 1977 period compared to the January-February 1978 period, adjusting for differences in sex distribution of the participants. In addition, there were no significant associations of P_{50} with change of equilibrating gas tanks or with pressure changes in the tanks.

Means and standard deviations of $MeCl_2$ exposure, standard P_{50} and CO-free P_{50} determinations are given in Table 2. P_{50} group means were lower in the study plant than the comparison plant, and within the study plant, there was a decrease in P_{50} during the shift as one might anticipate in view of the relationship between P_{50} and COHb. Smokers also had lower oxygen half-saturation pressures than nonsmokers, and men had lower oxygen half-saturation pressures than women.

Multiple linear regression analysis was employed to help sort out the interrelationships among half-saturation pressures

and other covariates. Both standard P_{50} and P_{50} corrected for the presence of COHb were taken to be dependent variables. Covariates considered in the analysis as either independent factors or stratification variables included sex, race, age, smoking history, before versus after-shift, COHb, hemoglobin, location of plant and $MeCl_2$ and acetone exposure concentrations. The regression results summarized in Table 3 indicate sex, age, location, hemoglobin, race and COHb are each independently associated with P_{50}.

The empirical equation developed by Collier to account for the COHb induced shift in P_{50} was based on a slope of -0.3 relating change in COHb to P_{50}.(3) Our findings of a before-shift slope of -.30 and post workshift slope of -.23 for relating change in COHb to P_{50} agree well with the slope estimates derived by Collier.

Group analysis of oxygen half-saturation pressures were also undertaken separately for men and women subcategorized by cigarette smoking status. Recognizing that the P_{50} differences between the comparison and study population may be related to geographic location as well as $MeCl_2$ exposure, emphasis was placed on comparison among the three $MeCl_2$ exposure categories within the study plant. From Table 4 a left shift in the ODC is apparent both in the morning (evidently due to residual percent COHb saturation) and after the work day. Among individuals

exposed to 300 + ppm $MeCl_2$ the P_{50} decreased by 1 to 2 mm Hg during the work day. Thus, the calculated decreases in P_{50} comparing post-shift averages for persons exposed to 300 + ppm $MeCl_2$ to pre-shift averages for persons exposed to less than 100 ppm $MeCl_2$ were between 2 and 4 mm Hg.

DISCUSSION

The relationship between percent COHb saturation, alveolar CO, and $MeCl_2$ exposure as determined in this observational study agree reasonably well with experimental findings reported by DiVincenzo and Kaplan.[6] For example, these investigators found that COHb increased from 1% at 0 ppm $MeCl_2$ to 3.4% at 100 ppm $MeCl_2$ and 6.8% at 200 ppm $MeCl_2$ for 14 nonsmokers (single day exposure). With exposures repeated over five consecutive days in a single volunteer, baseline COHb saturations rose to about 2% at 200 ppm $MeCl_2$. These authors also reported a decline in percent COHb saturation within 2 hours of last exposure (the decrease was about 2% COHb at 200 ppm $MeCl_2$). In the present study, COHb was measured after the employees had finished work and reported to the Medical Department. Since a number of employees were evaluated each day, the interval between last exposure and venipuncture could have been as much as one hour. In addition, exposures in the work setting were variable rather than constant. Thus, COHb saturations of 0.1% at 0 ppm $MeCl_2$, 2.8% at 100 ppm $MeCl_2$,

and 5.1% at 200 ppm $MeCl_2$ are in line with those arrived at experimentally. The low levels of methanol present in this study were insufficient to affect any changes on COHb formation rates based on experimental findings by Ciuchta *et al.*(2)

Evaluation of P_{50} and CO-free P_{50} revealed an expected dose-dependent effect of $MeCl_2$ on P_{50} mediated through the presence of COHb. The magnitude of the effect in employees exposed to over 300 ppm $MeCl_2$ was on the order of 2 to 4 mm Hg. Because of the rapid elimination of $MeCl_2$ from blood,(6) the present investigation could not reliably assess the direct effects of $MeCl_2$ on hemoglobin function. However, it has been shown experimentally that low partial pressures of $MeCl_2$ do not influence CO binding to hemoglobin.(4,5) Also, regression analyses with CO-free P_{50} as the dependent variable did not reveal a significant independent effect for $MeCl_2$. Thus, the presumed effects of $MeCl_2$ on tissue oxygenation would be those projected on the basis of equivalent COHb saturations derived from CO exposure.

Apparent compensatory changes in oxygen transport were observed for one subgroup of employees from the study plant. Hematologic findings for this employee subgroup, women who smoked cigarettes and were assigned to work areas of high $MeCl_2$ use, included increased red cell counts, hemoglobin and hematocrits.(12) These observations are supported by the higher carboxyhemoglobin and lower P_{50} findings for these women in the present study.

Because COHb elevations may reduce oxygen availability to the heart muscle, it has been postulated that the risk of ischemic events could be increased by exposure to $MeCl_2$. Death rates due to ischemic heart disease in the study plant were not significantly elevated relative to the corresponding U.S. population (14 observed versus 13.2 expected deaths for active and inactive employees and 8 observed versus 7.4 expected deaths among active employees only).[11] Long-term follow-up of another industrial population exposed to 30 to 125 ppm $MeCl_2$ also did not reveal an excess risk for ischemic heart disease.[7] Thus, other aspects of the present health surveillance program indicate that physiological adaptions may have occurred but that evidence of adverse cardiovascular outcomes is lacking in the present and in one other occupational cohort exposed to $MeCl_2$.

ACKNOWLEDGEMENTS

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TABLE 1

DISTRIBUTION OF STUDY PARTICIPANTS BY
SEX, RACE, SMOKING HABITS AND PLANT LOCATION

Sex, Race and Smoking Habits	Study Plant (136 Participants)	Comparison Plant (132 Participants)
WOMEN - TOTAL	81	70
WHITE		
Nonsmokers	29	41
Smokers	39	26
NONWHITE		
Nonsmokers	6	1
Smokers	7	2
MEN - TOTAL	55	62
WHITE		
Nonsmokers	23	27
Smokers	19	33
NONWHITE		
Nonsmokers	8	1
Smokers	5	1

TABLE 2

MEAN AND STANDARD DEVIATION OF STANDARD P_{50} and CO-FREE P_{50} BEFORE AND AFTER WORK SHIFT BY SMOKING STATUS, SEX AND PLANT

Population Subgroup	$MeCl_2$ ppm TWA	Standard P_{50} (mm Hg) Before	Standard P_{50} (mm Hg) After	CO-Free P_{50} (mm Hg) Before	CO-Free P_{50} (mm Hg) After
COMPARISON PLANT					
WOMEN					
Nonsmokers (42)	------	26.9±0.8	27.1±0.7	27.1±0.8	27.2±0.7
Smokers (28)	------	24.8±1.2	24.9±1.0	25.9±1.1	25.9±1.0
MEN					
Nonsmokers (26)	------	26.1±0.8	26.4±0.8	26.2±0.7	26.5±0.8
Smokers (34)	------	24.3±0.9	24.6±1.0	25.1±0.8	25.4±0.8
STUDY PLANT					
WOMEN					
Nonsmokers (35)	261±170	25.8±1.3	24.8±1.3	26.1±1.2	26.0±1.2
Smokers (46)	225±151	24.7±1.2	23.6±1.4	26.0±1.0	25.8±1.3
MEN					
Nonsmokers (31)	252±201	25.1±1.2	23.9±1.5	25.5±1.0	25.1±1.3
Smokers (24)	220±167	24.3±1.1	23.3±1.4	25.4±1.2	25.5±1.3

T A B L E 3

MULTIPLE REGRESSION ANALYSIS OF P_{50} AND CO-FREE P_{50}

Covariate	Dependent Variable: P_{50} (mm Hg) Slope	P_{50} (mm Hg) p-Value	CO-Free P_{50} (mm Hg) Slope	CO-Free P_{50} (mm Hg) p-Value
BEFORE SHIFT				
SEX (Female = 2, Male = 1)	.41	.011(a)	.41	.015
AGE (Years)	.021	.0002	.021	.0003
LOCATION (Comparison Plant = 2) (Study Plant = 1)	.21	.177	.35	.041
HEMOGLOBIN (g/100 ml)	-.25	.0001	-.29	.0001
RACE (Nonwhite = 2, White = 1)	.53	.006	.58	.005
COHb (% Saturation)	-.30	.0001	-.04	.071
ACETONE (ppm TWA)	.00004	.772	.00005	.761
$MeCl_2$ (ppm TWA)	-.0007	.180	-.0007	.176
AFTER SHIFT				
SEX (Female = 2, Male = 1)	.49	.021	.49	.010
AGE (Years)	.024	.0001	.023	.0006
LOCATION (Comparison Plant = 2) (Study Plant = 1)	.52	.005	.56	.003
HEMOGLOBIN (g/100 ml)	-.23	.0001	-.24	.0001
RACE (Nonwhite = 2, White = 1)	.22	.302	.26	.259
COHb AFTER-SHIFT (% Saturation)	-.23	.0001	-.05	.064
ACETONE (ppm TWA)	-.0001	.426	-.0002	.226
$MeCl_2$ (ppm TWA)	-.0012	.035	-.0011	.066

(a) Significance levels determined based on each covariate being the last entered into the equation.

TABLE 4

MEAN AND STANDARD DEVIATION OF BEFORE AND AFTER-SHIFT P_{50} AND CO-FREE P_{50} BY SEX, SMOKING HISTORY, AND $MeCl_2$ EXPOSURE INTENSITY

Population Subgroup	Number of Participants	Average $MeCl_2$ Exposure (ppm TWA)	P_{50} Before (mm Hg)	P_{50} After (mm Hg)	CO-Free P_{50} Before (mm Hg)	CO-Free P_{50} After (mm Hg)
NONSMOKING MEN						
Comparison	28	—	26.1±0.8	26.4±0.8	26.2±0.7	26.5±0.8
<100 ppm $MeCl_2$	7	44	25.8±1.1	25.6±1.1	25.8±1.2	25.8±1.5
100–299 ppm $MeCl_2$	14	179	25.3±1.2	23.9±1.3	25.5±1.1	25.2±0.9
300+ ppm $MeCl_2$	10	501	24.4±0.9	22.7±1.0	25.2±0.8	24.6±1.6
MEN WHO SMOKE						
Comparison	34	—	24.3±0.9	24.6±1.0	25.1±0.8	25.4±0.8
<100 ppm $MeCl_2$	9	47	24.7±1.0	24.4±1.0	25.4±1.2	25.9±1.1
100–299 ppm $MeCl_2$	5	172	23.9±1.6	23.1±1.4	25.5±1.5	25.1±0.9
300+ ppm $MeCl_2$	10	400	24.3±1.0	22.5±1.0	25.3±1.0	25.3±1.5
NONSMOKING WOMEN						
Comparison	42	—	26.9±0.8	27.1±0.7	27.1±0.8	27.2±0.7
<100 ppm $MeCl_2$	2	25	27.0±1.0	26.2±0.5	27.0±1.0	26.6±0.1
100–299 ppm $MeCl_2$	20	154	26.0±0.8	25.2±0.9	26.2±0.9	26.0±1.0
300+ ppm $MeCl_2$	13	461	25.3±1.7	24.1±1.4	25.9±1.6	26.0±1.4
WOMEN WHO SMOKE						
Comparison	28	—	24.8±1.2	24.9±1.0	25.9±1.1	25.9±1.0
<100 ppm $MeCl_2$	4	47	26.1±0.8	25.5±0.9	26.9±0.5	26.6±0.7
100–299 ppm $MeCl_2$	29	152	24.9±1.1	23.9±1.3	26.0±0.9	25.9±1.4
300+ ppm $MeCl_2$	13	444	23.8±1.0	22.3±0.8	25.5±1.0	25.3±0.9

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Figure 1.
DISTRIBUTION OF EMPLOYEE EXPOSURES TO $MeCl_2$ ON DAY OF P_{50} DETERMINATION

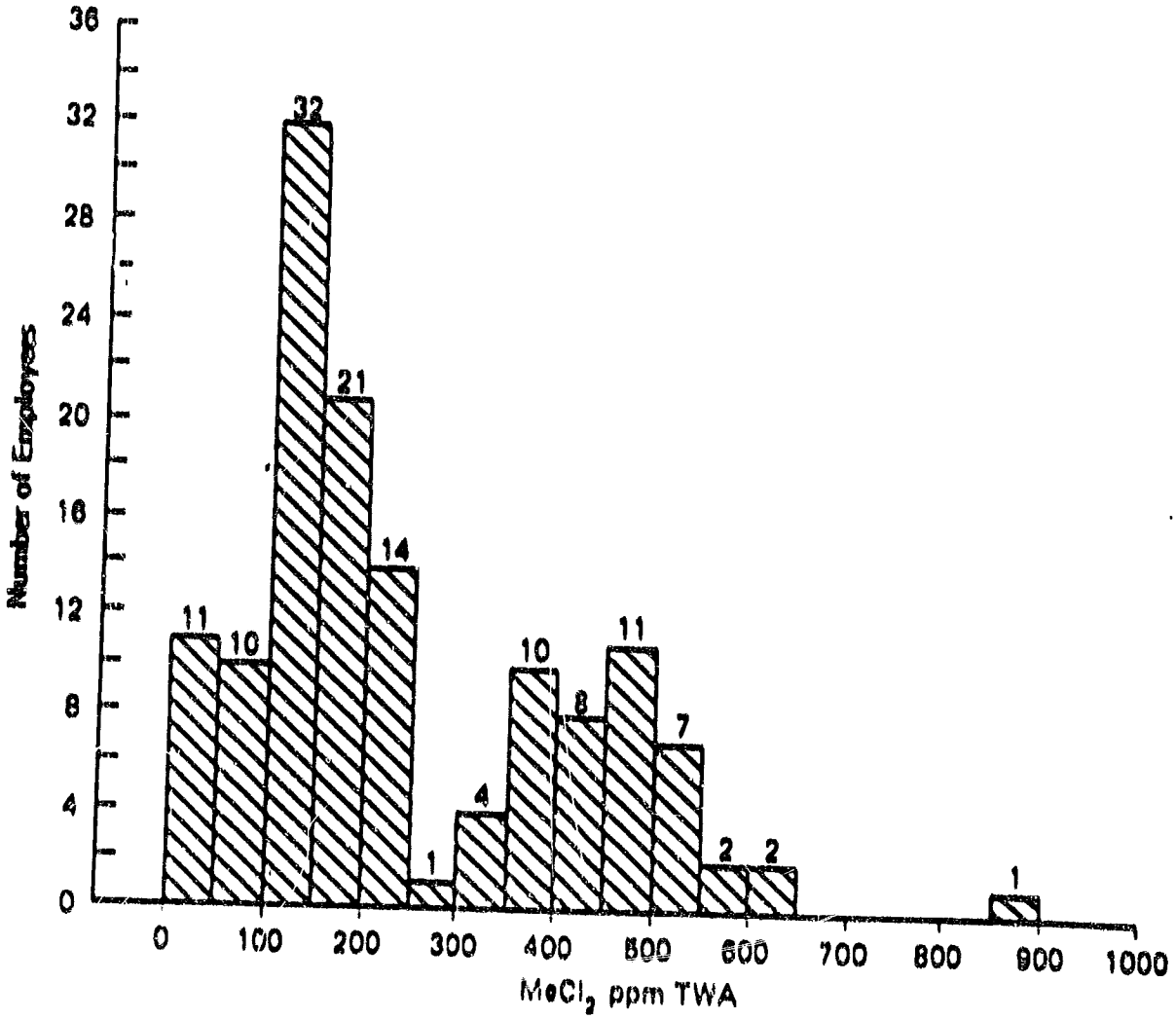

Figure 2.

RELATIONSHIP OF BEFORE-SHIFT AND POST-SHIFT COHb TO $MeCl_2$ EXPOSURE IN SMOKERS AND NONSMOKERS

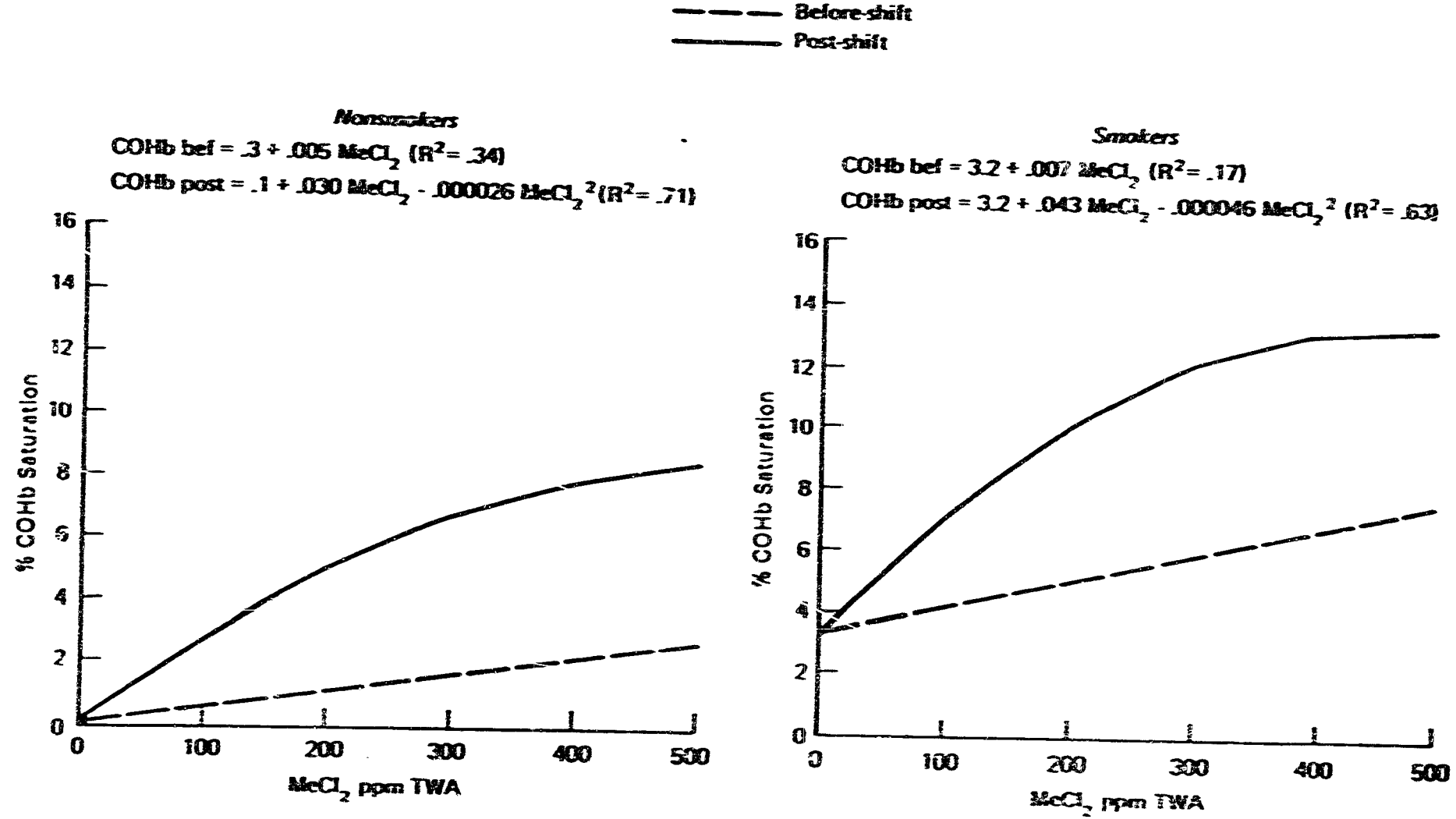

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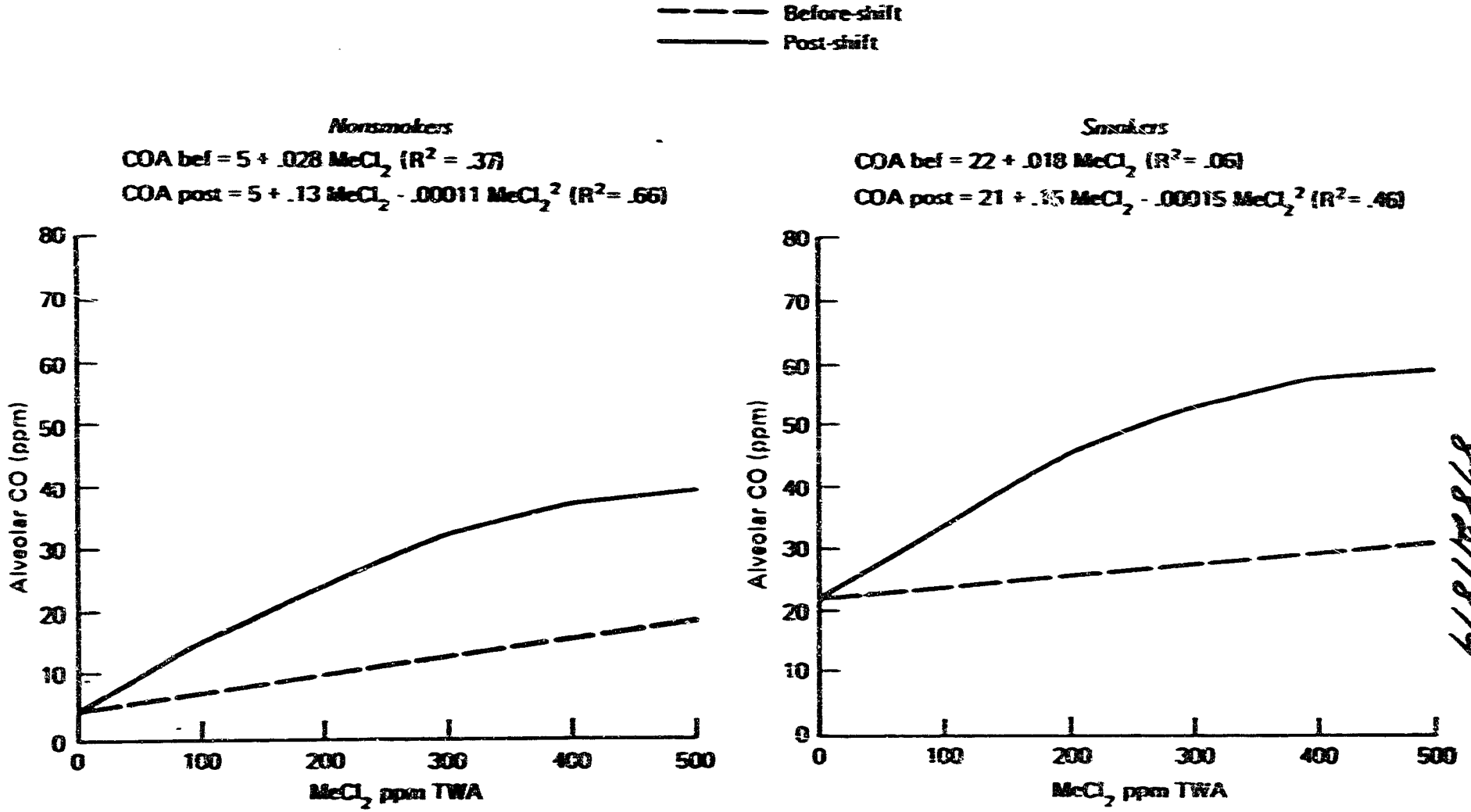
Figure 3.

RELATIONSHIP OF BEFORE-SHIFT AND POST-SHIFT ALVEOLAR CO TO $MeCl_2$ EXPOSURE IN SMOKERS AND NONSMOKERS



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Chemical Name (300 per name)	25	CAS No. (10)	24
METHYLENE CHLORIDE		75-09-2	

LK 6 pgs

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D-634

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July 9, 1982

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: CLINICAL LABORATORY EVALUATION

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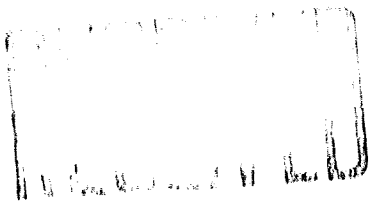

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CLINICAL LABORATORY EVALUATION

KEYWORDS

Carboxyhemoglobin

Hematopoietic Effects

Methylene Chloride

Occupational Exposure

Smoking

A B S T R A C T

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: CLINICAL LABORATORY EVALUATION

Ott MG, Skory LR, Holder BB, Bronson JM, and Williams PR

Hematopoietic effects seen in heavy cigarette smokers have been attributed to carbon monoxide present in the smoke. Since methylene chloride is partially metabolized in vivo to form carboxyhemoglobin, we investigated the pattern of hematologic changes relative to both methylene chloride exposure and cigarette smoking status in employees of two fiber production plants. Six serum constituents, changes in which could indicate liver injury, were also examined in relation to methylene chloride exposure.

Increased red cell counts, hemoglobin, and hematocrit observed among women but not among men exposed to about 475 ppm of methylene chloride were suggestive of a compensatory hematopoietic effect. Similar changes have been observed in cigarette smokers, but not necessarily restricted to women. Another frequently reported change in the hematology of smokers, increased mean corpuscular volume, was not seen with methylene chloride exposure. These findings suggest that some but not all hematologic changes observed in cigarette smokers may be explained on the basis of carbon monoxide alone. A dose-related increase in serum bilirubin was observed for both men and women exposed to methylene chloride. This appears to be an isolated finding as no corresponding pattern of dose-related changes in other serum and blood constituents, consistent with either liver injury or hemolysis, was observed.

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: CLINICAL LABORATORY EVALUATION

This report is the third in a series of five papers on the health of employees of two fiber production plants. Employees of the study plant were exposed to methylene chloride ($MeCl_2$), acetone, and methanol, the methanol being present in a ratio to $MeCl_2$ of approximately 1 to 10. Employees of the comparison plant were exposed to similar concentrations of acetone but were not exposed to $MeCl_2$ or methanol.

The health evaluation portion of the surveillance program was primarily undertaken to provide the employes with a general health examination. However, it was recognized that laboratory data obtained as a routine component of the evaluation could be useful in examining hypotheses concerning adaptive or toxic responses related to increased carboxyhemoglobin in $MeCl_2$ exposed employees. Certain hematologic and blood volume changes seen in heavy cigarette smokers, (increased hematocrit, increased red-cell volume as measured by chromium^{51} labelling, and decreased plasma volume), have been attributed to the carbon monoxide associated with cigarette smoke.(11,13) Several investigators have reported other hematologic changes in cigarette smokers relative to nonsmokers among clinic patients undergoing routine examinations or in working populations.(1,2,7) These changes include increased hemoglobin, mean corpuscular hemoglobin and mean

corpuscular volume. Incrimination of carbon monoxide as the principle cause of hematologic changes seen in smokers has been disputed, partly on the basis of the multiple chemical ingredients found in cigarette smoke.(6,8)

Experimental studies involving chronic exposure of dogs and monkeys to high concentrations of carbon monoxide (COHb levels of 32 to 40%), produced marked erythrocytosis, increased red blood cell mass, and increased hematocrit and hemoglobin.(14) However, these COHb saturations are considerably higher than those associated with cigarette smoking.

Because of the metabolism of $MeCl_2$ to form carboxyhemoglobin, non-smoking employees in the present study would have experienced elevations of carboxyhemoglobin in the absence of exposure to agents present in cigarette smoke or often attending environmental carbon monoxide exposures (e.g. motor vehicle exhaust fumes). Thus, carboxyhemoglobin-related effects on the hematopoietic system could be assessed without interference of many of the substances commonly associated with carbon monoxide. In addition, possible effects of $MeCl_2$ exposure could be assessed in both cigarette smokers and nonsmokers.

Methylene chloride is a chlorinated hydrocarbon and is metabolized by the liver. Therefore, serum constituents in the clinical chemistry battery that could indicate liver dysfunction were also examined in relation to exposure.

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METHODS

The target population and logistical details for the health evaluation are described in a methodological paper covering all aspects of the health surveillance project.(9) The study plant was located in Rock Hill, South Carolina and the comparison plant in Narrows, Virginia. Analyses of blood constituents for both plants were performed by Diagnostic Laboratories in Charlotte, North Carolina. In the study plant, blood samples were drawn with the subject in a sitting position, whereas, in the comparison plant, the subject was recumbent. The effect of posture on concentrations of blood constituents has been experimentally examined by several investigators.(4,12) The effect of being in a recumbent position is to lower most serum and blood constituents (3% for cellular constituents and up to 6.5% for serum constitutents after at least 30 minutes in that position). This effect thus represents a relatively minor source of bias in comparisons between plants.(12) Elevation differences between the two plants was another factor that could have an effect on the hematologic parameters. The study plant was located in a flat geographical area at an altitude of 169 meters above sea level. The comparison plant was located in a mountainous region (altitude of plant site was 483 meters with surrounding elevations of 1280 meters). The altitude differentials between the two sites translate into differences of from 5 to 14% in partial oxygen pressures.

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All blood samples were refrigerated after collection and were delivered to the laboratory daily. Samples obtained from comparison plant employees were flown to Charlotte. Cellular constituents were analyzed with a Coulter Model S, carboxyhemoglobin with a Co-Oximeter IL 182 and serum constituents with a Technicon SMAC biochemical analyzer except for serum alanine aminotransferase which was assayed using a Gilford System 3500. The health evaluations were conducted from October 25th through February 13th with 71% taking place between November 7th and December 3rd. Nearly all of the examinations in the study plant (264 of 266) were conducted during the morning and noon hours, whereas, about 23% (58 of 251) of the evaluations in the comparison plant were conducted later in the day to accommodate production schedules. Participation was on a volunteer basis and was estimated to be about 61% in the study plant and 55% in the comparison plant.

The blood constituents examined were red cell count (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), carboxyhemoglobin (COHb), aspartate aminotransferase (SGO-T), alanine aminotransferase (SGP-T), lactate dehydrogenase (LDH), alkaline phosphatase, total bilirubin and albumin.

The results of industrial hygiene monitoring of the work environment are detailed in a prior report.(9) Median time-weighted average (TWA) concentrations of $MeCl_2$ for an 8-hour day

were approximately 60 parts-per-million (ppm) for 11 employees who worked in jet assembly; 140 ppm TWA for 162 employees who worked in Block I extrusion jobs or on one job in the fiber preparation area; 475 ppm TWA for 65 employees assigned to Block II, cellulose triacetate production, or related activities in the preparation area; and 280 ppm TWA for 28 employees whose work station was intermediate to the two blocks or whose work assignments involved time in both Block I and Block II. Acetone exposures were lowest in Block II and in jet assembly (approximately 110 ppm). In Block I and many areas of the comparison plant median acetone exposures were about 1000 ppm TWA.

Statistical analyses were based on a multiple regression approach (the software package was SAS 79.5 supplied by SAS Institute Inc., Raleigh, NC). Covariates used in the analyses were sex, race, age, cigarette smoking history, time of venipuncture, and current intensity of $MoCl_2$ exposure. Additional analyses indicated that date of examination and intensity of acetone exposure within the comparison plant were not important covariates for any of the blood constituents. Separate analyses were performed for each of the four sex-by-race subgroups in the study population. Direct contrasts of laboratory findings between the study and comparison plant participants were given less emphasis because of previously outlined differences in the collection and handling of blood specimens.

RESULTS

The distribution of health evaluation participants with respect to sex, race, age and cigarette smoking history is given for both the study and comparison plants in Table 1. The considerable disparity in the composition of the total employee populations of each plant, particularly in regard to sex and race, is reflected in the differences seen in the table. The distribution of cigarette smoking varied with sex and race; however, there were no apparent differences between the study and comparison plant participants within sex-by-race subgroups. Among study plant volunteers, only 9 of 266 (3.4%) had been employed less than one year and 169 (63.5%) had been employed for more than five years at the time of examination. In the comparison plant, the percentages were 13.9 and 55.1% respectively.

In Table 2, the mean and standard deviation of each blood constituent is reported by plant, sex, and race. Fisher's method of combining probabilities from tests of significance[5] was used to compare means of the blood constituents for the two plants (white men and women only were compared because there were few nonwhites in the comparison plant) after stratification by current smoking status, sex, and age (<45 vs. 45+). Statistically significant differences between the two plant populations were observed for carboxyhemoglobin ($p < .0001$), serum alanine aminotransferase ($p < .0001$), total bilirubin ($P < .01$), and MCHC ($P < .05$).

Separate multiple regression analyses for the four sex-by-race subgroups of the study population were performed for each of the blood constituents. Independent covariates included smoking category, age, and time of venipuncture in addition to intensity of $MeCl_2$ exposure. A summary of the statistical findings in regard to intensity of $MeCl_2$ exposure is presented in Table 3. The positive association of carboxyhemoglobin with $MeCl_2$ exposure in all four subgroups was anticipated. Further analysis indicated that $MeCl_2$, cigarette smoking history, and time of venipuncture were important predictors of carboxyhemoglobin within the study plant, but that sex, race and age of subject were unimportant in predicting carboxyhemoglobin. A more rigorous treatment of carboxyhemoglobin in relation to $MeCl_2$ exposure is available in a separate paper.(10) A consistent positive association between total bilirubin and $MeCl_2$ exposure was also found in three of the four population subgroups. In addition, the mean bilirubin in the study group was statistically higher than that of the comparison group. These relationships have been depicted in Figure 1 via frequency plots of total bilirubin by $MeCl_2$ exposure. The differences in total bilirubin are less apparent among women than men. Among men, there was a shift in mean total bilirubin and also a flattening of the distribution curve with increased intensity of current exposure.

The findings obtained for alanine aminotransferase were inconsistent, depending on whether or not results from the comparison plant were considered. The mean serum alanine aminotransferase was statistically higher in the study than the comparison group. However, no association with increased $MoCl_2$ exposure was observed when only the study plant was examined. These relationships are summarized in Figure II.

There was a statistical increase in red cell count with intensity of exposure among white women of the study plant, but not among white men. Parallel increases in hematocrit, hemoglobin, and aspartate aminotransferase were also found among white women but not among white men or nonwhites of either sex. The distributions of red cell count by $MoCl_2$ exposure are summarized in Figure III. For the women there is a higher mean red cell count and increased spread in the distribution curve with increased exposure. No similar trend was evident among the men. The relative increase in red cell count with exposure was greater among women cigarette smokers than nonsmokers, particularly in the under 45 age group. Based on a fitted regression equation for white women under age 43 who smoked (study plant only), mean red cell counts were 4.39 million cells/ml for 0 ppm $MoCl_2$, 4.53 million cells/ml for 140 ppm $MoCl_2$ and 4.88 million cells/ml for 475 ppm $MoCl_2$. The corresponding estimates for nonsmokers were 4.39 million cells/ml for 0 ppm $MoCl_2$, 4.44 million cells/ml for 140 ppm $MoCl_2$ and 4.55 million cells/ml for 475 ppm $MoCl_2$.

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DISCUSSION

Carboxyhemoglobin concentrations showed the expected rise with both cigarette smoking and increased $MeCl_2$ exposure. A number of the other associations found in this investigation were not anticipated.

Serum alanine aminotransferase was statistically increased for employees of the study plant relative to the comparison plant, but was not related to intensity of $MeCl_2$ exposure within the study plant population. The shift of between 3.4 and 7.3 IU (see Table 2), is probably due to differences in collection and handling of blood specimens between the two plants particularly since it was not supported by a dose-response relationship in the study plant. Total bilirubin was positively correlated with $MeCl_2$ exposure among both men and women. This finding, if observed in conjunction with other positive indicators, could point to either liver injury or hemolysis. However, neither concomitant elevations of alkaline phosphatase nor the transaminases were found on a group basis or among the six study plant individuals (four white and two nonwhite) with total bilirubin in excess of 1.4 mg%. In addition, no employees from the study plant reported a history of cholestasis. Furthermore, supporting evidence for increased red cell turnover was also lacking. No dose-response relationship was found for LDH and there was no decline in RBC, hemoglobin or hematocrit either in the highest exposed group or among persons with high serum bilirubin.

Unfortunately, additional tests which could have clarified the situation further (e.g., direct bilirubin, and reticulocyte counts) were not performed.

Among white women in the study plant, red cell count, hematocrit and hemoglobin were increased in the group exposed to the highest $MeCl_2$ concentrations; however, similar results were not obtained for white men in the study plant. The findings among the women, especially those who smoked cigarettes, are consistent with a compensatory response similar to that observed with high altitude hypoxia. The partial pressure of oxygen at which hemoglobin is 50% saturated (P_{50}) was measured in 132 comparison plant and 136 study plant employees as part of the present research.(10) Interestingly, there was a slight differential sex effect in P_{50} relative to $MeCl_2$ exposure and cigarette smoking. The difference occurred because nonexposed nonsmoking women had average P_{50} determinations about 0.8 mm Hg higher than nonexposed, nonsmoking men whereas, among cigarette smokers in the highest $MeCl_2$ exposure group, there was no difference in P_{50} between men and women. It has been suggested that men have higher baseline hemoglobin which is at least partly compensated for by a greater oxygen-releasing capacity of the red cells of women under standard conditions.(3) The apparent loss of this compensatory advantage as suggested by the P_{50} measurements may offer an explanation for the hematologic changes observed in the exposed women, but not men.

The hematologic patterns associated with $MeCl_2$ exposure among women were similar to those seen with cigarette smoking except that MCV and MCH findings were not elevated with $MeCl_2$ exposure. Based on parallel analyses in the study and comparison plant, MCV was significantly increased among cigarette smokers for white men and women in both plants. Hematocrit, hemoglobin and MCH, were also increased in each subgroup among cigarette smokers. Our observations in regard to cigarette smoking are consistent with those reported in other studies of cigarette smokers using the Coulter Model S. Thus, there are differences in the hematologic profiles, particularly in regard to MCV and MCH, between cigarette smokers and individuals exposed to $MeCl_2$.

In conclusion, there were the expected changes in carboxyhemoglobin. There were also changes in serum and blood constituents, suggestive of a hematopoietic effect as evidenced by increased red cell counts in white women exposed to $MeCl_2$ concentrations of approximately 475 ppm TWA. The bilirubin findings were statistically significant but were not supported by parallel changes in other serum and blood constituents.

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TABLE 1

AGE AND SMOKING HISTORY BY SEX, RACE AND STUDY GROUP

Age and Smoking History	White Men				White Women				Nonwhite Men*		Nonwhite Women*	
	Study		Comparison		Study		Comparison		Study		Study	
	Nbr.	%	Nbr.	%	Nbr.	%	Nbr.	%	Nbr.	%	Nbr.	%
AGE	63		168		107		77		20		76	
<35	12	19.0	53	31.5	23	21.5	40	51.9	11	55.0	61	80.3
35-44	11	17.5	31	18.5	39	36.4	21	27.3	5	25.0	14	18.4
45-54	29	46.0	23	13.7	41	38.3	12	15.6	4	20.0	1	1.3
55-64	11	17.5	61	36.3	4	3.7	4	5.2	0	0.0	0	0.0
SMOKING HISTORY												
Never Smoked	17	27.0	32	19.0	48	44.9	37	48.1	9	45.0	46	60.5
Former Smoker	21	33.3	56	33.3	6	5.6	7	9.1	4	20.0	2	2.6
Current Smoker												
<1 pk/day	2	3.2	5	3.0	10	9.3	6	7.8	1	5.0	15	19.7
1+ pk/day	23	36.5	75	44.6	43	40.2	27	35.1	6	30.0	13	17.1

*There were only 3 nonwhite men and 3 nonwhite women in the comparison population.

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TABLE 2

MEAN AND STANDARD DEVIATION OF BLOOD CONSTITUENTS AMONG HEALTH EVALUATION PARTICIPANTS BY SEX, RACE, AND STUDY GROUP

System and Component	STUDY GROUP				COMPARISON GROUP	
	White Men $\bar{X} \pm SD$	Nonwhite Men $\bar{X} \pm SD$	White Women $\bar{X} \pm SD$	Nonwhite Women $\bar{X} \pm SD$	White Men $\bar{X} \pm SD$	White Women $\bar{X} \pm SD$
NUMBER OF EMPLOYEES	63	20	107	76	168	77
AGE	43.3±10.7	34.4±8.7	41.3±9.1	29.6±5.6	43.8±13.9	34.9±11.3
SERUM						
Alkaline phosphatase (IU)[a]	69.7±19.4	66.1±21.9	60.6±18.2	53.1±17.0	70.6±19.5	61.8±17.9
Lactate dehydrogenase (IU)	191.4±31.2	189.5±29.2	188.1±33.6	189.3±44.9	195.0±39.0	194.6±38.0
Alanine aminotransferase (IU)	20.4±13.2	20.5±11.9	11.0± 6.0	11.7±11.6	13.1± 8.7	7.6± 7.2
Aspartate aminotransferase (IU)	22.2±12.6	19.9± 3.7	16.7± 6.3	16.4± 6.5	20.6± 6.6	16.7± 5.9
Total bilirubin (mgZ)	0.7± 0.4	0.5± 0.3	0.5± 0.3	0.4± 0.2	0.5± 0.2	0.5± 0.2
Albumin (gZ)	4.2± 0.2	4.2± 0.2	4.1± 0.2	4.0± 0.3	4.2± 0.2	4.1± 0.3
BLOOD						
RBC (10^6/ l)	5.1± 0.3	5.2± 0.7	4.6± 0.4	4.5± 0.3	5.1± 0.4	4.6± 0.3
Hemoglobin (g/100 ml)	15.8± 0.9	15.0± 1.0	14.0± 1.2	12.9± 1.1	16.0± 1.1	14.0± 1.0
Hematocrit (Z)	45.7± 2.7	44.7± 3.5	41.4± 3.3	38.7± 2.8	46.8± 3.2	41.6± 2.8
MCV (fl)	90.6± 4.3	86.3± 7.7	90.5± 5.8	86.6± 5.8	91.7± 4.9	91.1± 4.5
MCH (pg/cell)	31.0± 1.6	28.9± 3.4	30.6± 2.2	29.0± 2.3	31.2± 1.7	30.8± 1.6
MCHC (g/100 ml)	34.6± 1.0	33.8± 1.6	34.1± 0.9	33.5± 0.9	34.3± 0.9	34.0± 0.8
COHb (sat.Z)	4.9± 4.1	5.1± 4.8	5.2± 3.6	4.9± 3.8	1.5± 2.1	1.1± 1.6

[a] IU - International Units

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TABLE 3

SUMMARY OF ASSOCIATIONS BETWEEN INTENSITY OF $MeCl_2$ EXPOSURE AND 13 BLOOD CONSTITUENTS BASED ON MULTIPLE REGRESSION ANALYSES

Population Subgroup	Blood Constituent	Slope[A]	Significance Level
Non-white men (20)	Carboxyhemoglobin (%)	2.1	(p = .005)
Non-white women (76)	Carboxyhemoglobin (%)	1.0	(p = .003)
	Total bilirubin (mg %)	0.06	(p = .020)
White men (63)	Carboxyhemoglobin (%)	1.0	(p = .0003)
	Total bilirubin (mg %)	0.08	(p = .012)
White Women (107)	Carboxyhemoglobin (%)	0.7	(p = .0002)
	Total bilirubin (mg %)	0.05	(p = .0007)
	Red cell count (10^6/ml)	0.06	(p = .004)
	Hematocrit (%)	0.47	(p = .016)
	Hemoglobin (g/100 ml)	0.16	(p = .028)
	Aspartate Aminotransferase (IU)	0.78	(p = .046)

[A]Units of change in blood constituents per 100 ppm $MeCl_2$

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Figure 1

TOTAL BILIRUBIN BY EXPOSURE GROUP
AMONG WHITE MEN AND WOMEN

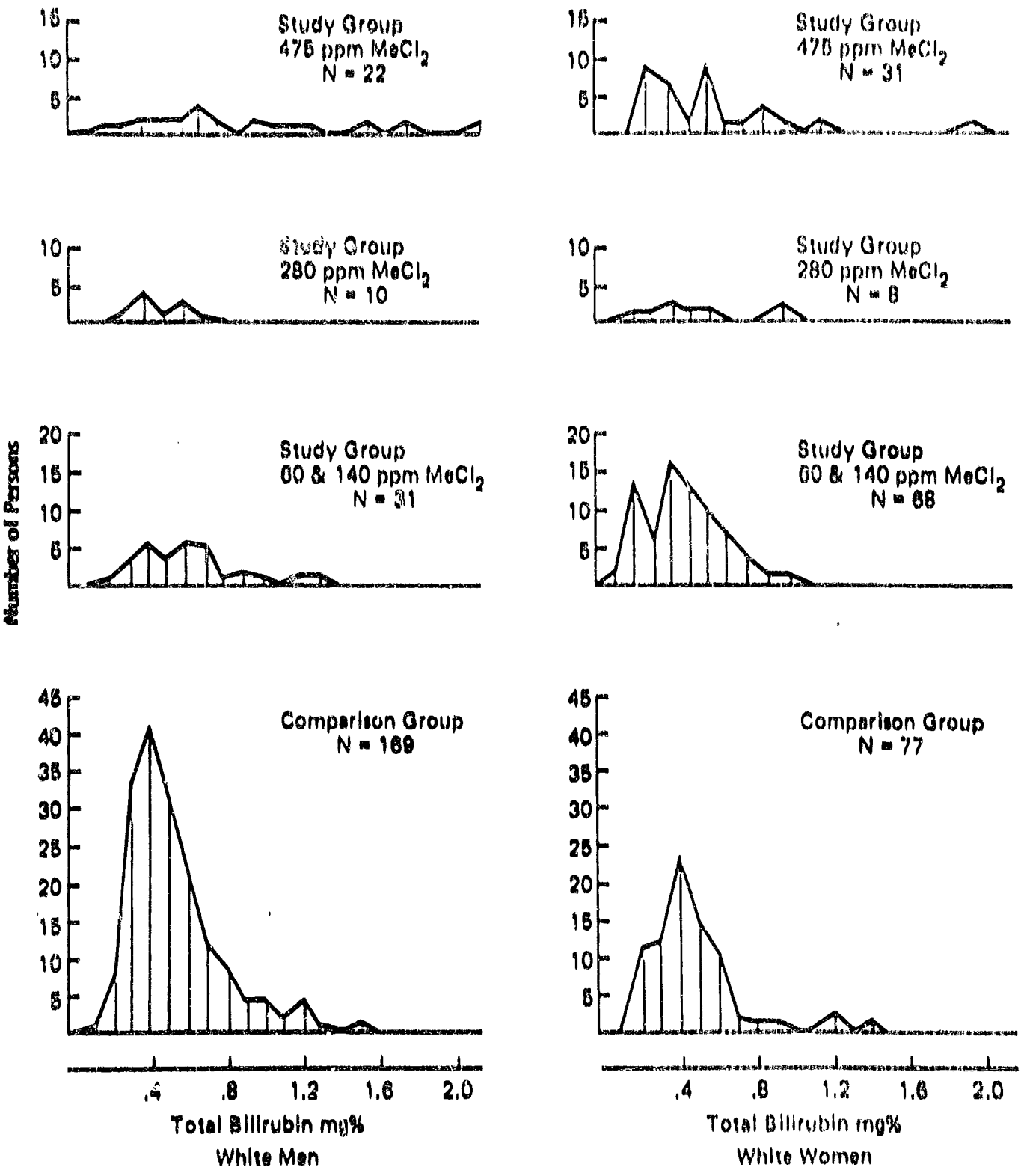

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Figure II

SERUM ALANINE AMINOTRANSFERASE BY EXPOSURE GROUP AMONG WHITE MEN AND WOMEN

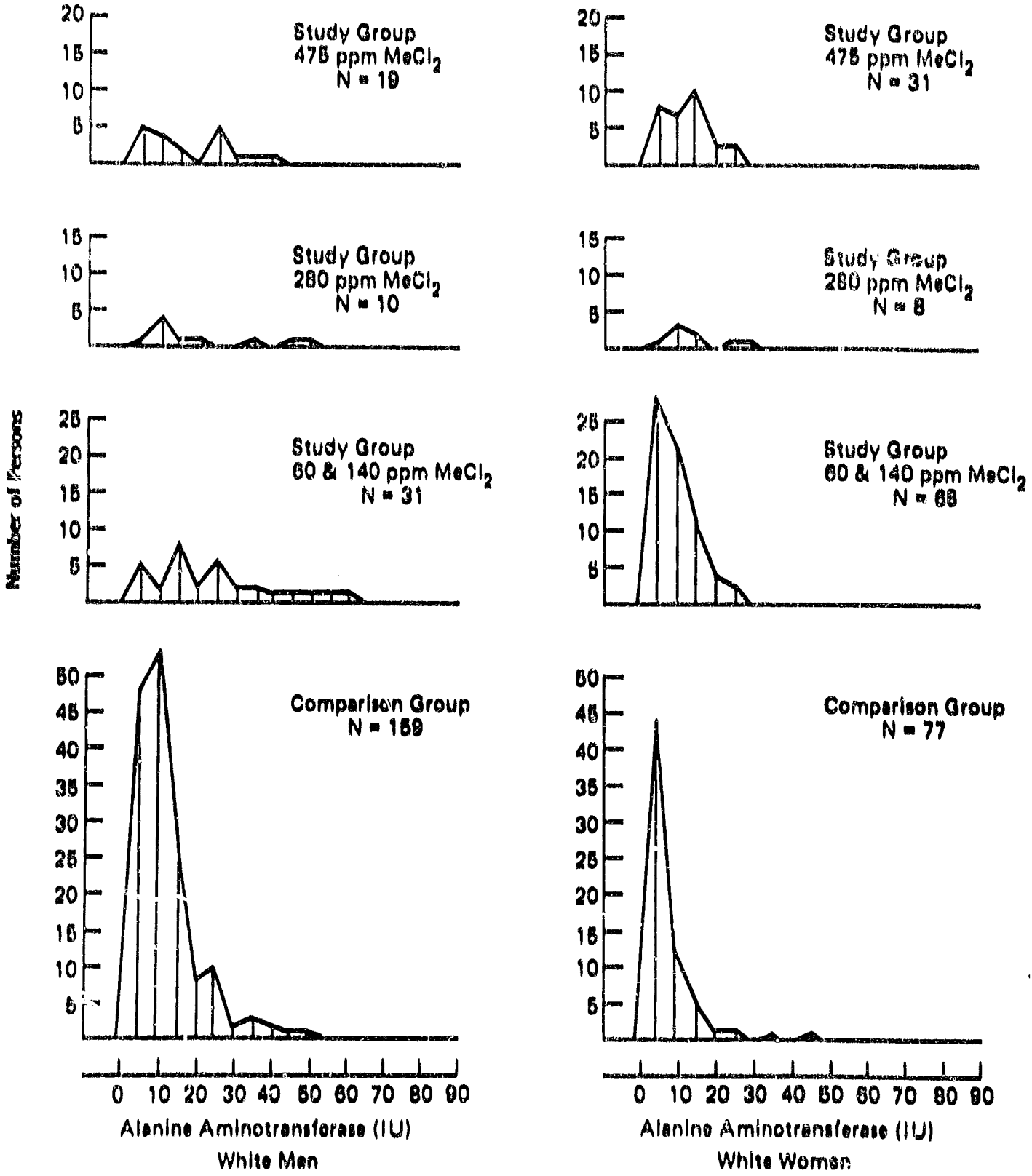

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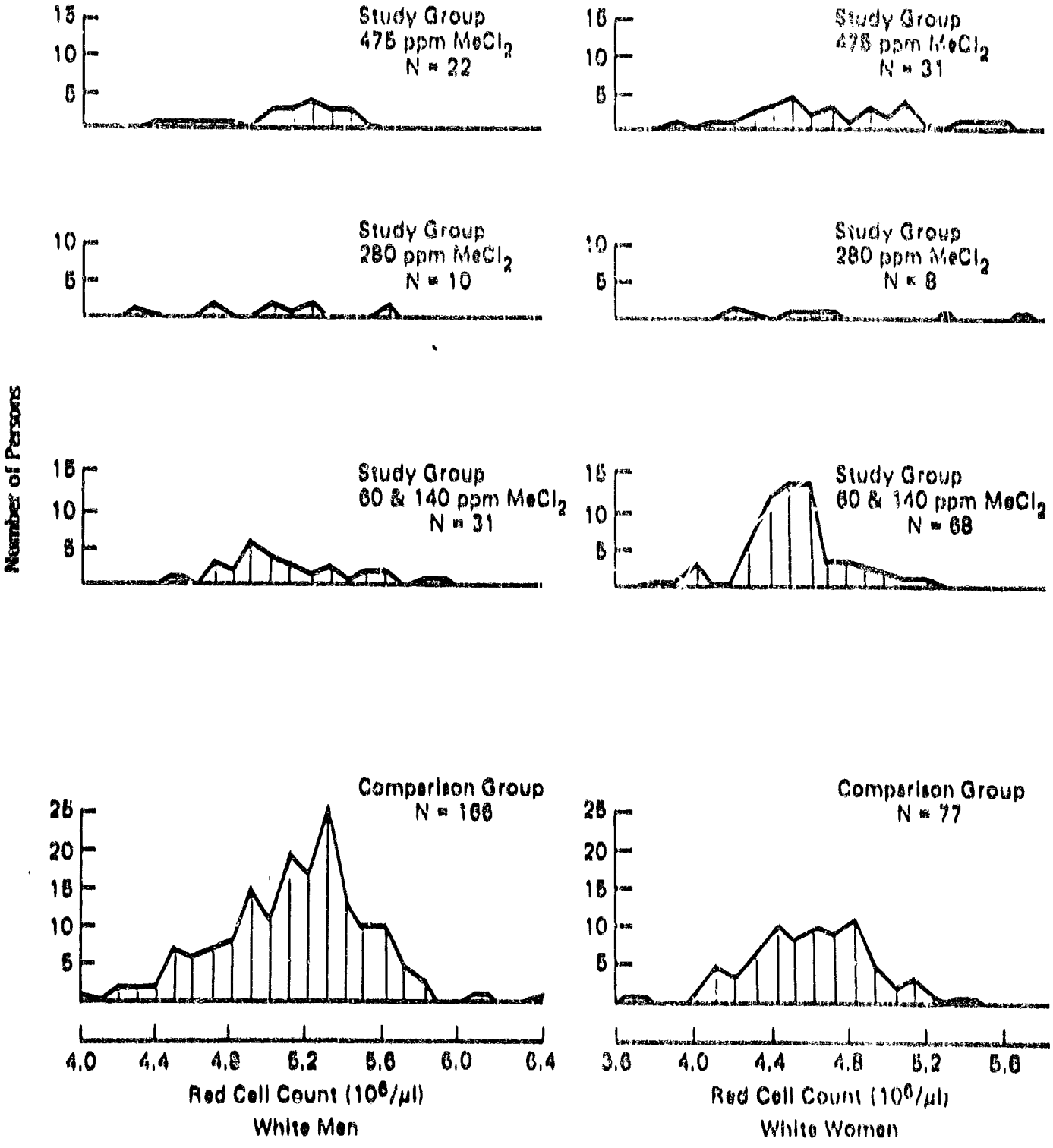
Figure III

RED CELL COUNTS BY EXPOSURE GROUP
AMONG WHITE MEN AND WOMEN



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HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: MORTALITY

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METHYLENE CHLORIDE	75-Ø9-2

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July 9, 1982

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: MORTALITY

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M O R T A L I T Y

KEYWORDS

Mortality

Methylene Chloride

Occupational Exposure

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A B S T R A C T

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: MORTALITY

Ott MG, Skory LK, Holder BB, Bronson JM, and Williams PR

Methylene chloride is partially metabolized to form carboxyhemoglobin resulting in probable reduction in oxygen availability to the myocardium. Thus, among exposed individuals there could be the potential for an increased risk of ischemic events, particularly among those with advanced coronary artery disease. The present retrospective cohort study focused on ischemic heart disease deaths among employees of a fiber production plant in which methylene chloride was used as a general purpose solvent.

Exposures to methylene chloride ranged from approximately 140 parts-per-million (ppm) time-weighted-average (TWA) to 475 ppm TWA. Relative to the United States general population, there was no significant increase in overall mortality or in deaths due to ischemic heart disease. Further analyses of ischemic heart disease mortality by employment status, duration of exposure, and follow-up interval also did not reveal an exposure-related effect. Thus, given qualifications relative to statistical power and possible confounding bias (e.g., healthy worker effect) attendant with this study, no mortality effects attributable to methylene chloride exposure were found.

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HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: MORTALITY

Concerns regarding possible health hazards of methylene chloride ($MeCl_2$) have been raised on the basis of its metabolism to form carboxyhemoglobin.[8,15] Increased blood carboxyhemoglobin potentially reduces oxygen availability to the heart and thereby could increase the risk of ischemic events, particularly in persons with advanced coronary artery disease. The interest in adverse cardiovascular outcomes relative to $MeCl_2$ exposure also stems in part, from the possibility that very high acute exposures to $MeCl_2$ (e.g. greater than 2%) may directly sensitize the myocardium to epinephrine.[13] However, excursion exposures of this magnitude are extremely unlikely.

The present cohort mortality study was conducted in conjunction with a detailed health evaluation offered to the current work force of two fiber production plants. The plant of primary interest was one which used $MeCl_2$; the second plant was included for comparison purposes. For the reasons stated above, attention was focused on cardiovascular disease deaths, particularly those in which the underlying cause was ischemic heart disease and which occurred among the active workforce. However, overall mortality and deaths due to malignant neoplasms and external causes were also investigated.

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In a prior study, Friedlander et al, had found that mortality among men occupationally exposed to $MeCl_2$ was consistent with that of industrial controls and was less than general population mortality.[5,6] Furthermore, examination of specific causes of death did not reveal excess risks for ischemic heart disease or site-specific malignant neoplasms. The population studied was a moderately large male population, whose $MeCl_2$ exposures were judged to have been in the range of 30 to 125 ppm. In contrast to the Friedlander study, the present cohort includes both men and women. $MeCl_2$ exposure intensities were higher in the present cohort; however, duration of exposure was generally less since $MeCl_2$ use in the study plant began in 1954, whereas, its use in operations covered by the Friedlander study predates the 1950's.

METHODS

The overall study design and objectives of this research as well as environmental considerations are contained in a prior report.[10] The cohorts of interest consisted of production employees who worked for at least three months in the preparation or extrusion areas of either plant subsequent to January 1, 1954 and prior to January 1, 1977. The preparation and extrusion departments were chosen because of the high potential for exposure to the solvent system used at each location. In the study plant, located in Rock Hill, South Carolina, both cellulose diacetate (acetate) and cellulose triacetate (CTA) fibers were manufactured in adjacent work areas. $MeCl_2$ was the major component

of the solvent system used in CTA production, whereas, acetone was the solvent of choice in acetate production. Only acetone was present in the comparison plant (located in Narrows, Virginia), as production was limited to acetate fibers. $MeCl_2$ exposure concentrations in the study plant were estimated to be 475 parts-per-million (ppm) time-weighted average (TWA) for an 8-hour day in areas of high $MeCl_2$ use (CTA production) and 140 ppm TWA in areas of low $MeCl_2$ use (mixed acetate and CTA production).(10) Some employees worked in intermediate exposure areas or intermittently in areas of both high and low $MeCl_2$ use. Approximately 25% of the work force was assigned to a high use area of the study plant. Methanol was a component of the solvent system in CTA production and was present in approximately a 1 to 10 ratio to $MeCl_2$. Acetone exposures in both plants ranged from about 100 ppm TWA to over 1000 ppm TWA, depending on work area. Generally, acetone exposures were lowest in areas of high $MeCl_2$ use. Details concerning the environmental exposure assessment may be found in our prior report.(10) Service employees (machine cleaners and janitors) were included in the study, but not the comparison cohort. In the comparison plant, service employees were assigned work throughout the plant complex, and, therefore, no records were available to determine which of these employees had worked in the preparation or extrusion departments.

Vital status of employees in the two cohorts was established through company records, by telephone and mail contact, by motor vehicle registration searches and, finally, through a submission

of names to the Social Security Administration. The data obtained through the Social Security submission gave positive identification of decedents, but did not distinguish between alive and unknown status for other employees. In general, Social Security identification of deceased men appears to have been reasonably complete since the 1950's.(11,12) In the present study, analyses were performed both assuming the persons not identified as deceased by the Social Security Administration to be living and to be lost-to-follow-up. The latter assumption would likely lead to a slight upward bias in risk ratios while the former assumption leads to a slight downward bias.

The mortality experience of the two cohorts was followed from three months after entry date through June 1977. Expected deaths were computed by the indirect method using United States white male, nonwhite male, and white female mortality data (5-year age intervals and 5-year calendar periods of time) with 1975 being the most recent national data available. Expected deaths were not computed for nonwhite women as no deaths were observed among the 108 nonwhite women in the study. Person years were accumulated on a month-by-month basis and comparisons in the study cohort were based on Poisson distribution assumptions using the tables of Bailar and Ederer.(1) Direct comparisons of standardized mortality ratios (SMRs) between the two cohorts were not made. Miettinen has pointed out the potential problems of such comparisons.(9) In particular, SMR estimates are internally standardized to each subgroup and, therefore, are not mutually

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comparable when differences exist in the age-sex-race-specific distributions of the subgroups. A solution to this difficulty used in the present investigation, was to compute stratum-specific numbers of observed and expected deaths for each subgroup and to compare the subgroups, conditioned on equating total observed and expected deaths within each stratum. Stratification was based on age at entry, years since entry and length of employment. A summary statistic was calculated that is analogous to the Mantel-Haenszel statistic except that external information has been incorporated into the estimation procedure and a multinomial model replaces the hypergeometric. Confirmatory analyses were also carried out based on Cox's regression model ignoring external information.[2] Both approaches presume an underlying multiplicative hazard model.

RESULTS

Vital and employment status of both the study and comparison cohorts are presented in Table 1. The study cohort was larger than the comparison cohort (1271 vs. 948 persons) and differed in sex and racial composition. There were more women and non-whites in the study cohort, and more white men in the comparison cohort. Of the 1271 individuals in the study cohort, 701 (55%) left employment between 1954 and 1977, whereas, 279 (29%) persons in the comparison cohort left employment during those years.

Vital status ascertainment for the 980 employees who left the company is summarized in Table 2. Initial follow-up through

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personal contact of former employees or their relatives led to verification of 611 living persons and 15 decedents. A list of the remaining 354 former employees was submitted to the Social Security Administration for further follow-up. Positive identification of 16 additional decedents was obtained. Thus, although exception reporting was complete for both cohorts, confirmative information on vital status was not available for 226 (18%) persons in the study cohort and 112 (12%) individuals in the comparison cohort. However, few deaths were apt to have been missed based on previous experience with Social Security follow-up.

The distribution of deaths relative to the number of persons at risk is given by year of first exposure and duration of exposure for men (Table 3) and women (Table 4) in both cohorts. Data for service only employees of the study plant are presented separately, since employees in the comparison plant with similar work assignments were not included in the study.

Observed and expected deaths by cause are summarized for the study cohort in Table 5. Expected deaths were computed under two separate assumptions, firstly, by assuming individuals not identified as deceased by the Social Security Administration to be alive, and secondly, by assuming those individuals were lost-to-follow-up although accepting identification of deaths by the Social Security Administration. Overall mortality was comparable to that of the corresponding United States population. There was

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a relative excess of deaths due to external causes in each of the three sex-by-race subgroups of the cohort. When observed and expected deaths were summed across the three subgroups, and the conservative estimates of expected deaths were used for comparison, a significant difference was attained for All External Causes (18 observed vs. 10.2 expected, $P<.05$) and the subcategory Accidents (13 observed vs. 6.4 expected, $P<.05$). Review of the 10 deaths due to accidents among men, revealed that four involved automobile collisions, and one each was described as a pedestrian-automobile accident, an industrial accident (past employee of the study plant who was working for another company at the time of the accident), a house fire, a drowning, a home accident and, finally, one death in which the circumstances of the accident were unknown. Four of the men had left employment at least two years prior to death and a fifth man had been working in another area of the plant complex prior to his death. The three accidental deaths among women occurred at least 10 years after last $MeCl_2$ exposure.

Among study plant employees, 7 deaths due to malignant neoplasms were observed compared to 12.4 expected deaths. The site-specific distribution of cancer deaths was also similar to expected; among men the sites were lung (2), pancreas, bladder and blood forming organs (acute monocytic leukemia); among women the sites were lung and primary unknown.

Mortality contrasts between the study and comparison cohorts are examined in Table 6. For white women, there were no differences in cause-specific mortality between the two cohorts. Among white men, statistical differences in risk were observed for All Causes, risk ratio 2.2 (P<.01), Diseases of the Circulatory System, risk ratio 2.2 (P<.05), and All External Causes, risk ratio 2.5 (P<.05), based on conditional risk estimates incorporating external information. Similar risk estimates were obtained based on Cox Regression analyses using age-at-entry as the only covariate. A significant risk ratio was also observed for the subcategory Ischemic Heart Disease (P<.05), but not for Accidents (P = .08). Observed deaths were considerably less than expected (United States mortality experience) in the comparison group for each of the categories in which significant differences between cohorts had been found.

Mortality due to ischemic heart disease among study plant participants was examined by duration of exposure, length of follow-up, and employment status in Table 7. Overall, there were 14 observed compared to 13.2 expected deaths. With a subcategorization of this detail, the expected numbers of deaths in any given cell of the table were small, and thus, weak associations would be difficult to detect. There were, however, no definite trends in the data with respect to duration of exposure, follow-up interval or employment status. Of particular interest was the relationship between employment status and ischemic heart disease. Eight of the fourteen deaths due to ischemic heart

disease had occurred among actively working employees. This compares with 7.4 expected deaths based on United States mortality experience. Similar analyses with respect to All Causes of death and other cause of death categories revealed no trends indicative of an exposure effect.

DISCUSSION

The mortality experience of the study cohort was comparable to that of the corresponding United States population with the possible exception of deaths due to External Causes. Review of those deaths in the study plant did not reveal a pattern suggestive of an occupational etiology.

Since there were apparent differences in the mortality experience of white men in the study and comparison cohorts, possible reasons for these differences were explored. We first examined available mortality data and population characteristics for the counties in which the two plants were located. Population characteristics, as determined in the 1960 United States census, affirmed our impression of urban-rural differences between the two counties (study plant county, 48% rural; comparison plant county, 85% rural). An investigation of the geographic patterns of cardiovascular disease mortality (1968-1971) found the Southeastern United States (area includes the study plant county) to have the highest standardized death rates due to cardiovascular disease of any area of the country.[3] Based on further communications with the authors of that report,

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cardiovascular disease mortality in the study plant county was determined to have been 31% above the national average. Cardiovascular disease mortality in the comparison plant county was 11% above the national average. In light of these data, the mortality trends observed for the study plant are in a consistent direction with the expected geographic differences in mortality, namely, higher cardiovascular disease mortality in the study plant county relative to the comparison plant county, and in the study plant county relative to the United States population. Furthermore, a mortality study of two cotton mills in the neighboring state of North Carolina and involving similar operations but not $MeCl_2$ exposure, reported standardized mortality ratios of 98 for men and 84 for women with cause-specific standardized mortality ratios for arteriosclerotic heart disease being 103 for white men and 128 for white women.(8) These SMRs are similar to those of the study plant and not the comparison plant. The low death rates for the comparison cohort are not explained by either the geographical mortality patterns or other occupational data.

Selection factors (e.g., hiring practices) and employee record keeping procedures were also investigated in relation to the mortality differences between white men in the two cohorts. As both plants belonged to the same parent company, no differences were anticipated in the record retention policy between the two locations. Discussions with the respective personnel managers from each plant did not uncover any differences in retention of work history records for former employees. Hiring practices were

indirectly examined by comparing the distribution of white men by age at hire. Of the 696 white men in the comparison cohort, 71.4% (497) were under age 25 at hire and only 5.0% (35) were at least 35 years of age. In contrast, only 44.2% (217 of 491) of the white men in the study cohort were under age 25 at hire and 16.3% (80) were 35 years of age or older. These differences together with the higher employee turnover rates in the study plant indicate that hiring practices were dissimilar between the two plants. The apparent lack of comparability in hiring practices also indicates a need for caution in comparing mortality between two cohorts. One possible explanation for the very low death rates in the comparison plant is that the company, being the only large scale employer in that area, was able to be more selective in hiring.

Mortality data from the present study do not indicate that exposure to $MeCl_2$, low concentrations of methanol and variable concentrations of acetone has had an adverse impact on the health of exposed employees. This interpretation requires qualification, in part, because of the small numbers of observed and expected deaths for contrasts of particular interest. For example, only 7.4 deaths due to ischamic heart disease were expected among active employees of the study plant based on U.S. death rates. Eight deaths were actually observed. Thus, considering the healthier than average status of typical working populations[4] and the low power for detecting moderately elevated risks, our results do not exclude the possibility of

increased health risks in this population. With this qualification in mind, the findings of the present study, are in agreement with the Friedlander study previously discussed, but do not preclude the need for mortality studies of other working populations exposed to $MoCl_2$. The present study extends the coverage of exposed populations to both men and women, and the conditions of exposure to higher TWA concentrations of $MoCl_2$ than in the previous reported study.

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T A B L E 1

VITAL AND EMPLOYMENT STATUS OF STUDY AND COMPARISON POPULATION AS OF JUNE 30, 1977

Population Group by Sex and Race	Total	Working	Retired	Deceased (Company Records)	Left Employment	
					Deceased	Alive per Social Security and/or Personal Follow-up
Study Population						
Total Group	1271	513	30	27	27	674
White						
Men	487	174	19	21	16	257
Women	615	195	10	1	10	399
Nonwhite						
Men	64	47	1	5	1	10
Women	105	97	0	0	0	8
Comparison Population						
Total Group	948	584	62	23	4	275
White						
Men	696	432	47	22	2	193
Women	248	148	15	1	2	82
Nonwhite						
Men	1	1	0	0	0	0
Women	3	3	0	0	0	0

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TABLE 2

VITAL STATUS FOLLOW-UP OF 980 EMPLOYEES WHO LEFT EMPLOYMENT PRIOR TO JUNE 30, 1977

Population Group by Sex and Date Left	Total	Known Deceased[a]	Known Alive Personal Contact	Social Security Search Only
Study Population	701	27	448	226
Men				
Prior to 1960	84	7	39	38
1960–1969	113	9	69	35
1970+	87	1	66	20
Women				
Prior to 1960	93	5	54	34
1960–1969	186	4	119	63
1970+	138	1	111	26
Comparison Population	279	4	163	112
Men				
Prior to 1960	7	0	6	1
1960–1969	89	2	52	35
1970+	99	0	59	40
Women				
Prior to 1960	18	2	6	10
1960–1969	44	0	29	15
1970+	22	0	11	11

[a]Includes 15 deaths identified through personal followup and 16 deaths identified through Social Security Administration records.

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TABLE 3

NUMBER OF DEATHS AND NUMBER OF MEN AT RISK IN STUDY AND COMPARISON COHORTS BY YEAR OF FIRST EXPOSURE AND DURATION OF EXPOSURE

Year of First Exposure	Duration of Exposure				
	Total Deaths/Risk	<1 Year Deaths/Risk	1-4 Years Deaths/Risk	5-9 Years Deaths/Risk	10+ Years Deaths/Risk
STUDY COHORT PRODUCTION					
Total	22/390	5/88	6/154	6/41	5/107
1954-1959	18/215	3/40	4/60	6/27	5/88
1960-1969	1/75	0/8	1/34	0/14	0/19
1970-1976	3/100	2/40	1/60	0/0	-/-
SERVICE					
Total	21/161	0/14	4/47	5/24	12/76
1954-1959	17/95	0/6	4/22	4/13	9/54
1960-1969	4/48	0/6	0/10	1/10	3/22
1970-1976	0/18	0/2	0/15	0/1	-/-
COMPARISON COHORT[a] PRODUCTION					
Total	24/697	0/96	2/195	3/93	19/313
1954-1959	22/247	0/4	1/12	3/13	18/218
1960-1969	2/309	0/39	1/98	0/77	1/95
1970-1976	0/141	0/53	0/85	0/3	-/-

[a]Year of first exposure is the earliest date subsequent to 1953 that the employee worked in the preparation or extrusion area of the comparison plant. Solvent exposure was only to acetone.

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TABLE 4

NUMBER OF DEATHS AND NUMBER OF WOMEN AT RISK IN STUDY AND COMPARISON COHORTS BY YEAR OF FIRST EXPOSURE AND DURATION OF EXPOSURE

Year of First Exposure	Duration of Exposure				
	Total Deaths/Risk	<1 Year Deaths/Risk	1–4 Years Deaths/Risk	5–9 Years Deaths/Risk	10+ Years Deaths/Risk
STUDY COHORT PRODUCTION					
Total	11/712	2/116	4/290	1/137	4/169
1954–1959	10/260	2/37	3/69	1/41	4/113
1960–1969	1/270	0/40	1/101	0/73	0/56
1970–1976	0/182	0/39	0/120	0/23	-/—
SERVICE					
Total	0/8	0/0	0/5	0/3	0/0
1954–1959	0/2	0/0	0/0	0/2	0/0
1960–1969	0/1	0/0	0/1	0/0	0/0
1970–1976	0/5	0/0	0/4	0/1	-/—
COMPARISON COHORT[a] PRODUCTION					
Total	3/251	0/47	3/110	0/36	0/58
1954–1959	2/91	0/9	2/18	0/18	0/46
1960–1969	0/50	0/10	0/15	0/13	0/12
1970–1976	1/110	0/28	1/77	0/5	-/—

[a]Year of first exposure is the earliest date subsequent to 1953 that the employee worked in the preparation or extrusion area of the comparison plant. Solvent exposure was only to acetone.

TABLE 5

OBSERVED AND EXPECTED DEATHS[a] BY CAUSE, SEX, AND RACE
FOR STUDY COHORT, 1954 - JUNE 1977

Cause of Death Category	White Men			Nonwhite Men			White Women		
	Obs	Exp[b]	Exp[c]	Obs	Exp[b]	Exp[c]	Obs	Exp[b]	Exp[c]
All Causes	37	34.9	30.4	6	6.4	5.6	11	15.9	13.5
Malignant Neoplasms	5	6.3	5.6	0	0.9	0.9	2	5.2	4.5
Diseases of Circulatory System	11	14.0	12.4	4	2.2	2.0	3	3.7	3.2
Ischemic Heart Disease	10	10.5	9.4	2	1.1	1.1	2	1.6	1.4
Symptoms, Senility and Ill-Defined Conditions	2	0.5	0.5	0	0.2	0.2	0	0.3	0.2
All External Causes	12	7.9	6.5	2	1.6	1.3	4	2.9	2.4
Accidents	9	5.4	4.4	1	0.8	0.6	3	1.7	1.4
Suicide	2	1.7	1.4	0	0.1	0.1	1	0.9	0.7
Residual	4	6.2	5.4	0	1.5	1.2	1	3.8	3.2
Unable to Locate Death Certificate	3	—	—	0	—	—	1	—	—

[a]Expected deaths calculated from United States, death rates for white men, nonwhite men and white women respectively - There were no deaths among 107 nonwhite women in the study cohort.

[b]Individuals presumed living through end of follow-up period if not identified by Social Security as deceased.

[c]Individuals presumed lost-to-follow-up if not contacted and known to be living.

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TABLE 6

MORTALITY CONTRASTS BETWEEN STUDY AND COMPARISON COHORTS
BY CAUSE OF DEATH CATEGORY, WHITE MEN AND WOMEN

Cause of Death Category	Observed/Expected Deaths				Risk Estimates (Study/Comparison)			
	Study Cohort		Comparison Cohort		Conditional Risk		Cox Regression Analysis	
	Men	Women	Men	Women	Men	Women	Men	Women
All Causes	37/34.9	11/15.9	24/53.8	3/6.7	2.2**	1.3	2.1**	1.5
Malignant Neoplasms	5/ 6.3	2/ 5.2	5/10.0	2/2.3	1.2	!	1.3	!
Diseases of Circulatory System	11/14.0	3/ 3.7	9/23.0	1/1.9	2.2*	!	1.9	!
Ischemic Heart Disease	10/10.5	2/ 1.6	6/17.4	1/0.9	3.1*	!	2.6	!
Symptoms, Senility and Ill-Defined Conditions	2/ 0.5	0/ 0.3	1/ 0.8	0/0.1	!	!	!	!
All External Causes	12/ 7.9	4/ 2.9	5/10.6	0/1.0	2.5*	!	2.6*	!
Accidents	9/ 5.4	3/ 1.7	4/ 7.2	0/0.6	2.5	!	2.7	!
Suicide	2/ 1.7	1/ 0.9	1/ 2.3	0/0.3	!	!	!	!
Residual	4/ 6.2	1/ 3.8	4/ 9.4	0/1.4	!	!	!	!
Unable to Locate Death Certificate	3/-	1/-	0/-	0/0-				

! Not computed due to small number of deaths.

*P< .05

** P<.01

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TABLE 7

OBSERVED AND EXPECTED DEATHS DUE TO ISCHEMIC HEART DISEASE IN STUDY COHORT BY DURATION OF EXPOSURE, SUBSEQUENT FOLLOW-UP INTERVAL AND EMPLOYMENT STATUS, 1954 - June 1977

Duration of Exposure and Follow-up Interval	Total Cohort			Actively Working for Company		
	White Men Obs/Exp	Nonwhite Men Obs/Exp	White Women Obs/Exp	White Men Obs/Exp	Nonwhite Men Obs/Exp	White Women Obs/Exp
Total	10/10.5†	2/1.1	2/1.6	7/5.9	1/0.8	0/0.7
<5 Years Exposure						
<5 Years	1/ 0.9	0/0.2	0/ 0.2	0/ 0.7	0/0.2	0/0.1
5-9 Years	0/ 0.4	0/0.1	0/ 0.1	0/ 0.1	0/0.0	0/0.0
10+ Years	1/ 2.2	1/0.1	0/ 0.3	0/ 0.1	0/0.0	0/0.0
5-9 Years Exposure						
<5 Years	2/ 1.1	0/0.2	0/ 0.2	2/ 1.1	0/0.2	0/0.1
5-9 Years	0/ 0.2	0/0.1	0/ 0.1	0/ 0.0	0/0.0	0/0.0
10+ Years	0/ 0.3	0/0.1	0/ 0.1	0/ 0.0	0/0.0	0/0.0
10+ Years Exposure						
<5 Years	2/ 1.6	0/0.1	0/ 0.2	2/ 1.5	0/0.1	0/0.1
5-9 Years	3/ 2.1	0/0.2	2/ 0.2	3/ 1.5	0/0.2	0/0.2
10+ Years	1/ 1.6	1/0.1	0/ 0.2	0/ 0.8	1/0.1	0/0.1

† Expected numbers of deaths may not be additive due to round-off errors and presumes Social Security follow-up is complete.

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Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-Ø9-2

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July 9, 1982

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: GENERAL STUDY DESIGN AND ENVIRONMENTAL CONSIDERATIONS

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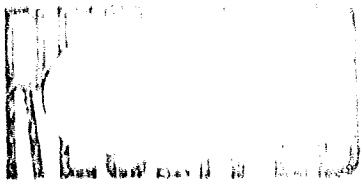

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GENERAL STUDY DESIGN AND ENVIRONMENTAL CONSIDERATIONS

KEYWORDS

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Methylene Chloride

Occupational Exposure

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A B S T R A C T

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: GENERAL STUDY DESIGN AND ENVIRONMENTAL CONSIDERATIONS

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Recent concern regarding health hazards of methylene chloride stem primarily from the discovery of its metabolism to form carboxyhemoglobin. In this report, a research program is described, the purpose of which was to assess potential health effects of methylene chloride exposure in an occupational setting. Particular attention was given to evaluating possible direct and carboxyhemoglobin-mediated effects on the hematopoetic and circulatory systems.

The study involved one fiber production plant which used a methylene chloride/methanol mixture and acetone as solvents and a second fiber production plant that used acetone alone. The research design included a retrospective cohort mortality study and several health evaluation studies as well as an environmental assessment of the two plants.

Industrial hygiene monitoring indicated that typical methylene chloride exposures ranged from 140 parts-per-million (ppm) time-weighted-average for an 8-hour day in areas of low methylene chloride use to 475 ppm time-weighted-average in areas of high methylene chloride use and that methanol was present in about a one to ten ratio to methylene chloride. Acetone exposures in both plants ranged from 100 to over 1000 ppm time-weighted-average.

HEALTH EVALUATION OF EMPLOYEES OCCUPATIONALLY EXPOSED TO METHYLENE CHLORIDE: GENERAL STUDY DESIGN AND ENVIRONMENTAL CONSIDERATIONS

INTRODUCTION

Methylene chloride ($MeCl_2$) is an important commodity chemical with a variety of end uses as a non-flammable industrial solvent. Its toxicity has generally been regarded to be low relative to other chlorinated methanes, a principle concern being anesthetic effects at high concentrations.(7)

In 1972, a finding of increased carboxyhemoglobin (COHb) was reported among persons exposed to the solvent in an experimental setting.(10) This observation was subsequently confirmed and was determined to be due to the metabolism of $MeCl_2$ to form COHb.(3,8,9) A second metabolic pathway for the degradation of $MeCl_2$ has also been identified.(1) Of the two routes of metabolism, the first-mentioned oxidative pathway has received more attention than the glutathione-dependent pathway to carbon dioxide. Further studies on the uptake, metabolism and elimination of $MeCl_2$ in humans have been reported by DiVincenzo and Kaplan.(4,5)

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Discovery of the oxidative pathway led to reconsideration of the potential hazards of $MeCl_2$ exposure, particularly in regard to COHb related effects on the cardiovascular system. Increased COHb has been associated with decreased maximal aerobic capacity, a decrease in time to onset of angina during exercise in men with ischemic heart disease, decrement in visual function, and at high levels, severe headaches.(2,6,11) Although an extensive literature exists regarding carbon monoxide (CO) toxicity, Turino recently indicated that adequate data were not yet available to assess the effects of chronic CO exposure resulting in 5-10% COHb saturations.(12)

The present research was undertaken to develop a better understanding of any effects of $MeCl_2$ exposure in an employee population, particularly those that might be mediated through the metabolism of $MeCl_2$ to form COHb. This research included an industrial hygiene assessment of the work environment, a retrospective cohort mortality study and several health evaluation studies.

OVERALL STUDY DESIGN

Initially a search was made of industrial users of $MeCl_2$ to identify a work setting in which the agent was present at concentrations approaching the existing OSHA standard of 500 parts per million (ppm) time-weighted-average for an 8-hour day (TWA) (29 CFR Par 1910.1000, Table Z-2) and in which multiple chemical exposures were not considered a major complicating factor. An additional consideration was that a comparison plant in which $MeCl_2$ was not present could be found.

The plant selected for study was located in Rock Hill, South Carolina, and was part of a multi-plant complex that had been in operation since 1948. Both cellulose diacetate (acetate) and cellulose triacetate (CTA) fibers were manufactured in adjacent work areas of the study plant with CTA production having been initiated in 1954. Methylene chloride was the major component and methanol was a lesser constituent of the solvent system used in CTA production, whereas, acetone was the only solvent used in acetate production. Thus, potential for exposure to $MeCl_2$, methanol, and acetone existed in the study plant. The existing OSHA standard for acetone was 1000 ppm TWA and for methanol was 200 ppm TWA (29 CFR par 1910.1000, Table Z-1).

We were not able to identify a comparison plant that was both similar in operation, except for the presence of $MeCl_2$, and geographically close to the study plant. However, it was possible to select a comparison plant with similar operations. This plant was located in Narrows, Virginia (about 260 kilometers from the study plant), was part of a similar, although older multi-plant complex, and was owned by the same company as the study plant. The operations were of the same design as existed at the study plant except that no $MeCl_2$ was used because only acetate fibers were manufactured.

There were five aspects to the research project:

1) a comprehensive industrial hygiene survey of both the study and comparison plant;

2) a mortality study of both the exposed and comparison populations using a retrospective cohort design including follow-up of employees who left the company;

3) a health evaluation offered to employees of the exposed and comparison populations (the evaluation included a health history questionnaire, a physical examination, and laboratory assessment of blood and urine constituents);

4) an analysis of continuous ambulatory electrocardiograms recorded from a subset of men over age 34, and

5) an evaluation of $MeCl_2$ metabolism to form COHb and related effects on the oxyhemoglobin dissociation curve.

ENVIRONMENTAL CONSIDERATIONS

Comprehensive industrial hygiene surveys of the two plants were conducted from September 12, 1977 through February 7, 1978. An initial visit to each plant was undertaken to identify chemicals present in the work environment, establish exposure ranges (8-hour) TWA, and peak concentrations), and lay the ground work for more detailed personnel sampling to be conducted in conjunction with later medical testing. The second visit included personnel monitoring, with additional area and short-term excursion sampling. More than 350 personnel, 170 area and 20 excursion samples were

obtained by industral hygienists during the 3.5 month survey period. A summary of the industrial hygiene methods is contained in the Appendix.

The environmental studies focused on the preparation and extrusion departments of both plants. These departments were selected because of the high potential for exposure to the solvent system used at each location. In the preparation area, the solvent is mixed with either acetate or CTA flake. The mixture is then passed through a series of filtration steps to remove undissolved polymer. Finally, polymer in solution is transferred to the extrusion areas where filaments are dried and wound on bobbins. The extrusion areas of both plants also included operations for producing cigarette tow (filter packing) from the acetate polymer.

Results

At both plants, CO concentrations in general work area samples (excluding cigarette smoking areas) were found to be less than 3 ppm and, therefore, this agent was assumed to be a negligible factor in the exposure profile of employees. Oil mist exposure in the extrusion area was also judged to be a negligible factor since measured exposures were less than 5 mg/m^3 TWA. The oil mist was due to a finish oil, which contained mineral and vegetable oils, surfactants, and antifoam and antistat agents.

As previously mentioned, the solvent system employed in CTA production was a mixture of $MeCl_2$ and methanol. Personnel monitoring showed a ratio of less than 1 to 10 for methanol to $MeCl_2$ concentrations in the air and, secondly, that methanol concentrations were predictable from and highly correlated with $MeCl_2$ concentrations. Thus, the exposure profile for each employee could be described in terms of acetone and $MeCl_2$ with the understanding that methanol accompanied $MeCl_2$ in an approximate 1 to 10 ratio.

The categorization of exposure with respect to $MeCl_2$ and acetone is given in Table 1 for both the study and comparison plants. In general, acetone exposures in the study plant were lowest where $MeCl_2$ exposures were highest. Solvent exposure was less variable in extrusion than in preparation areas of each plant. However, ambient air concentrations of solvents were generally higher in extrusion, because of the continuous nature of the operation. The study plant extrusion area was divided into two blocks; Block I, mixed acetate and CTA extrusion, and Block II, CTA extrusion only. In Block I, median $MeCl_2$ concentrations (84 personnel samples) were about 140 ppm TWA with a range of 60-350 ppm. Individuals who worked in tow production were exposed to somewhat higher $MeCl_2$ concentrations because of the proximity of these operations to Block II. Based on 19 personnel samples, a range of 50 to 470 ppm $MeCl_2$ was observed. In Block II $MeCl_2$ concentrations ranged from 210 to 690 ppm TWA (63 samples). Median TWA exposures were approximately 475 ppm.

Acetone exposures in the extrusion department of the study plant were lowest for individuals whose work assignments were in Block II or involved tow production. For example, 59 of 63 personnel samples for acetone were below 300 ppm TWA in Block II and 14 of 19 samples were below 300 ppm TWA in tow production. Acetone exposures were much higher in Block I extrusion and were comparable to those found in the comparison plant extrusion areas. The respective ranges were 250 to 1600 ppm TWA (84 samples) in Block I extrusion and 260 to 1750 ppm TWA (83 samples) in the comparison plant.

Personnel sampling in the preparation area of the study plant (26 samples) revealed 8-hour TWA concentrations of $MeCl_2$ from 5 ppm to 380 ppm with two uncharacteristically high readings of 860 and 900 ppm respectively. TWA concentrations depended both on job assignment and whether or not the mixture being prepared was for use in CTA or acetate extrusion. Peak concentrations occurred during stripping and redressing of filter presses and charging of CTA and acetate residue tanks. TWA concentrations of acetone ranged from 10 to 800 ppm in the study plant. In the preparation area of the comparison plant, acetone TWAs (16 samples) varied from 50 to 980 ppm with one sample of 1470 ppm.

Exposures measured during the surveys were believed to be representative of exposure throughout the history of the plant, based on review of process changes and limited prior sampling

data. To summarize, the exposure profile across both plants included acetone alone at low and high TWA concentrations, low $MeCl_2$ TWAs in the presence of high acetone TWAs and intermediate and high $MeCl_2$ TWAs concomitant with low acetone exposure intensities.

GENERAL DESIGN OF MORTALITY STUDY

The target population for the mortality study consisted of production and service employees who had worked for at least three months in the preparation or extrusion areas of either the study or comparison plant subsequent to January 1, 1954 and prior to 1977. In the study plant, service employees (e.g., machine cleaners and janitors) were specifically assigned to preparation or extrusion areas and, therefore, were included in the study. In contrast, many service employees from the comparison plant were assigned work throughout the plant complex rather than in specific departments, thus, since no record was kept of the day-by-day assignments, there was no mechanism for identifying these employees and including them in the study. All work history records at each plant location were reviewed to identify the individuals who satisfied the target population definition. A total of 1271 employees from the study plant and 948 employees from the comparison plant met these criteria and were included in the mortality study.

The following items of information were obtained for each individual: name, employee identification number (and other

information pertinent to tracing former employees), sex, race, birthdate, employment status, a listing of each job held and the employment dates on each job. Vital status of former company employees was established by telephone and mail contact, searching motor vehicle registrations, and through a submission of names to the Social Security Administration.

Causes of death were determined from copies of death certificates obtained through company insurance records or state vital statistics agencies, and were coded by a nosologist according to the Revision of the International Classification of Diseases in force at the time of death. Mortality within the study plant cohort was compared with that of both the corresponding U.S. population and the comparison plant. Outcomes of a priori interest were deaths due to ischemic heart disease and malignant neoplasms.

GENERAL DESIGN OF HEALTH EVALUATION STUDIES

The health evaluation portion of the overall surveillance program included a general health examination that was offered to personnel currently assigned to the preparation or extrusion work areas and several additional studies undertaken for subsets of the employee population. Following verbal and written explanations of the study objectives, self-administered "intent to participate" forms were completed by employees of both plants and returned on a voluntary basis. Based on the replies, examination

dates were then scheduled. The evaluation was conducted from October, 1977 to February, 1978, specifically from October 25th through December 19th in the study plant and from November 7th through February 13th in the comparison plant.

Because the program was voluntary, we sought to estimate participation in the health evaluation through knowledge of the actively employed population defined in the mortality study. Using comparable job classifications, 61 and 55% of the work force in the study and comparison plants, respectively, took part in the program. Participation was highest among non-white men and women (76%), most of whom worked in the study plant. Among white women, participation was 60% in the study plant and 57% in the comparison plant; among white men, 43% in the study plant and 54% in the comparison plant. Acceptance in both plants was slightly higher among whites under the age of 45 than over that age, 60% vs. 48% in the study plant and 57% vs. 52% in the comparison plant. Thus, similar participation rates albeit lower than desirable, were obtained in both plants. Nonwhites were somewhat over-represented, and white males under-represented, relative to the total study plant population. The questionnaire portion of the health evaluation included information on environmental exposures (alcohol and cigarette smoking history, medications, part-time jobs and prior job history). These data were used, in conjunction with selected laboratory tests, to determine evidence or lack of evidence of liver injury and to determine if certain hematologic changes observed in cigarette smokers and

attributed by some researchers to carbon monoxide exposure were also seen with $MeCl_2$ exposure.

Additional studies were performed for several subsets of the health evaluation participants. In one study, 24-hour electrocardiographic (EKG) monitoring was conducted on 50 older men, 24 from the study plant and 26 from the comparison plant. The objective of this monitoring was to determine whether or not $MeCl_2$ exposure concentrations existing in the present work environment were associated with increased ventricular or supraventricular ectopic activity or ST-segment depression.

In a second investigation, measurements of blood COHb saturation, alveolar air CO and P_{50} (the partial pressure at which hemoglobin is 50% saturated with oxygen) both before and after shift, were made in conjunction with personal monitoring of $MeCl_2$ exposure. This evaluation was performed for 268 employees, 136 from the study plant and 132 from the comparison plant. The objective was to quantitate the relationships between these variables in a large employee population and to provide an additional perspective for interpreting the overall study findings.

SUMMARY

The primary objective of the present research was to assess the potential health effects of $MeCl_2$ exposure in an occupational setting with particular attention given to possible COHb related effects on the cardiovascular system.

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Recognizing that evaluation of the current workforce alone would leave unanswered important questions regarding selection in and out of the studied population, a retrospective cohort mortality study was incorporated into the overall study design.

Industrial hygiene monitoring of the study and comparison plants was carried out over a 3.5 month period. The industrial hygiene surveys were scheduled, in part, to coincide with biological monitoring and medical testing to detect shifts in the oxyhemoglobin dissociation curve. Continuous ambulatory EKG monitoring was conducted for a subset of 50 male employees. Finally, selected blood constituents obtained during the health evaluation were examined to determine the presence or absence of hematologic changes or liver dysfunction relative to exposure.

The environmental studies indicated that, on the whole, acetone exposures were similar in both plants. In the study plant, about 25% of the personnel were assigned to jobs for which typical $MeCl_2$ exposures were 475 ppm TWA. Most other employees in the study plant population experienced $MeCl_2$ exposures of about 140 ppm TWA. However, some individuals were either assigned to jobs of intermediate exposure or were intermittently working in areas of relatively high (475 ppm) and low (140 ppm) exposure. Acetone exposures in the study plant were, in general, inversely correlated with $MeCl_2$ exposures.

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A P P E N D I X

INDUSTRIAL HYGIENE METHODS

During the initial visit, personnel samples for acetone, methanol and $MeCl_2$ were collected using two hand packed 700 mg silica gel tubes and one Pittsburg Coconut Base (PCB) one-gram charcoal tube in series. At the comparison plant, two PCB one-gram charcoal tubes in series were employed in collecting acetone. Sampling times were four hours at a flow rate of about 50 cc/minute on MSA, SKC and Sipin equipment; thus, employees were monitored with a second sampling train during the last four hours of the shift. Onsite testing indicated that one silica gel tube was adequate for second visit sampling at the study plant and that eight-hour sampling could be conducted at the comparison plant. Acetone and $MeCl_2$ were adsorbed on both the silica gel and activated charcoal and, therefore, were analyzed from both media using two different columns. Methanol was collected on the silica gel tube. Measurements were based on flame ionized detection chromatography. Area and excursion samples were collected with high flow Bendix and MSA pumps at a rate of 240 ml/min for 15 minutes with similar adsorbent configurations as in personal sampling.

APPENDIX

(1)

Industrial Hygiene Methods

Background carbon monoxide concentrations were measured with an Energetics Science, Incorporated, 2000 Series Carbon Monoxide Ecolyzer. Area oil mist samples were collected at both plants using high flow Bendix and MSA pumps at flow rates of 2 - 2.5 liters/minute for one hour. The oil mist was collected on Gelman 47 mm (.08 micron pore size) filter paper and was weighed on an analytical balance.

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TABLE 1

CATEGORIZATION OF OCCUPATIONAL EXPOSURE TO METHYLENE CHLORIDE AND ACETONE

Plant and Job Category	$MeCl_2$ Median TWA	Acetone Median TWA
Study Plant[a]		
Category 1 - Low $MeCl_2$ and High Acetone (Most production jobs in Block I extrusion and in preparation)[b]	140 ppm	1080 ppm
Category 2 - Moderate $MeCl_2$ and low Acetone (Jobs in tow extrusion and where time was spent in both Block I and Block I & Block II (e.g. service[c]	280 ppm	110 ppm
Category 3 - High $MeCl_2$ and Low Acetone (Jobs in Block II extrusion and one job in preparation)	475 ppm	110 ppm
Comparison Plant		
Category - 1 Low Acetone (Supervisors, jobs in tow production and some jobs in preparation)	0 ppm	380 ppm
Category - 2 Moderate Acetone (Inspectors and service jobs in filament)	0 ppm	770 ppm
Category - 3 High Acetone (Operator jobs in filament extrusion)	0 ppm	1070 ppm

(a) Eleven employees assigned to production support jobs had low exposure to both $MeCl_2$ and acetone (median $MeCl_2$ TWA was about 60 ppm and median acetone TWA was 110 ppm) and were included in health evaluation studies at their request.

(b) One exception was a job in preparation which was categorized as low $MeCl_2$ exposure (140 ppm) and low rather than high acetone exposure (110 ppm as opposed to 1080 ppm).

(c) Several service jobs were classified as $MeCl_2$ - 280 ppm and acetone - 1080 ppm.

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METABOLISM OF DIHALOMETHANE TO CABON MONOXIDE PREPARED BY UNIV OF MINNESOTA WITH COVER LETTER

LK

Chemical Name (300 per name)	25	CAS No. (10)	24
DICHLOROMETHANE		75-Ø9-2	

1A

Mech

D-639

UNIVERSITY OF MINNESOTA
TWIN CITIES

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Medical School
105 Millard Hall
Minneapolis, Minnesota 55455
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8182118823

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July 3, 1973

RECEIVED

Dr. Perry J. Gehring
Dow Chemical Company
Midland, Michigan 48642

Dear Perry:

I am enclosing herewith a preliminary report describing our studies on the in vivo metabolism of dihalomethanes. We are now initiating our in vitro studies in an attempt to unravel the enzymatic mechanisms involved in the conversion of these compounds to carbon monoxide. As I indicated during our telephone conversation, these studies could be markedly simplified if ^{36}Cl-dichloromethane were available. I appreciate any help you can give us in obtaining the labelled compound for our experiments.

See you in East Lansing. Best personal regards.

Sincerely yours,

MWA

M. W. Anders, Ph.D.

MWA/jdr
enclosure

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Metabolism of Dihalomethanes to Carbon Monoxide

I. In Vivo Studies

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Department of Pediatrics
University of Minnesota
Minneapolis, Minnesota 55455

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INTRODUCTION

Halogenated hydrocarbons have been widely employed as solvents for a variety of purposes in both industry and commerce. With particular reference to the halogenated methane derivatives, both carbon tetrachloride and dichloromethane have been extensively used. However, since the ability of carbon tetrachloride to produce liver damage is well documented, the use of this solvent has been largely discontinued in this country and it has been consequently replaced by dichloromethane. In general, it has been felt that the toxicity of dichloromethane is substantially lower than that of carbon tetrachloride, particularly with respect to the production of liver damage. It should be noted however that Svibarly et al (7) reported that the acute toxicity of chlorobromomethane was considerably higher than that of either dichloromethane or carbon tetrachloride. These measurements were made 8 hours after the acute inhalational exposure to the solvents.

We initiated our investigation into the metabolism of dihalomethanes following the reports of Stewart et al. (5,6,) who showed that humans exposed to dichloromethane vapors exhibited high carboxyhemoglobin levels. Although the source of the carbon monoxide was not determined, these authors suggested that dichloromethane may be metabolized to carbon monoxide. To establish conclusively the source of carbon monoxide, we initiated experiments using rats as experimental animals. The results of these experiments conclusively demonstrate that dihalomethanes are metabolized to carbon monoxide.

EXPERIMENTAL SECTION

Animals: Male rats weighing 200 to 350 grams were employed. Dihalomethanes were injected intraperitoneally in corn oil solution. Other drugs were given intraperitoneally in either saline or corn oil solution.

Analytical Methods: Blood carbon monoxide levels were determined gas chromatographically according to the method of Collison et al (2). In some experiments, ^{13}C-labelled dichloromethane was administered and carboxyhemoglobin levels were measured both by the gas chromatographic technique and by the infrared spectrophotometric method of Barlow et al (1). Infrared spectra were recorded on a Perkin Elmer model 180 spectrophotometer from 2000 to 1850 cm^{-1} in the absorbance mode with a spectral slit width of 2.6 cm^{-1}. A calcium chloride cell with a 0.05 mm pathlength was used for the sample and a variable pathlength calcium fluoride cell containing water was used as a reference.

RESULTS

Fig. 1 shows the result of an experiment designed to establish the dose-response curve for production of carboxyhemoglobin following the administration of various doses of dichloromethane to rats. Fig. 1 shows that as the dose of dichloromethane was increased, higher levels of carboxyhemoglobin were produced and that the duration of action persisted for a longer period of time. On the basis of this study a dose of 3 mmoles/kg was chosen for use in future experiments. Fig. 2 illustrates the production of carboxyhemoglobin following the administration of diiodomethane. It can be seen here that a maximum saturation of approximately 8.5% carboxyhemoglobin was produced approximately 3 hours following the administration of diiodomethane at a dose of 3 mmoles/kg. Fig. 3 shows the production of carboxyhemoglobin following the administration of bromochloromethane. In this case maximal production of carboxyhemoglobin occurred 4 hours following the administration of the solvent and the maximal level produced was approximately 5% saturation. Fig. 4 shows the results obtained with dibromomethane. This compound produced substantially higher levels of carboxyhemoglobin than the other dihalomethanes studied, the maximum saturation being approximately 14% and occurring about 5 hours following the administration of the solvent. The following compounds were also tested and produced no elevation in blood carboxyhemoglobin levels: carbon tetrachloride, chloroform, chloromethane, carbon disulfide, methanol, formaldehyde, dimethoxymethane, Freon 11 (trichlorofluoro-

methane), and Freon 12 (dichlorodifluoromethane).

Experiments were also conducted using two known enzyme inducers, phenobarbital and 3-methylcholanthrene, to determine their effect on the metabolism of dichloromethane. Figure 5 illustrates the results obtained with phenobarbital treatment. Phenobarbital was administered intraperitoneally at a dose of 50 mg/kg for four days prior to treatment with dichloromethane. This treatment produced only minimal alterations in the metabolism of dichloromethane. Figure 6 shows the results obtained with 3-methylcholanthrene which was given in a single dose of 40 mg/kg 24 hours before administration of dichloromethane. 3-Methylcholanthrene treatment also produced no significant alteration in the metabolism of dichloromethane. The effect of the repeated administration of dichloromethane was also studied. Dichloromethane was given at a dose of 3 mmoles/kg for four days and the experiment conducted 24 hours after the last dose. As can be seen in Figure 7, the repeated administration of dichloromethane produced a substantial increase in levels of carboxyhemoglobin. A similar experiment was conducted using dibromomethane given at a dose of 3 mmoles/kg for four days. As can be seen in Figure 8, the repeated administration of dibromomethane led to no appreciable changes in levels of carboxyhemoglobin.

The effect of a known inhibitor of drug metabolism, SKF 525-A (2-diethylamino-ethyl 2,2-diphenylvalerate HCl), on the metabolism of dichloromethane was also studied. In this experiment, SKF 525-A was administered intraperitoneally at a dose of 0.1 mmoles/kg 30 minutes before the administration of dichloromethane. Figure 9 shows that SKF 525-A treatment produced only minimal alterations in the metabolism of dichloromethane.

To establish conclusively that the increased levels of carboxyhemoglobin seen after the administration of dihalomethanes were indeed derived from these compounds, experiments were conducted using ^{13}C-labelled dichloromethane. Figure 10a shows the infrared spectrum of blood from a control rat that has been gassed with ^{13}C-carbon monoxide. This blood sample showed the characteristic peaks of both ^{12}C- and ^{13}C-carbon monoxide. Figure 10b shows the infrared spectrum of blood from

given ^{13}C-dichloromethane. It can be seen here that the sample clearly shows a peak attributable to ^{13}C-carbon monoxide providing that dichloromethane was metabolised to carbon monoxide.

DISCUSSION

The results of these experiments show that of a variety of substituted methanes studied, only the dihalomethanes were metabolised to carbon monoxide in the intact rat. That the source of carbon monoxide in these cases is indeed the dihalomethanes was established conclusively by the experiments using ^{13}C-labelled dichloromethane (Figure 10b). It should be noted that these results stand in conflict with those of Heppel and Porterfield (4) who reported that stoichiometric amounts of halide ion and formaldehyde were produced from dibromomethane or bromochloromethane when these solvents were incubated with slices from various organs of rats. Further evidence that the Heppel and Porterfield pathway is not functioning is found in our observation that formaldehyde, even if it had been produced from the dihalomethanes, was not converted to carbon monoxide.

FUTURE DIRECTIONS

Future experiments concerning the metabolism of dihalomethanes will deal with the enzymatic mechanisms involved in the formation of carbon monoxide. These experiments will be conducted using isolated subcellular fractions of rat liver supplemented with co-factors.

These experiments will be monitored by following the production of carbon monoxide and release of halide ions. It is possible that carbon monoxide can be quantitated using cytochrome P-450 as a reagent. With dibromomethane as the substrate the release of inorganic bromide can be measured. However, the methods for measuring bromide ions are tedious (3). A more facile approach would be to employ ^{36}Cl-dichloromethane as a substrate. The radioactive inorganic chloride could then be measured using liquid scintillation techniques (8).

It should be noted that these proposed experiments are of considerable fundamental and practical importance. The only precedent for production of carbon monoxide in the mammalian systems is the degradation of heme by the heme oxygenase pathway. Therefore, the enzymatic mechanisms leading to the conversion of dihalomethanes to carbon monoxide are most interesting. Furthermore, the finding that a widely used chlorinated hydrocarbon solvent is converted to carbon monoxide is of considerable toxicological importance particularly in view of the widespread use of these compounds in commerce and industry.

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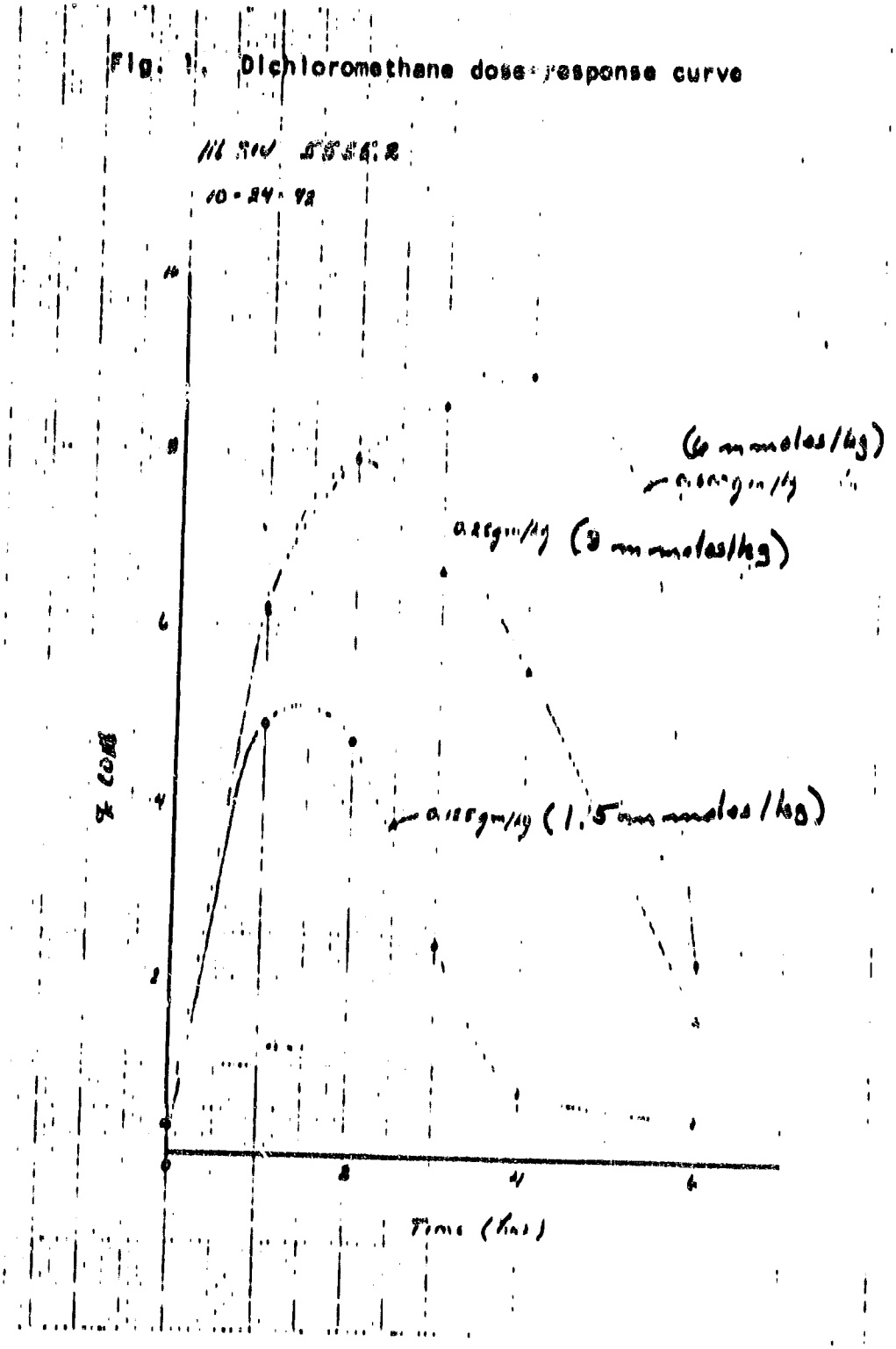
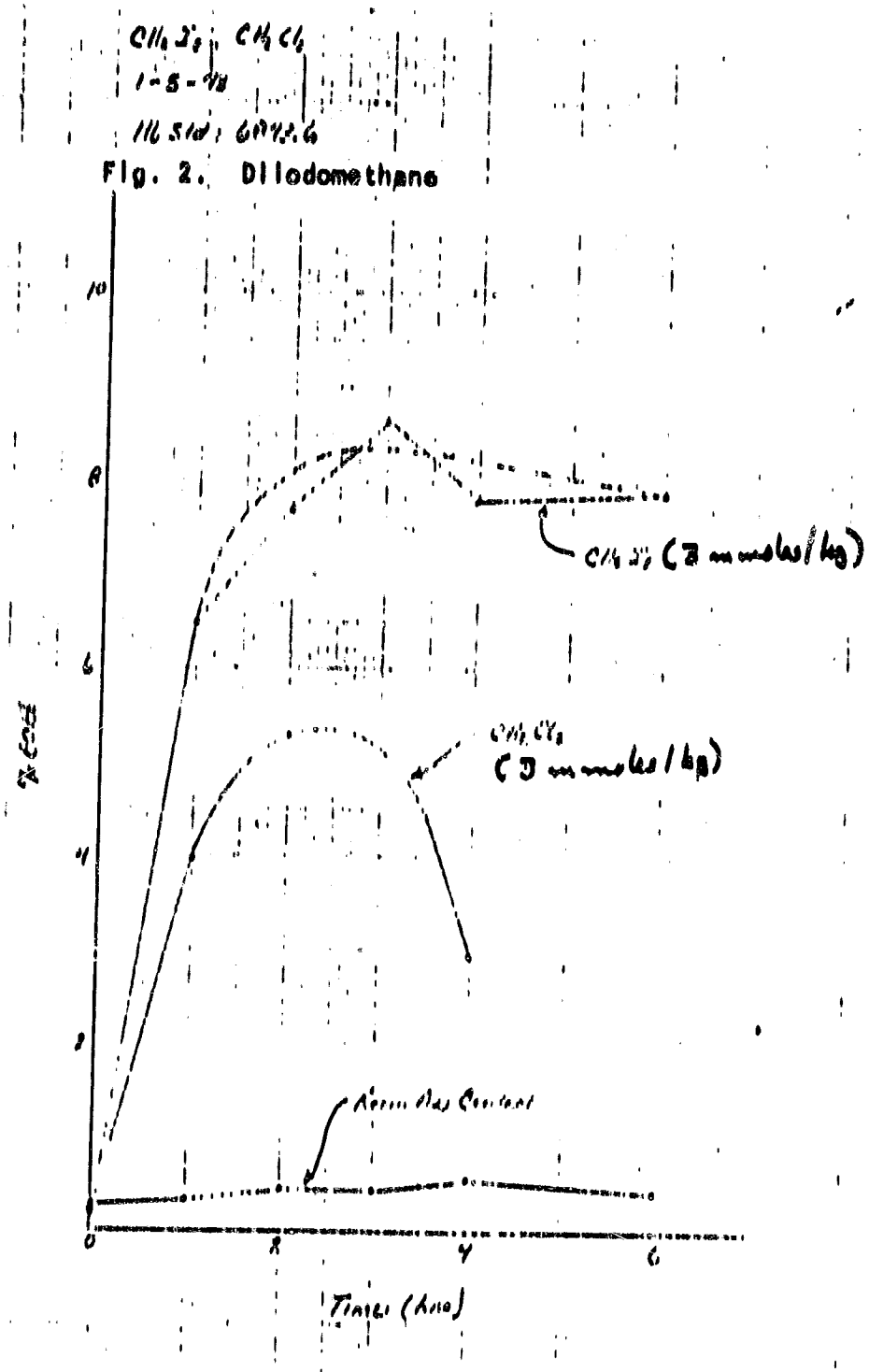
Fig. 1. Dichloromethane dose-response curve





CH_2I_2, CH_2Cl_2
1-5-78
Fig. 2. Diiodomethane
10
8
6
4
2
0
2
4
6
% Cold
Times (hrs)
CH_2I_2 (3 mmoles/kg)
CH_2Cl_2 (3 mmoles/kg)
Room Air Content

CH_2ClBr, CH_2Cl_2

E 21-78

[illegible]

Fig. 3. Bromochloromethane

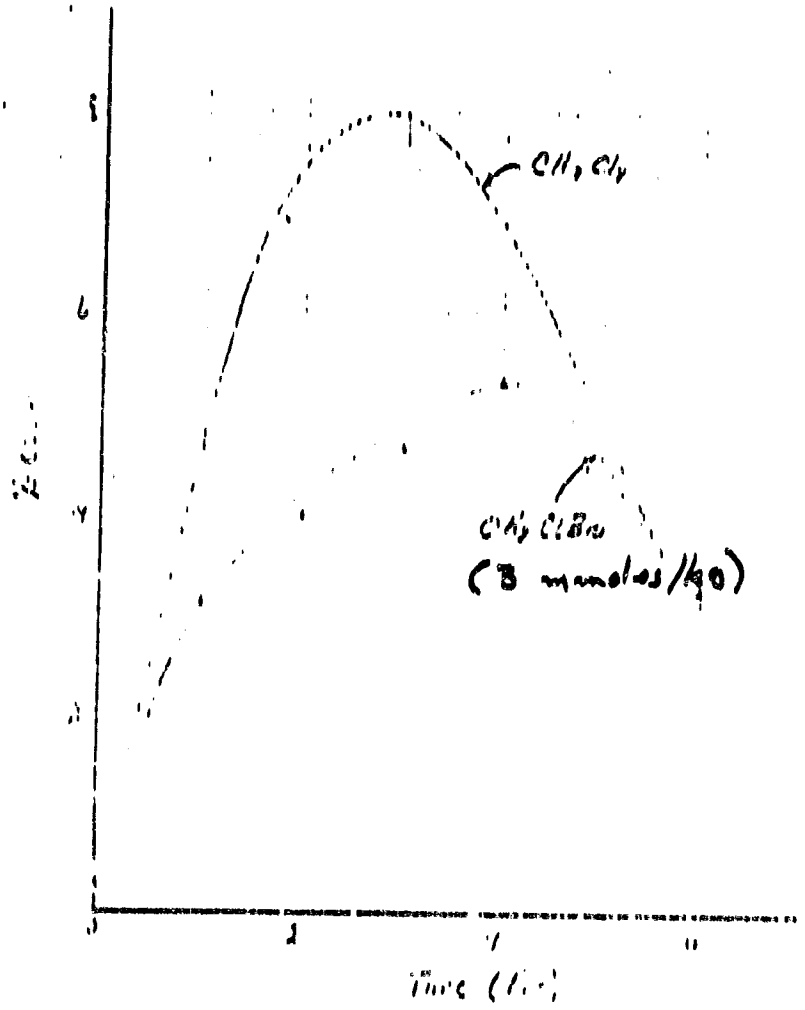


Fig. 4. Dibromomethane

CH_2Br_2, $CHCl_3$, Power Air Control

4-9-78

[illegible] (0-6 hrs); [illegible] (6-10 hrs)

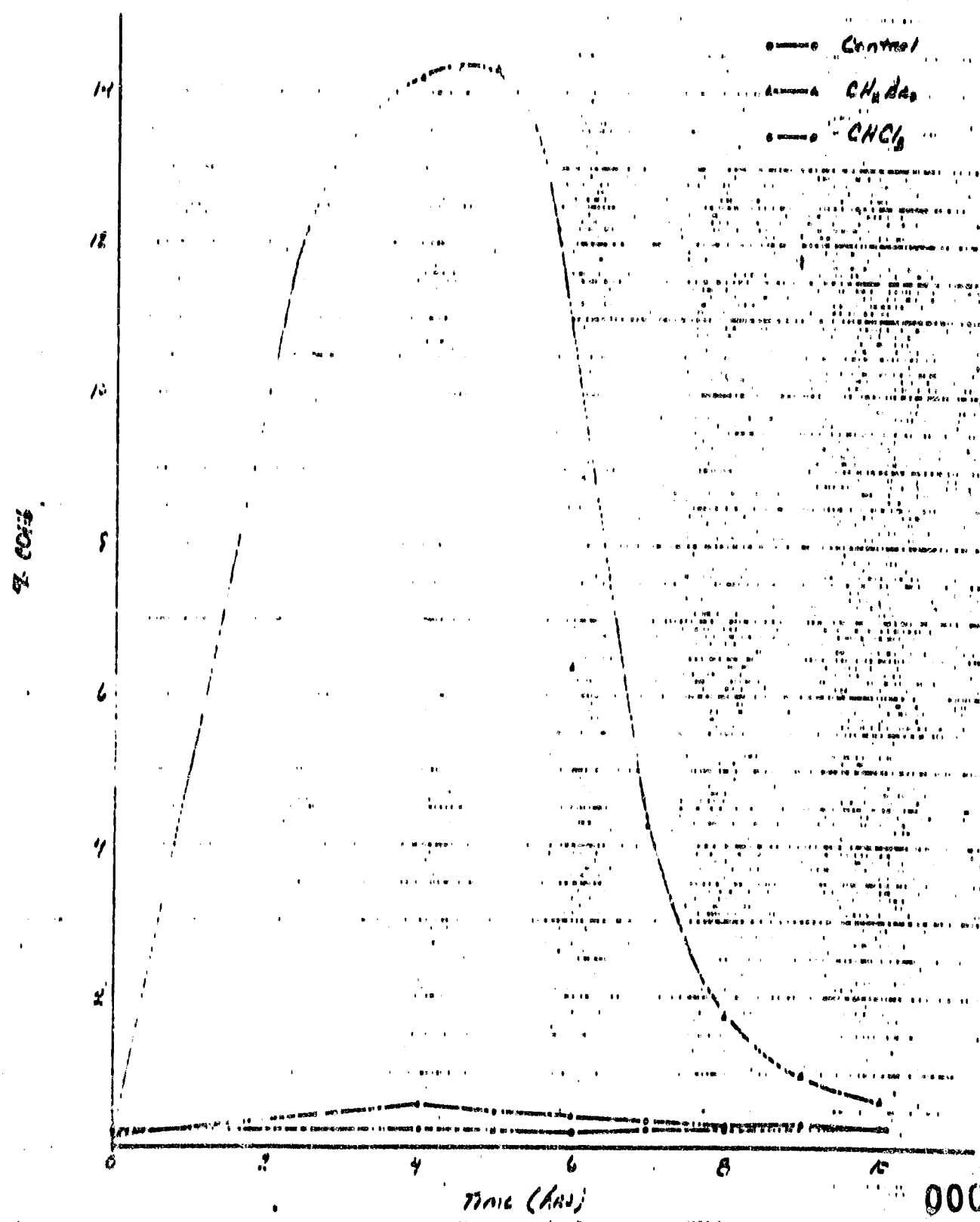


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Fig. 5. Effect of phenobarbital treatment.

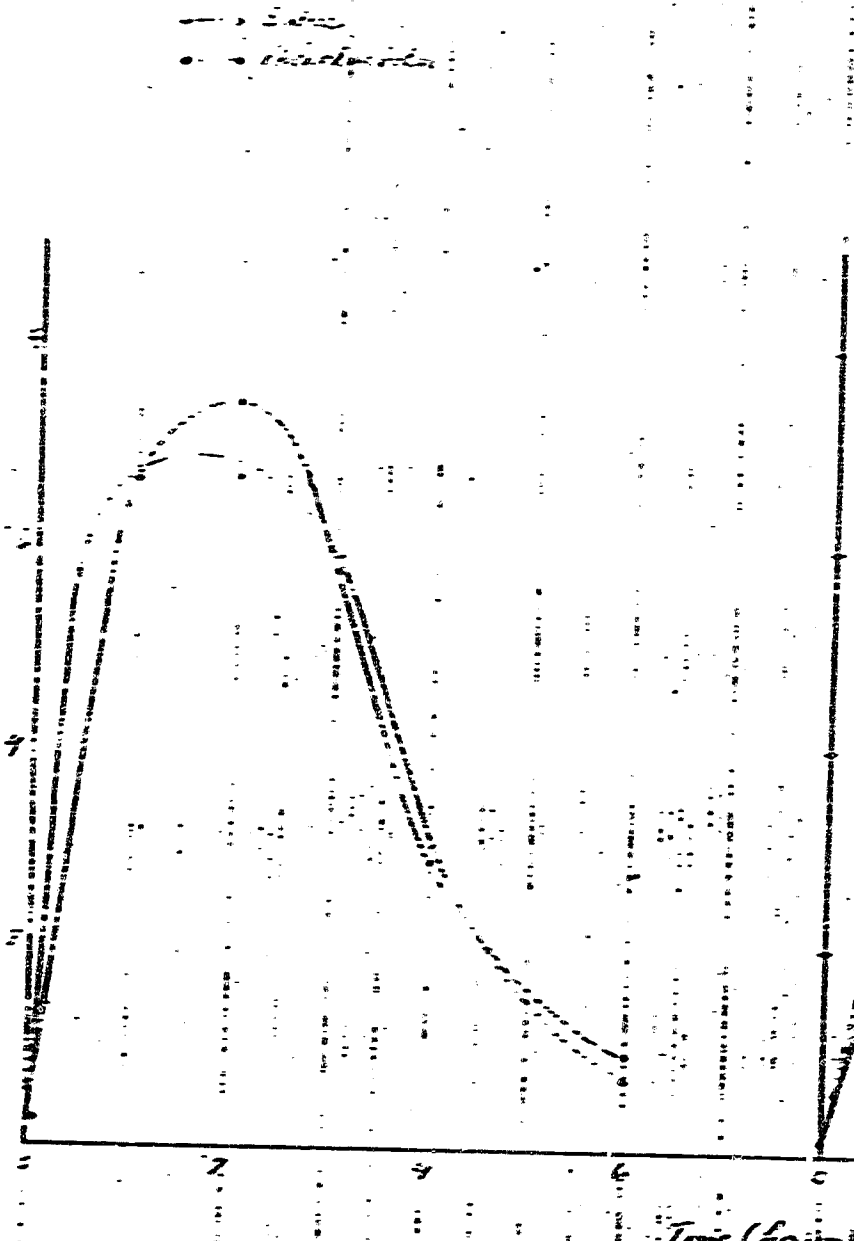
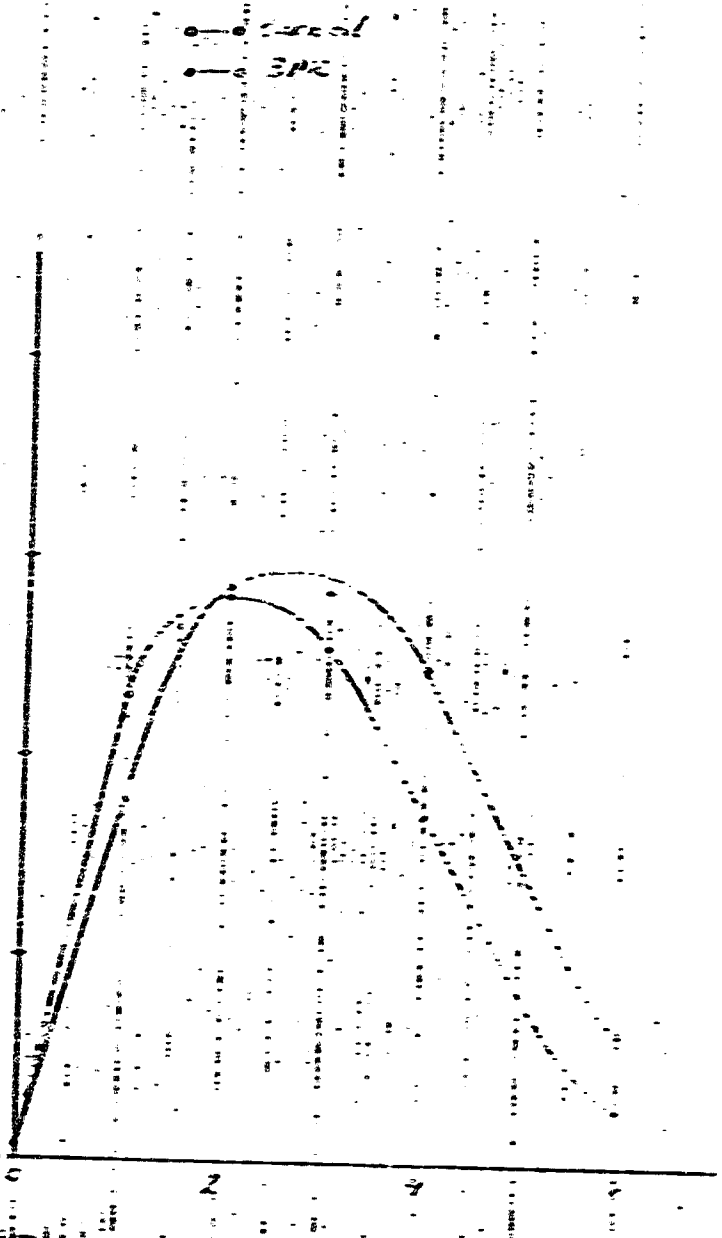


Fig. 6. Effect of 3-methylcholanthrene treatment.



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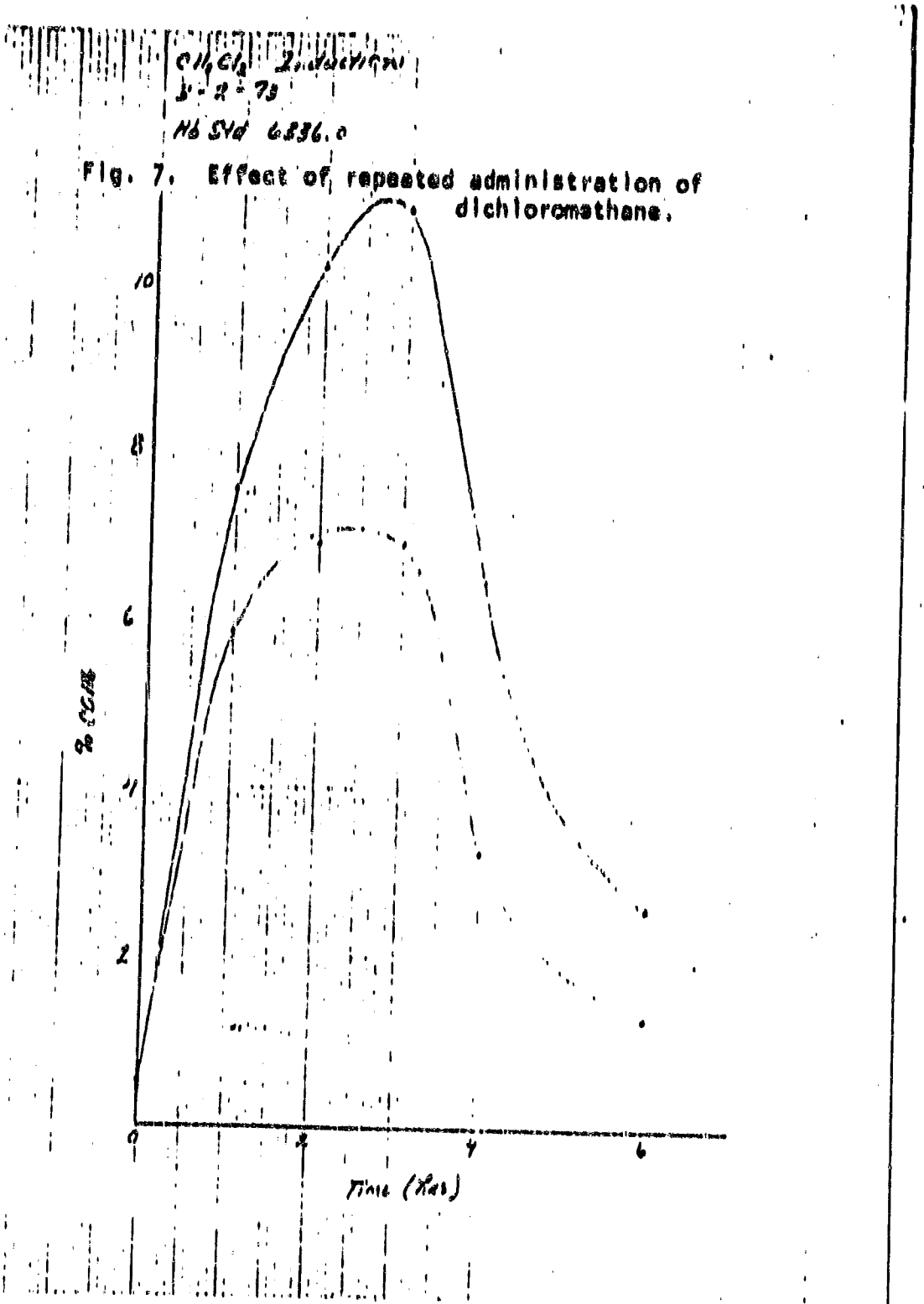


Fig. 7. Effect of repeated administration of dichloromethane.

Fig. 8. Effect of repeated administration of dibromomethane.

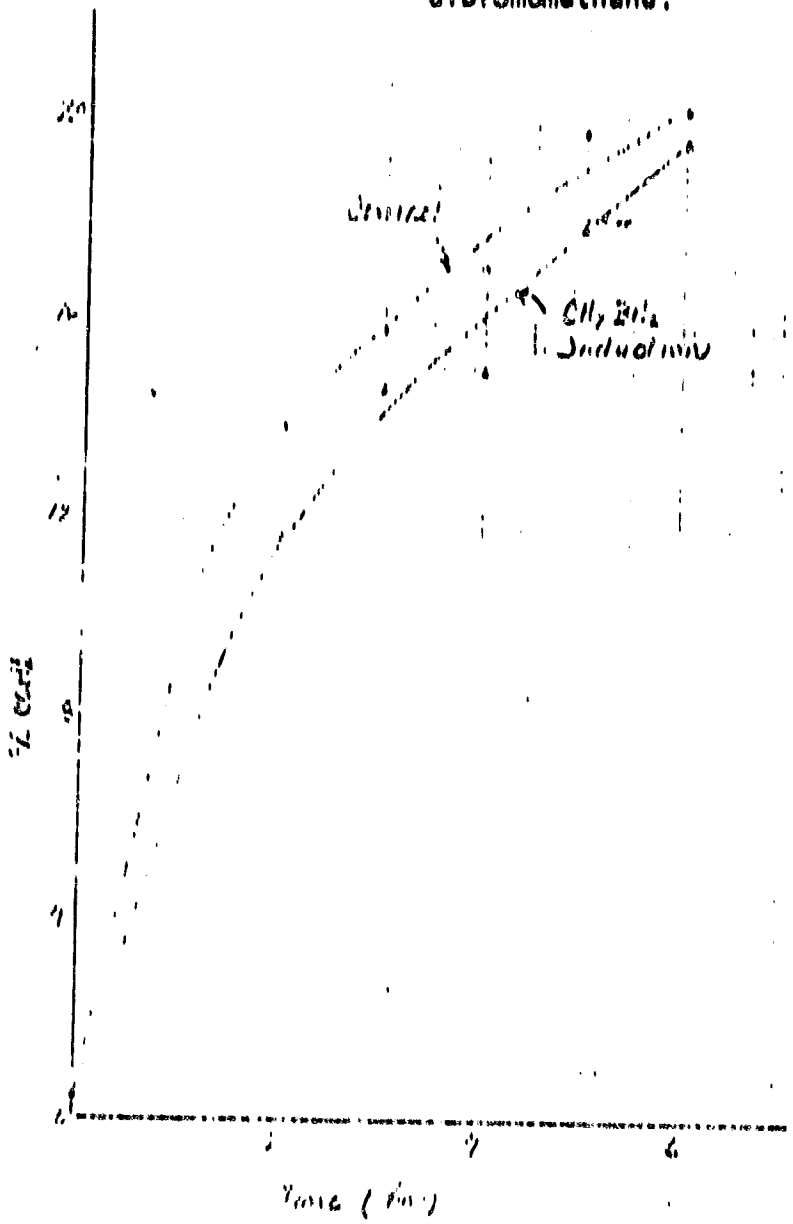
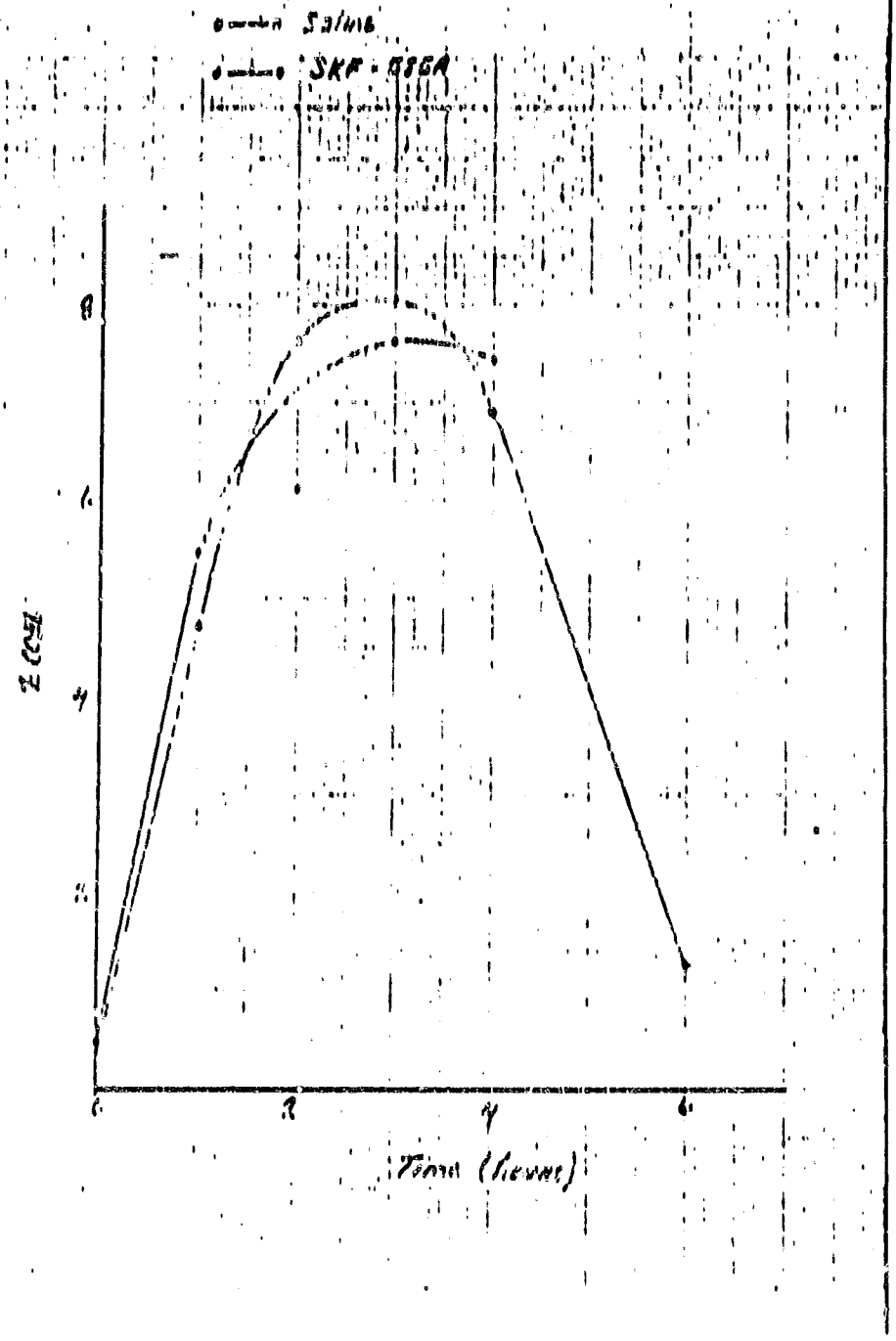
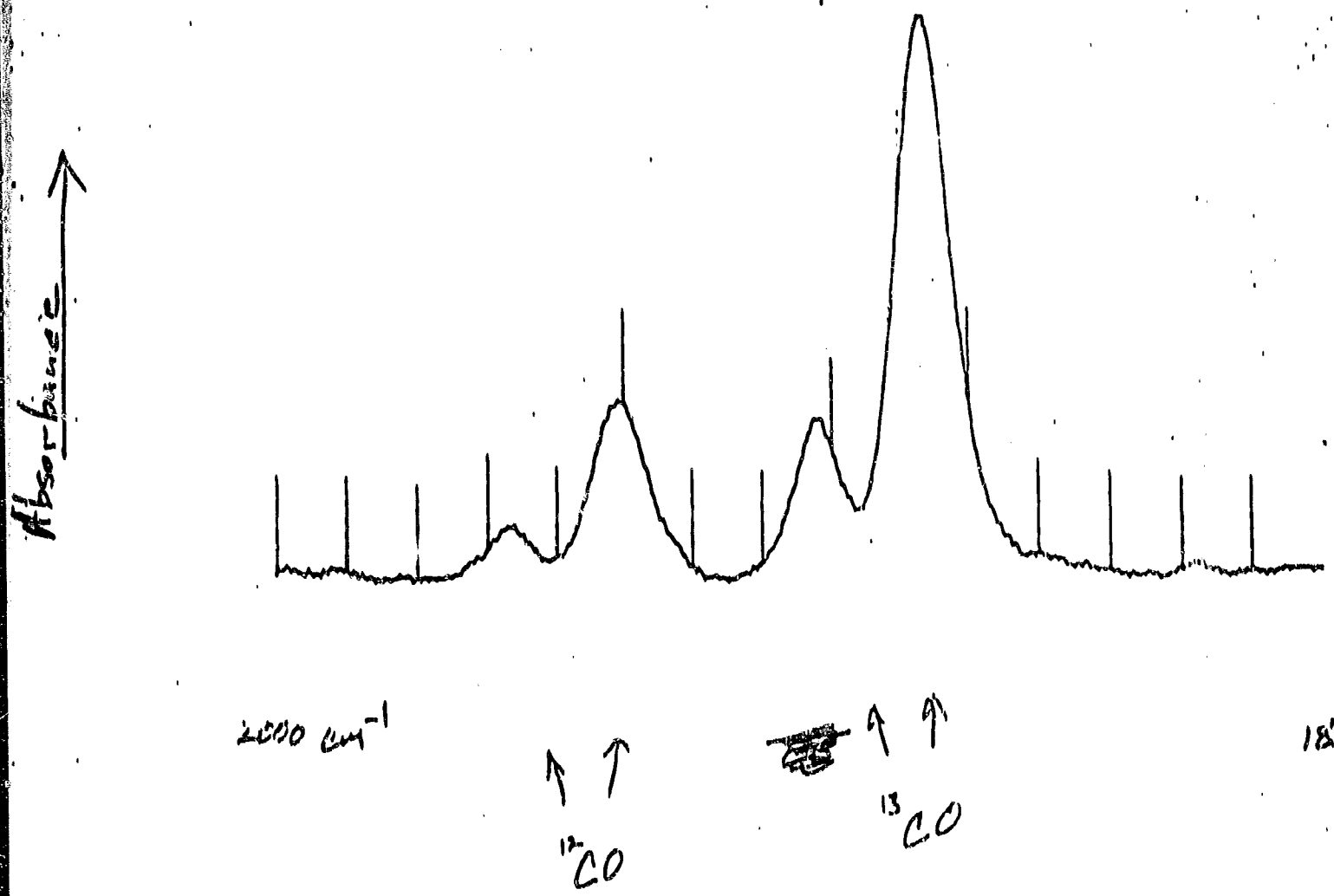


Fig. 9. Effect of SKF 525-A treatment on the metabolism of dichloromethane.



^{13}CO treated rat

Fig. 10.a. Infrared spectrum of blood gassed with ^{13}C-carbon monoxide.



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MARKERS ARE 10 cm^{-1}

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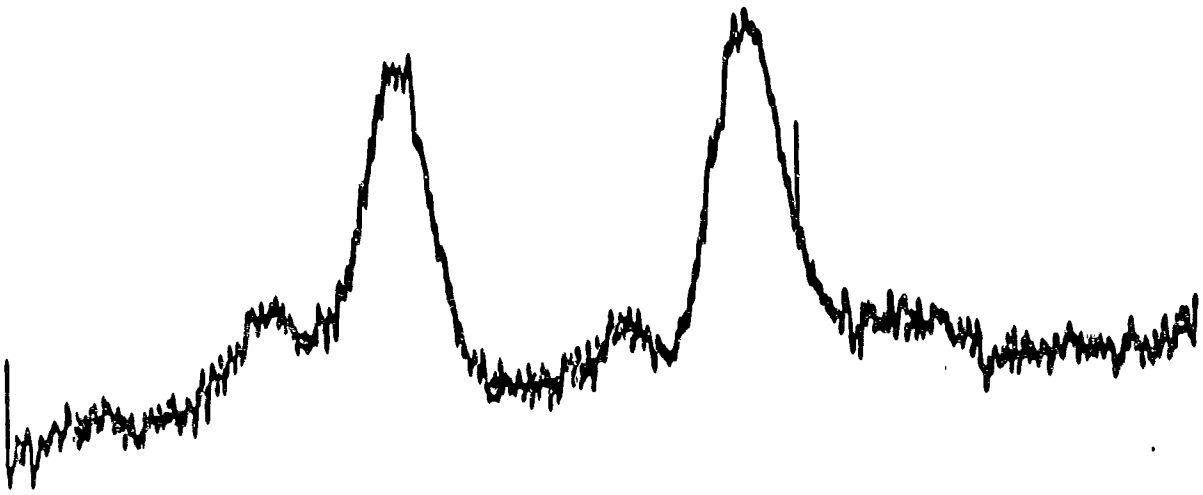
Fig. 10.b. Infrared spectrum of blood from rat given ^{13}C-dichloromethane.



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Doc Type: • RI • UP • HEAS D 8D SU HS FN

Doc Title: 26-WEEK PROGRESS REPORT 27-MONTH CHRONIC TOXICITY AND ONCOGENICITY STUDY PREPARED BY HAZLETON LABS AMER INC

Chemical Name (300 per name)	CAS No. (10)
METHYLENE CHLORIDE	75-Ø9-2

LK 61

1A

8792111824
D-640

HAZLETON
LABORATORIES AMERICA, INC.

SPONSOR: National Coffee Association

DATE: May 28, 1980

MATERIAL: Methylene Chloride

SUBJECT: 26-WEEK PROGRESS REPORT
27-Month Chronic Toxicity and Oncogenicity Study
Project No. 2112-101

RECEIVED

SUMMARY

This report presents the findings from the first twenty-six weeks of a 27-month study designed to evaluate the chronic toxicity and carcinogenic potential of methylene chloride when administered in the drinking water to Fischer 344 rats (Charles River Breeding Laboratories, Portage, Michigan). The study was initiated on September 24-25, 1979 (for technical convenience the males were initiated on September 24 and the females on September 25), and completed twenty-six weeks of treatment as of March 24, 1980. Terminal sacrifice is scheduled for December of 1981.

Suppression of weight gain was noted between Weeks 0 through 6 and Weeks 0 through 13 in male Groups 5, 6, and 7 as compared to Group 1. Suppression of weight gain was also noted during Weeks 0 through 13 in male Groups 6 and 7 as compared to Group 2. These findings were not apparent by Week 26. Food consumption by male Groups 2, 3, 4, 5, 6, and 7 was significantly lower than Group 1; male Groups 4, 5, and 6 were significantly lower than Group 2. Mean water consumption by Group 5 and 6 animals of both sexes were consistently lower than Groups 1 and 2. Relative adrenal weights were somewhat elevated in a dose-related pattern in treated groups of both sexes.

MALES

TABLE 1 - CONTINUED

GROUP		1	2	3	4	5
LEVEL		.00	.00	5.00	50.00	100.00
		******	******	******	******	******
WEEKS ON STUDY						
**********						
20	MEAN (GRAMS)	357.7	350.0	359.4	343.7	343.9
	STD.DEV.	18.88	17.95	18.48	21.94	22.77
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	88/ 88
21	MEAN (GRAMS)	361.8	350.8	359.7	359.8	349.4
	STD.DEV.	18.03	16.78	18.45	22.79	22.78
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	84/ 88
22	MEAN (GRAMS)	368.8	357.8	358.7	355.3	353.8
	STD.DEV.	18.18	17.33	18.82	23.38	22.87
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	84/ 88
23	MEAN (GRAMS)	369.8	363.1	363.2	358.7	358.7
	STD.DEV.	18.11	17.88	18.88	23.83	23.87
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	84/ 88
24	MEAN (GRAMS)	371.3	363.8	364.4	360.4	358.7
	STD.DEV.	18.49	17.87	18.88	23.94	23.83
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	84/ 88
25	MEAN (GRAMS)	373.8	367.7	366.8	362.8	361.7
	STD.DEV.	19.01	17.88	17.74	23.18	23.86
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	84/ 88
26	MEAN (GRAMS)	378.8	369.8	369.8	364.8	363.9
	STD.DEV.	18.61	17.48	18.81	23.48	24.13
	ADJUSTED SURVIVAL	84/ 88	88/ 88	84/ 88	84/ 84	84/ 88

TABLE 1 - CONTINUED

GROUP		MALES	
		0	1
LEVEL		250.00	250.00
		******	******
AGENTS IN STUDY			
	MEAN (GRAMS)	333.6	339.5
00	STD.DEV.	22.83	20.49
	ADJUSTED SURVIVAL	94/ 98	89/ 89
	MEAN (GRAMS)	341.3	343.7
01	STD.DEV.	23.34	21.16
	ADJUSTED SURVIVAL	94/ 98	89/ 89
	MEAN (GRAMS)	344.3	346.9
02	STD.DEV.	23.67	21.84
	ADJUSTED SURVIVAL	94/ 98	89/ 89
	MEAN (GRAMS)	347.2	349.4
03	STD.DEV.	24.81	21.38
	ADJUSTED SURVIVAL	94/ 98	89/ 89
	MEAN (GRAMS)	348.7	351.0
04	STD.DEV.	23.14	21.40
	ADJUSTED SURVIVAL	94/ 98	89/ 89
	MEAN (GRAMS)	352.4	356.6
05	STD.DEV.	25.40	23.08
	ADJUSTED SURVIVAL	94/ 98	89/ 89
	MEAN (GRAMS)	358.2	359.5
06	STD.DEV.	23.67	21.84
	ADJUSTED SURVIVAL	94/ 98	89/ 89

FEMALES

TABLE 1 - CONTINUED

GROUP		1	2	3	4	5
LEVEL		.30	.90	9.00	90.00	135.00
WEEKS ON STUDY						
20	MEAN (GRAMS)	205.4	205.8	206.1	204.3	201.3
	STD.DEV.	12.42	10.86	10.89	12.40	8.36
	ADJUSTED SURVIVAL	88/ 88	93/ 90	88/ 88	88/ 88	88/ 88
81	MEAN (GRAMS)	206.0	207.8	207.6	205.8	204.7
	STD.DEV.	12.66	9.89	10.17	12.32	8.29
	ADJUSTED SURVIVAL	88/ 88	92/ 90	88/ 88	88/ 88	88/ 88
88	MEAN (GRAMS)	208.8	209.6	209.8	206.8	205.1
	STD.DEV.	12.74	9.68	10.45	12.40	8.07
	ADJUSTED SURVIVAL	88/ 88	93/ 88	88/ 88	88/ 88	88/ 88
83	MEAN (GRAMS)	209.8	210.6	210.6	208.8	207.8
	STD.DEV.	12.79	9.36	10.41	12.99	9.99
	ADJUSTED SURVIVAL	88/ 88	90/ 93	88/ 88	88/ 88	88/ 88
84	MEAN (GRAMS)	209.4	210.7	210.4	208.8	207.4
	STD.DEV.	13.40	9.84	10.85	11.97	8.83
	ADJUSTED SURVIVAL	88/ 88	90/ 95	88/ 88	88/ 88	88/ 88
89	MEAN (GRAMS)	210.6	210.8	211.0	211.0	210.3
	STD.DEV.	12.73	9.80	10.35	12.12	8.86
	ADJUSTED SURVIVAL	88/ 88	90/ 90	88/ 88	88/ 88	88/ 88
86	MEAN (GRAMS)	210.8	210.1	211.9	211.8	210.7
	STD.DEV.	12.48	9.38	10.01	11.97	8.48
	ADJUSTED SURVIVAL	88/ 88	90/ 90	88/ 88	88/ 88	88/ 88

FEMALES TABLE 1 - CONTINUED

GROUP		5	7
LEVEL		250.00	500.00
WEEKS ON STUDY			
19	MEAN (GRAMS)	300.4	309.2
	STD.DEV.	11.22	12.32
	ADJUSTED SURVIVAL	84/ 88	26/ 29
21	MEAN (GRAMS)	302.4	308.4
	STD.DEV.	11.43	12.40
	ADJUSTED SURVIVAL	84/ 88	26/ 29
23	MEAN (GRAMS)	303.1	303.8
	STD.DEV.	11.16	11.46
	ADJUSTED SURVIVAL	84/ 88	26/ 29
25	MEAN (GRAMS)	304.1	304.1
	STD.DEV.	10.79	10.83
	ADJUSTED SURVIVAL	84/ 88	26/ 29
28	MEAN (GRAMS)	305.8	306.3
	STD.DEV.	11.01	11.29
	ADJUSTED SURVIVAL	84/ 88	26/ 29
29	MEAN (GRAMS)	307.8	307.8
	STD.DEV.	10.66	10.82
	ADJUSTED SURVIVAL	84/ 88	26/ 29
36	MEAN (GRAMS)	307.5	308.7
	STD.DEV.	10.82	11.79
	ADJUSTED SURVIVAL	84/ 88	26/ 29

MALES TABLE 1 - CONTINUED

GROUP		1	2	3	4	5
LEVEL		.00	.00	5.00	50.00	105.00
WEEKS ON STUDY						
10	MEAN (GM/WEEK)	126.2	125.7	123.1	122.9	126.6
	STD.DEV.	7.91	7.38	10.84	10.46	9.39
	ALL SURVIVORS	85	80	85	84	85
11	MEAN (GM/WEEK)	124.1	125.0	123.3	122.8	126.7
	STD.DEV.	8.17	8.74	10.97	10.59	9.82
	ALL SURVIVORS	85	80	85	84	85
12	MEAN (GM/WEEK)	121.3	125.0	121.3	122.6	125.8
	STD.DEV.	8.34	8.11	10.63	10.58	9.36
	ALL SURVIVORS	85	80	85	84	85
13	MEAN (GM/WEEK)	123.3	125.4	121.7	125.1	126.1
	STD.DEV.	8.67	8.29	11.41	11.59	9.46
	ALL SURVIVORS	85	80	85	84	85
	TOTAL MEAN	1743.2	1719.0 $^{S=}$	1685.1 $^{S=}$	1652.6 $^{S=,0=}$	1679.5 $^{S=,0=}$
	S.D.	109.3	95.9	133.3	143.9	115.8

TABLE 1 - CONTINUED

		MALES	
GROUP		6	7
LEVEL		250.00	250.00
WEEKS IN STUDY			
10	MEAN (GM/WEEK)	133.9	128.0
	STD.DEV.	11.63	12.05
	ALL SURVIVORS	05	05
11	MEAN (GM/WEEK)	130.4	117.3
	STD.DEV.	18.64	10.02
	ALL SURVIVORS	05	05
12	MEAN (GM/WEEK)	128.9	110.0
	STD.DEV.	12.71	8.16
	ALL SURVIVORS	05	05
13	MEAN (GM/WEEK)	121.8	110.0
	STD.DEV.	10.30	8.94
	ALL SURVIVORS	04	05
TOTAL MEAN		1617.7	1532.8
S.D.		144.3	148.8

FEMALES TABLE 3 - CONTINUED

GROUP		1	2	3	4	5
LEVEL		.00	.00	5.00	10.00	100.00
WEEKS ON STUDY						
10	MEAN (GM/WEEK)	88.9	89.8	91.3	89.8	92.8
	STD.DEV.	12.18	7.88	10.81	8.92	8.46
	ALL SURVIVORS	88	90	89	88	88
11	MEAN (GM/WEEK)	88.7	89.9	90.4	91.8	92.6
	STD.DEV.	6.80	6.87	9.82	6.82	6.56
	ALL SURVIVORS	88	90	88	88	88
12	MEAN (GM/WEEK)	86.6	89.9	91.1	93.2	91.7
	STD.DEV.	14.73	6.92	9.78	6.86	7.68
	ALL SURVIVORS	88	90	88	88	88
13	MEAN (GM/WEEK)	88.8	93.8	88.3	90.8	90.8
	STD.DEV.	8.98	7.79	20.89	9.48	7.88
	ALL SURVIVORS	88	90	88	88	88
	TOTAL MEAN	1253.2	1246.8	1249.1	1237.9	1239.8
	S.D.	137.7	104.1	116.1	114.8	109.2

FEMALES TABLE 3 - CONTINUED

GROUP		6	7
LEVEL		250.00	500.00
WEEKS ON STUDY			
10	MEAN (GM/WEEK)	94.3	87.6
	STD.DEV.	9.84	6.90
	ALL SURVIVORS	86	28
11	MEAN (GM/WEEK)	91.6	79.1
	STD.DEV.	9.38	16.83
	ALL SURVIVORS	86	28
12	MEAN (GM/WEEK)	86.3	91.2
	STD.DEV.	7.98	8.68
	ALL SURVIVORS	86	28
13	MEAN (GM/WEEK)	87.6	89.9
	STD.DEV.	7.88	8.79
	ALL SURVIVORS	86	28
TOTAL MEAN		1221.2	1109.8
S.D.		111.1	83.6

Table 3
Mean Water Consumption Values
27-Month Chronic Toxicity and Oncogenicity Study

Rats

Week	Dose Level (mg/kg/day):	Milliliters/Week/Rat						
	Groups:	1	2	3	4	5	6	7
		0	0	5	50	125	250	250
1		188.4	176.5	177.7	168.6	165.4	174.5	151.1
2		189.1	185.2	179.4	173.4	172.5	162.0	157.6
3		191.9	185.8	181.0	175.3	169.2	144.6	177.6
4		186.4	177.6	179.9	170.1	165.9	160.6	126.5
5		187.6	177.7	177.3	174.0	166.2	161.2	170.1
6		182.9	171.4	171.7	170.0	166.7	162.2	162.6
7		181.0	173.5	169.7	168.5	157.9	161.1	170.6
8		175.8	158.1	166.2	162.7	152.6	152.6	161.1
9		175.6	171.6	168.9	171.6	156.6	155.1	161.1
10		177.7	162.0	161.6	166.1	155.0	151.0	149.9
11		175.9	159.5	166.1	166.7	155.8	156.1	165.8
12		171.0	165.6	166.2	162.1	150.1	152.8	166.8
13		169.2	156.6	158.9	158.1	156.7	162.6	168.1
14		165.2	155.0	158.2	157.7	160.9	170.6	161.9
15		167.6	155.8	167.7	155.6	176.6	172.9	167.8
16		161.9	157.5	165.5	155.6	160.0	170.8	169.9
17		169.0	158.9	161.6	156.6	151.7	161.3	158.9
18		160.1	159.2	166.7	160.1	160.5	165.9	172.1
19		170.0	161.8	166.1	170.6	170.0	172.7	173.1
20		167.1	161.5	165.0	169.0	161.1	159.0	151.1
21		165.8	162.0	161.6	171.9	160.9	157.6	155.6
22		160.6	160.2	167.0	166.5	166.2	152.0	151.9
23		170.5	171.2	171.7	166.1	165.2	155.6	157.6
24		166.6	166.7	166.6	160.1	166.2	157.6	155.6
25		175.0	177.5	172.0	171.9	161.1	156.5	156.6
26		170.0	176.9	169.9	171.2	166.1	161.6	166.1
Weeks 1-4	Mean	187.7	179.5	177.1[a]	170.6[a]	167.1[a,b]	162.0[a,b]	171.1
	S.D.	3.0	6.2	3.6	5.1	3.0	3.6	11.1
Weeks 1-13	Mean	181.6	173.1	170.6[a]	167.4[a]	161.1[a,b]	157.6[a,b]	167.1[a]
	S.D.	7.0	8.6	7.1	5.6	6.1	6.7	11.1
Weeks 1-26	Mean	175.1	168.6	167.0	166.2[a]	156.6[a,b]	153.6[a,b]	158.6[a,b]
	S.D.	8.6	9.1	9.1	6.1	10.7	8.1	10.2

Table 1 - Continued
Mean Water Consumption Values
22-Month Chronic Toxicity and Oncogenicity Study

Females

Week	Group: Dose Level (mg/kg/day):	1 0	2 8	3 [illegible]	4 40	5 200	6 [illegible]	7 [illegible]
		Milliliters/Week/Rat						
1		150.8	149.9	141.[illegible]	148.5	138.8	131.0	129.5
2		146.3	149.4	142.9	135.1	136.7	132.2	130.7
3		148.7	146.7	142.5	138.0	136.9	132.1	132.0
4		146.6	145.7	146.6	137.5	132.7	131.7	131.1
5		148.9	146.8	142.6	138.2	137.0	135.5	131.0
6		145.1	146.7	141.7	138.1	133.1	126.9	128.1
7		138.2	137.9	134.0	135.0	127.2	131.0	126.5
8		136.4	138.2	136.5	129.9	125.3	127.3	124.8
9		148.0	145.9	142.2	137.2	136.4	132.3	141.8
10		142.3	142.9	144.3	136.6	136.0	136.8	145.4
11		150.4	143.2	144.8	135.6	138.1	139.7	148.2
12		144.3	142.2	142.1	138.4	138.2	135.8	150.6
13		132.0	133.7	136.4	136.6	137.5	136.8	148.2
14		143.8	149.2	148.4	144.8	141.6	137.6	152.1
15		141.6	142.5	142.7	142.1	136.4	136.2	142.7
16		142.1	148.5	145.5	148.1	139.6	135.3	141.4
17		149.0	147.5	148.2	141.0	141.6	136.1	138.9
18		144.3	143.6	142.1	144.1	148.5	136.5	138.1
19		146.2	144.7	144.8	142.2	138.0	136.0	135.2
20		152.2	152.6	151.1	144.3	141.1	132.5	148.4
21		146.5	150.1	139.1	141.6	141.9	133.7	138.9
22		152.4	149.2	151.4	150.3	146.5	141.0	145.5
23		150.5	156.3	151.0	150.9	144.9	141.3	145.5
24		154.2	148.9	151.9	151.3	145.1	145.9	150.9
25		145.4	148.1	155.9	156.7	150.7	145.1	148.2
26		142.6	145.8	151.6	156.5	150.1	99.6	144.6
Weeks 1-6	Mean	147.4	147.3	142.6[illegible]	141.2	135.8[illegible]	136.1[illegible]	131.1[illegible]
	S.D.	1.8	2.4	1.6	8.2	2.5	1.9	1.9
Weeks 1-13	Mean	146.8	146.8	141.3	137.7	136.6[illegible]	135.8[illegible]	136.9
	S.D.	5.5	6.9	6.2	6.6	6.1	2.3	7.5
Weeks 1-26	Mean	148.2	148.5	146.2	142.6	138.8[illegible]	135.8[illegible]	140.8[illegible]
	S.D.	7.8	7.5	6.5	7.7	6.8	8.1	7.6

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Chemical Name (300 per name) 25	METHYLENE CHLORIDE
CAS No. (10) 24	75-09-2

D-6441 87821/825

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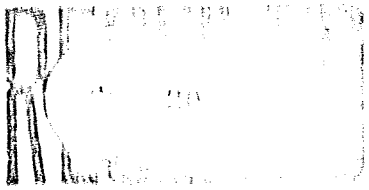

REPORT TO THE

NATIONAL COFFEE ASSOCIATION, INC.

July 19, 1978

000002

Arthur D Little Inc

BALB/c-3T3 NEOPLASTIC TRANSFORMATION ASSAY WITH METHYLENE CHLORIDE*

The BALB/c-3T3 neoplastic transformation assay is designed to measure the ability of chemical agents to induce alterations in a population of mammalian cells from a pattern of controlled monolayer growth to one exhibiting foci of disoriented, piled-up growth against a backgound monolayer. In general, the altered cells will grow in soft agar and produce tumors when injected into appropriate hosts so that a positive result in the cell culture assay system is a presumptive test for *in vivo* carcinogenicity. A negative test in this system would indicate a low probability of a positive response *in vivo* if the compound is a direct-acting agent or is converted biochemically to an active agent in cell culture as it is in the intact animal.

Experimental Procedures

The assay is performed generally as described by Kakunaga (Int. J. Cancer, 12:463, 1973). The cells employed in this assay are the 1-13 line of BALB/c-3T3 cells isolated by Kakunaga and transmitted to this laboratory in January, 7. The medium is Eagle's minimal essential medium with ten percent fetal calf serum; penicillin and streptomycin are employed at 50 units/ml and 50 µg/ml, respectively. Cells for the transformation assay are plated from frozen stocks at 10^4 cells per 60 mm Falcon plate. Twenty-four hours later, test material is added to the appropriate plates after direct dilution in culture medium. After the seventy-two hour treatment, the media containing the test chemical are removed, the plates washed and incubated for an additional four weeks. The medium, which is renewed twice weekly during this interval, does not contain any of the test chemical. The plates are then fixed with methanol stained with May-Grumwald-Giemsa, and Type III foci (Reznikoff *et al.*, Cancer Research, 33:3239, 1973) are counted in each plate using a dissection microscope. The impurities present in the sample of food grade

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* Food grade test specification

methylene chloride that we tested are shown in Table 1.

Results and Discussion

Exposure of BALB/c-3T3 cells for 72 hours to food grade methylene chloride did not result in a dose related increase in induction of Type III transformed foci with respect to either frequency of Type III foci per plate, the fraction of plates with Type III foci as compared to historical cumulative results with untreated controls (Table 2). The untreated controls in this experiment yielded substantially lower values for spontaneous transformation than the mean of cumulative control values that we have obtained over the last 18 months and are among the lowest we have ever observed.

The strong positive response with 3-methylcholanthrene (MCA) confirms that the cells were responsive with respect to transformation and that they were able to metabolize polycyclic aromatic hydrocarbons.

Our findings that food grade methylene chloride at a wide range of concentrations does not induce neoplastic transformation in BALB/c-3T3 cells are in contrast to those of Price et al. (In Vitro, 14:290-293, 1978) who reported transformation of a Fischer rat cell line chronically infected with Rauscher leukemia virus using a methylene chloride sample obtained from Fisher Scientific at concentrations of 1.6×10^{-3}M and 1.6×10^{-4}M.

The different responses in the two experiments may be due to the levels of impurities present in each of the samples. Support for this view comes from the finding by Price (personal communication) that no transformation was induced in cells of the virus-infected rat cell line by a methylene chloride sample identical to the one used in our test with BALB/c-3T3 cells.

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Table 1

Chemical Assay of Impurities Present in Food Grade Methylene Chloride Sample

Impurities	ppm
Cyclohexane	305
trans-Dichloroethylene	86
Vinylidene Chloride	33
Methyl Bromide	11
Chloroform	<10
Carbon Tetrachloride	< 5
Ethyl Chloride	3
Methyl Chloride	< 1
Trichloroethylene	< 1
Vinyl Chloride	< 1

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Table 2

Neoplastic Transformation Studies of Methylene Chloride†

		Methylene Chloride†				MCA	Dose Calculation
		0.00001	0.0001	0.001	0.01	0.0002	%**
		1.56×10^{-6}	1.56×10^{-5}	1.56×10^{-4}	1.56×10^{-3}	10^{-5}	Molarity
	Control*	0.13	1.33	13.3	133	2.0	µg/ml
Total Foci / Total Plates	2/18	5/18	8/17	2/18	6/15	48/13	
Foci per Plate ± Std. Error of Mean	0.11 ± 0.07	0.28 ± 0.14	0.47 ± 0.15	0.11 ± 0.08	0.40 ± 0.13	3.69 ± 0.52	
Total Plates with Foci / Total Plates	3/19	4/18	7/17	2/18	6/15	13/13	
Ratio	0.16	0.22	0.41	0.11	0.40	1.00	
Cell Survival T/C (3-day exposure)		0.60	0.58	0.71	0.66	0.29	

Comments: *One dish with multiple foci in this set; not possible to count. Historical negative control data based on 836 sample observations over a time period of 14 months are 0.39 foci/plate ± 0.01 and a ratio of total plates with foci/total plates of 0.26.

**Percent calculated as V/V for methylene chloride, W/V for 3-methylcholanthrene (MCA).

† Food grade test specification.

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Doc Title "SMOKERS' POLYCYTHEMIA" 23

Chemical Name (300 per name)	25	CAS No. (10)	24
METHYLENE CHLORIDE		75-09-2	

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"SMOKERS' POLYCYTHEMIA"

As part of our analysis of the laboratory data from the $MeCl_2$ exposure study we had occasion to evaluate the phenomenon of compensatory polycythemia known to occur in heavy smokers. There has been speculation that this reaction is not, as commonly believed, due to carbon monoxide effects, but rather to the irritative effect of some other smoke constituents on the lung.

The metabolism of $MeCl_2$ to CO causes a substantial increase in the body burden of CO without any interference from other metabolites. It would be expected, therefore, that if CO causes polycythemia this phenomenon would be much more pronounced in the study plant. The attached table shows that this is not the case. While there is a small, but significant, difference between smokers and nonsmokers, there is no difference between the two plants.

Thus we can conclude that $MeCl_2$ exposure does not cause or contribute to "smokers' polycythemia", an effect that is of great concern regarding the long-term effect of smoking on the cardiovascular system.

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TABLE: HEMOGLOBIN CONCENTRATION, GROUP MEAN COMPARISON

	Control Plant	Study Plant	Diff.	p
Female Nonsmoker	13.87	13.83	0.04	NS
Female Smoker	14.36	14.34	0.02	NS
Diff.	0.49	0.51		
p	<.05	<.05		
Male Nonsmoker	15.80	15.75	0.05	NS
Male Smoker	16.23	15.78	0.45	NS
Diff.	0.43	0.03		
p	<.05	NS		

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Doc Title 23

FATE AND EFFECTS OF METHYLENE CHLORIDE ACTIVATED SLUDGE

Chemical Name (300 per name) 25	CAS No. (10) 24
METHYLENE CHLORIDE	75-Ø9-2

87-8211827

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Fate and Effects of Methylene Chloride in Activated Sludge

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Activated sludge obtained from a municipal wastewater treatment plant was acclimated to methylene chloride at concentrations between 1 and 100 mg/liter by continuous exposure to the compound for 5 to 11 days. Acclimated cultures were shown to mineralize methylene chloride to carbon dioxide and chloride. Rates of methylene chloride degradation were 0.14, 2.3, and 7.4 mg of CH_2Cl_2 consumed per h per g of mixed-liquor suspended solids for cultures incubated in the presence of 1, 10, and 100 mg/liter, respectively. Concentrations of methylene chloride between 10 and 1,000 mg/liter had no significant effect on O_2 consumption or glucose metabolism by activated sludge. A hypothetical model was developed to examine the significance of volatilization and biodegradation for the removal of methylene chloride from an activated sludge reactor. Application of the model indicated that the rate of biodegradation was approximately 12 times greater than the rate of volatilization. Thus, biodegradation may be the predominant process determining the fate of methylene chloride in activated sludge systems continuously exposed to the compound.

Simple chlorinated hydrocarbons, such as methylene chloride, are widely used as solvents for cleaning and extraction and for a variety of other industrial applications. These compounds may enter wastewater treatment systems as a result of spills or through the routine discharge of wastes. The substantial use of chlorinated hydrocarbon solvents raises the question of the fate and effects of these compounds in the environment, and specifically on sewage treatment processes.

For many years, methylene chloride has been generally considered to be non-biodegradable. Results of the biochemical oxygen demand test for methylene chloride indicate that negligible oxygen is consumed over a 20-day test period (The Dow Chemical Co., unpublished results). However, recent evidence indicates that certain bacteria are capable of utilizing methylene chloride as a source of carbon for growth. Brunner and Leisinger (4) and Brunner et al. (5) reported the isolation of a *Pseudomonas* species able to grow on methylene chloride. Growth on methylene chloride resulted in the release of chloride from the substrate. Rittmann and McCarty (12) have also reported the utilization of methylene chloride by sewage microorganisms. Batch culture experiments indicated that methylene chloride degradation was dependent on the size of the microbial population. However, it was impossible to determine whether complete mineralization occurred, since neither chloride release nor CO_2 evolution was monitored.

The present study was conducted to investigate the fate and effects of methylene chloride in a system that simulates a municipal wastewater treatment plant. Experiments were conducted to examine the effect of methylene chloride concentration on both the acclimation of activated sludge and on the rate of biodegradation. Additional studies were performed to determine the effect of methylene chloride on the degradation of a readily utilizable test substrate.

MATERIALS AND METHODS

Biodegradation studies. Activated sludge was obtained from a local domestic wastewater treatment plant. Approximately 9 liters of activated sludge was adjusted to contain 2,500 ± 500 mg of mixed-liquor suspended solids (MLSS) per liter and placed into a laboratory-scale, semicontinuous activated sludge system. The unit was constructed from a glass cylinder (21 by 6 in. [ca. 53.3 by 15.2 cm]) similar to that described by the Soap and Detergent Association (13). The solids were allowed to settle in the chamber, and the upper 7 liters of waste liquor was removed and replaced with 6.5 liters of deionized water and 65 ml of synthetic sewage stock solution (14). The stock solution was composed of, per liter: glucose, 30 g; nutrient broth (Difco Laboratories, Detroit, Mich.), 30 g; K_2HPO_4, 1.5 g; and $(NH_4)_2SO_4$, 2.5 g. The system was aerated at a rate of 800 ml/min by means of a sparger placed in the bottom of the vessel. The unit was operated on a daily fill-and-draw cycle with synthetic sewage and incubated at ambient temperature (21°C).

A preliminary study indicated that acclimation of activated sludge was necessary for the biodegradation of methylene chloride. Consequently, during incuba-

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tion, the unit was supplemented with an appropriate concentration of methylene chloride. Methylene chloride volatilized from the system was continuously replaced by the addition of an air-methylene chloride mixture. The mixture was prepared by passing air at a slow rate (~5 ml/min) through methylene chloride contained in a gas-washing bottle. The outlet port of the bottle was connected to a sparger that was placed approximately 6 in. (ca. 15.2 cm) above the bottom of the activated sludge unit. Concentrations of methylene chloride and chloride ion in the culture were monitored routinely during the acclimation period as described below. The flow rate of methylene chloride-saturated air was adjusted to maintain the desired concentration. Acclimation of the activated sludge microorganisms was inferred by detecting the continuous release of chloride in the system. Once acclimation was noted, the unit was incubated for several days until the release of chloride in the culture reached a constant rate. The pH of the system was monitored periodically and adjusted to pH 7.2 ± 0.2 by the addition of 1 N NaOH. Subsequently, the acclimated sludge was used in closed-bottle respirometer studies to investigate the overall fate of methylene chloride in the system.

Biodegradation studies were conducted using an Oceanography International Corp. model 1/BOD respirometer system. The respirometer flasks were modified (Fig. 1) to facilitate the complete analysis of the transformation process. Each respirometer bottle (1.6 liters) is equipped with a manometric oxygen-generating system. As O_2 is consumed by the sample, it is regenerated electrolytically to maintain a constant atmosphere within the bottle. The apparatus is designed to generate 1 mg of O_2 per min, and a timing circuit records the amount consumed. A stopcock valve inserted between the O_2 generator and the bottle allowed isolation of the generator during removal of samples from the respirometer system. The bottles are equipped with a CO_2 trap fitted with a screw-capped septum port. CO_2 evolved during biodegradation is absorbed by an aliquot (5 ml) of 1 N NaOH in the CO_2 trap. The absorbant can be removed and replaced using a syringe. A long glass sampling tube and modified glass syringe are utilized for removing samples of mixed-liquor for analysis.

Before assembling the respirometers, the acclimated sludge was supplemented with synthetic sewage and aerated for an additional hour to remove residual methylene chloride. Subsequently, 1-liter samples of activated sludge were dispensed into six respirometer bottles. ^{14}C-labeled methylene chloride was added (neat) to four of the units. Two of the respirometers containing methylene chloride were treated with $HgCl_2$ (200 mg/liter) and served as killed controls for monitoring non-biological degradation. The remaining two respirometers did not receive methylene chloride and served as viable controls for monitoring endogenous metabolism. The respirometers were incubated for 30 h at ambient temperature (21°C). Samples of mixed-liquor were removed periodically for analysis. Methylene chloride concentration was determined by gas chromatography as described below. Chloride concentration in mixed-liquor samples was analyzed using an ion-specific electrode as described below. $^{14}CO_2$ evolution was monitored by removing the NaOH solution in the CO_2 trap and replacing it with a fresh aliquot. A remaining sample of activated sludge was analyzed by liquid scintillation counting for a material balance at each time period.

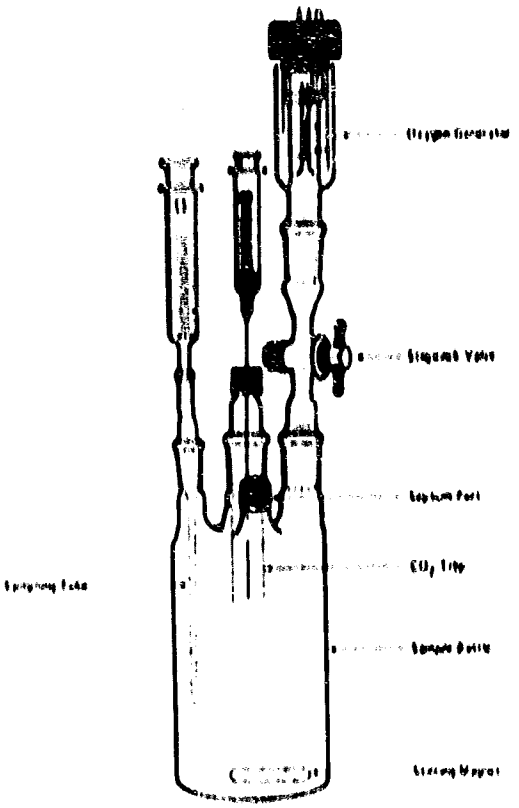

FIG. 1. Modified 1/BOD closed-bottle respirometer

Inhibition studies. Activated sludge was equilibrated as above for 3 days on a fill-and-draw cycle with a defined sewage medium. For these studies, the defined sewage stock solution was composed of, per liter: glucose, 40 g; K_2HPO_4, 13 g; and $(NH_4)_2SO_4$, 15 g. The laboratory-scale activated sludge unit was aerated at a rate of 900 ml/min and incubated at ambient temperature (21°C).

Inhibition studies were conducted in a manner analogous to the respirometer experiments previously described. One-liter aliquots of equilibrated sludge were dispensed into four respirometer bottles. Methylene chloride was added (neat) to three of the units at nominal concentrations of 10, 100, and 1,000 mg/liter. The remaining respirometer served as a control. The systems were incubated for 24 h at ambient temperature (21°C). Samples of mixed-liquor were removed periodically from each respirometer for analysis of total organic carbon as described below. O_2 consumption at each interval was read directly from the O_2 recorders.

Analytical methods. Concentration of methylene chloride in activated sludge was determined by gas chromatography. For experiments conducted at a methylene chloride concentration of 100 mg/liter, the analysis involved an isooctane extraction procedure similar to the method of Richard and Junk (11).

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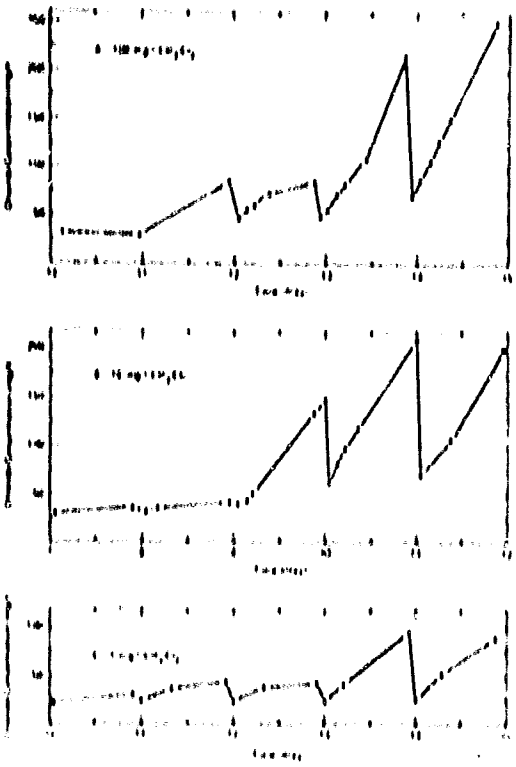

FIG. 3. Chloride release during acclimation of activated sludge to methylene chloride. Three activated sludge reactors (0.5 liters each) were operated for 2 weeks on a daily fill-and-draw cycle with synthetic sewage and continuously exposed to nominal concentrations of 1, 10, and 100 mg of methylene chloride per liter as described in the text. Samples of the cultures were removed periodically and analyzed for chloride concentration as described in the text. Note that the daily drop in chloride concentration was due to replacement of the culture medium.

ation from the expected stoichiometry for chloride release from the substrate was presumably due to error inherent in the measurement of chloride concentration in activated sludge. The rate of biodegradation was 2.3 mg of CH_2Cl_2 consumed h^{-1} g of MLSS $^{-1}$. Degradation of methylene chloride was not observed in killed-control respirometers, nor was the release of chloride detected in stable control samples incubated in the absence of methylene chloride. Biodegradation of ^{14}C-labeled methylene chloride resulted in the evolution of $^{14}CO_2$ (Fig. 3B). After 50 h, 49% of the parent compound was recovered as $^{14}CO_2$. The evolution of $^{14}CO_2$ was not observed in killed controls. Overall material balances for the experimental and killed-control samples were 91.7 and 92.2%, respectively.

Experiments were conducted in an identical manner to investigate the biodegradation of methylene chloride by activated sludge previously acclimated to concentrations of 1 and 100 mg of methylene chloride per liter. Respirometer studies conducted with activated sludge (2.24 g of MLSS per liter) previously acclimated to 100 mg of methylene chloride per liter indicated the disappearance of 100 mg of ^{14}C-labeled methylene chloride within 10 h, with a concomitant release of chloride. The rate of biodegradation was 7.4 mg of CH_2Cl_2 consumed h^{-1} g of MLSS $^{-1}$. After 50 h, 65% of the parent compound was recovered as $^{14}CO_2$. Respirometer studies conducted with activated sludge (2.58 g of MLSS per liter) previously acclimated to 1 mg of methylene chloride per liter indicated the disappearance of 1 mg of ^{14}C-labeled methylene chloride within 3 h. The rate of biodegradation was 0.14 mg of CH_2Cl_2 consumed h^{-1} g of MLSS $^{-1}$. After 50 h, 66% of the parent compound was recovered as $^{14}CO_2$.

Effect of methylene chloride on activated sludge. Inhibition studies were conducted to investigate the effect of methylene chloride on O_2 consumption and glucose metabolism by activated sludge (2.54 g of MLSS per liter). Results indicated that concentrations of methylene chloride between 10 and 1,000 mg/liter had no significant effect on O_2 consumption (Table 1) or total organic carbon removal (Table 2) by activated sludge over a 24-h period. Thus, inputs of methylene chloride would not be expected to adversely affect the microbial population of an aerobic wastewater treatment plant.

DISCUSSION

Microorganisms enriched from municipal activated sludge are capable of mineralizing methylene chloride to CO_2 and chloride. Acclimated cultures were shown to rapidly degrade methylene chloride under aerobic conditions. Rates of degradation were 0.14, 2.3, and 7.4 mg of CH_2Cl_2 consumed h^{-1} g of MLSS $^{-1}$ for cultures incubated in the presence of 1, 10, and 100 mg/liter, respectively. The biodegradation of methylene chloride by activated sludge is consistent with the previous reports of methylene chloride-utilizing bacteria (4, 5, 12) and indicates the possibility for biodegradation of the compound in the environment.

The biodegradation of methylene chloride by activated sludge requires acclimation of the microbial population by exposure to the compound for a period of time. The requirement for acclimation is consistent with the failure of the compound to degrade in tests of ready biodegradability, such as the biochemical oxygen demand test, in which acclimation may not occur. Acclimation of a microbial population is thought to involve one or more of the following: (i) induction or derepression of specific enzymes

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Samples (5 ml) of activated sludge were extracted with 5 ml of isooctane in 10-ml serum bottles. Aliquots (5 μl) of the isooctane extract were analyzed by direct injection as outlined below. Average recovery efficiencies for methylene chloride ranged from 89% at 10 mg/liter to 79% at 100 mg/liter. Concentrations of methylene chloride in activated sludge were not corrected for recovery efficiency.

For experiments conducted at concentrations of 1 and 10 mg/liter, methylene chloride in activated sludge was analyzed by a purge-and-trap procedure similar to the method of Bellar and Lichtenberg (2). A Hewlett-Packard model 7675A purge-and-trap sampler was utilized for the analysis. The outlet of the sampler was plumbed, using ⅛-in. (ca. 0.16-cm) stainless steel tubing, directly to the analytical column via a T-junction fitting. The extra port of the fitting was adapted for use as an injection port. Exposed areas of the line were wrapped with a flexible heating tape and maintained at 200°C. Activated sludge samples (5 ml) were placed into sampling tubes and purged with helium at a rate of 20 ml/min for 20 min at ambient temperature. Methylene chloride volatilized from the sample was trapped on a small column (3 by 0.25 in. [ca. 7.6 by 0.64 cm]) packed with Chromosorb 106 (Supelco, Inc., Bellefonte, Pa.). Adsorbed methylene chloride was injected directly into the gas chromatograph by thermal desorption of the trap column at 250°C for 5 min with helium as the carrier gas at a flow rate of 40 ml/min. Average recovery efficiencies for methylene chloride ranged from 88% at 1 mg/liter to 75% at 10 mg/liter. Methylene chloride concentrations in activated sludge were not corrected for recovery efficiency.

Gas chromatography was performed using a Hewlett-Packard model 5710A gas chromatograph with a flame ionization detector. The analytical column (glass; 6 ft by 0.25 in. [ca. 183 by 0.64 cm]) was packed with 1% SP-1000 on 60/80 Carbopak B (Supelco, Inc.). Compounds were eluted from the column isothermally at an oven temperature of 150°C with a helium carrier gas at a flow rate of 40 ml/min. Output of the flame ionization detector was connected to a Hewlett-Packard model 3390S recording integrator. All values were calculated from peak area measurements. The instrument was calibrated by injecting 5 μl of an external standard of methylene chloride prepared in isooctane. The detector response was linear over the concentration range of interest.

Chloride concentration in activated sludge was determined using an Orion model 94-17 chloride electrode and model 701 specific ion analyzer. Measurement of the chloride concentration of activated sludge was affected by the high levels of suspended solids. Consequently, a sample addition method was employed. Samples (10 ml) of mixed-liquor were transferred into 90 ml of a solution containing 10 mg of NaCl per liter. Ionic strength of the solution was adjusted before analysis by the addition of 2 ml of 5 M $NaNO_3$. The instrument was calibrated using standard NaCl solutions.

Total organic carbon was determined using a Beckman model 915B total carbon analyzer. Mixed-liquor samples were filtered through 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.). A 5-ml sample of the filtrate was transferred to a 10-ml serum bottle, diluted with 5 ml of Milli-Q continental water (Millipore Continental Water Systems Corp., El Paso, Tex.), and acidified by the addition of 50 μl of 20% (wt/vol) H_2SO_4. The bottle was sealed, and the sample was purged with air at a rate of 15 ml/min for 30 min. Aliquots (50 μl) of the sample were analyzed for total organic carbon. The instrument was calibrated using potassium biphthalate as the standard.

The concentration of MLSS in activated sludge was determined by gravimetric analysis (1).

Radioactivity associated with various fractions of the respirometers was monitored by liquid scintillation counting. Radioactivity in the mixed-liquor fraction was analyzed by transferring duplicate samples (1.0 ml) to 10 ml of Aquasol scintillation cocktail (New England Nuclear Corp., Boston, Mass.). Radioactivity evolved as $^{14}CO_2$ was determined at each interval by removing the absorbant in the CO_2 trap and replacing it with a fresh aliquot. Samples (50 μl) of the absorbant were transferred in duplicate into 10 ml of Aquasol scintillation cocktail. Samples were analyzed using a Packard model 2425 liquid scintillation counter. Radioassay data were corrected for background and quenching, and the distribution of radioactivity in the respirometers was calculated with respect to the total activity initially present in the mixed liquor.

Materials. ^{14}C-labeled methylene chloride was purchased from the New England Nuclear Corp. and was found to be 98% radiochemically pure. High-purity (Distilled in Glass Grade) methylene chloride and isooctane were obtained from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. Nutrient broth was from Difco Laboratories. All other materials were of the highest purity commercially available and were used without further purification.

RESULTS

Acclimation of activated sludge to methylene chloride. Preliminary studies indicated that acclimation of activated sludge was necessary for the biodegradation of methylene chloride. Three activated sludge reactors were operated for 2 weeks on a daily fill-and-draw cycle with synthetic sewage and continuously exposed to nominal concentrations of 1, 10, and 100 mg of methylene chloride per liter. Acclimation of the cultures was inferred by detecting the continuous release of chloride in the systems (Fig. 2). Maximum rates of chloride release were 1.3, 3.1, and 3.6 mg of chloride released h^{-1} g of $MLSS^{-1}$ for cultures exposed to methylene chloride concentrations of 1, 10, and 100 mg/liter, respectively.

Fate of methylene chloride in activated sludge. Closed-bottle respirometer studies were conducted using activated sludge previously acclimated to 10 mg of methylene chloride per liter. Biodegradation of ^{14}C-labeled methylene chloride (10 mg/liter) was monitored by observing the rate of disappearance of the parent compound, chloride release, and the evolution of $^{14}CO_2$ (Fig. 3). Disappearance of the substrate was observed within 4 h, with a concomitant release of chloride (Fig. 3A). The apparent devi-

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TABLE 2. Effect of methylene chloride on glucose metabolism by activated sludge

Time (h)	Total organic carbon concn (mg/liter) for the following CH_2Cl_2 system[a] in the respirometer			
	0	10	100	1,000
0	103.6	94.0	94.4	93.2
2	51.2	49.8	46.2	56.6
4	29.6	17.0	18.0	21.8
8	16.8	19.6	15.6	15.2
12	16.0	16.2	17.2	22.0
24	15.8	19.2	16.8	20.4

[a] CH_2Cl_2 concentrations are expressed in milligrams per liter.

ble intermediate, formyl chloride. A similar mechanism has been suggested for the oxidation of methylene chloride by the methane monooxygenase from *Methylococcus capsulatus* (16). Thus, the biodegradation of methylene chloride to CO_2 by activated sludge could conceivably involve either an initial attack by a monooxygenase, with the subsequent oxidation of carbon monoxide to CO_2, or an initial attack by a halidohydrolase, with the further oxidation of formaldehyde to CO_2. In view of the high yields of CO_2 recovered, it is likely that the latter mechanism is predominant in the system. Further studies would be necessary to clarify this point.

The relative importance of two processes, volatilization (air stripping) and biodegradation, expected to be the major factors determining the fate of methylene chloride in an activated sludge reactor, was examined with a hypothetical model. The model consisted of a completely mixed reactor, with a volume (V) of 1.6×10^7 liters, operating at steady state conditions. The hydraulic flow rate (Q_w) was assigned a value of 2.3×10^6 liters/h, and thus the hydraulic residence time of the system was approximately 7 h. The system was operated at a flow rate (Q_a) of 1.7×10^7 liters/h, based on a standard wastewater treatment design factor (air flow/hydraulic flow) of 7.5 (10). The model assumes that the rates of each process controlling the fate of methylene chloride in the reactor can be treated independently and that the results of laboratory experiments can be extrapolated to describe the process in the complete system. Thus, to a first approximation, a mass balance for the reactor would be described by the equation: $-V(dC/dt) = \text{Rate}_{\text{input}} - \text{Rate}_{\text{output}} - \text{Rate}_{\text{volatilization}} - \text{Rate}_{\text{biodegradation}}$. The rate of methylene chloride input (A_1) to the system would be a function of the hydraulic flow rate (Q_w) and the influent concentration (C_0). Correspondingly, the output rate (A_2) would be a function of flow rate (Q_w) and the average concentration of methylene chloride in the system (C). From the report of Mackay et al. (9), the rate of volatilization (air stripping) can be written as a function of the aeration rate (Q_a), the concentration of methylene chloride in the reactor (C), and a coefficient (P') describing the partitioning of methylene chloride between the air and water phases. The partition coefficient (P') is a dimensionless term and is related to the Henry's law constant by the equation, $P' = H/RT$, where H is the Henry's law constant (meter^3 atmosphere mole^{-1}), R is the gas constant (meter^3 atmosphere mole^{-1} degree Kelvin^{-1}), and T is the temperature (degree Kelvin). Dilling (6) has reported a value of 0.11 for the partition coefficient of methylene chloride at 25°C. Thus, the rate of volatilization (A_3) of methylene chloride from the activated sludge reactor is described by the equation: $A_3 = P'Q_aC$. For the purpose of the modeling exercise, the degradation of methylene chloride by activated sludge will be treated as a first-order reaction, where the rate of degradation is a function of the volume of the system (V), the concentration of methylene chloride in the reactor (C), and a constant (k_b) describing the biodegradation rate. This approach is supported by the results obtained from the respirometer studies conducted at 1 and 10 mg/liter; graphs of the logarithm of methylene chloride concentration versus time gave a straight line. The first-order rate constant calculated for the degradation of methylene chloride was 1.4 h^{-1}. Thus, the rate of biodegradation (A_4) would be expressed by the equation: $A_4 = k_bVC$. Therefore, the overall mass balance for the activated sludge reactor can be written as follows: $-V(dC/dt) = Q_wC_0 - Q_wC - P'Q_aC - k_bVC$. At steady state, the concentration of methylene chloride in the reactor (C) is constant, and thus dC/dt is equal to zero. If the influent concentration (C_0) to the reactor is assigned a value of 10 mg/liter, the average concentration (C) of methylene chloride in the system would be approximately 1 mg/liter. Thus, the rate of volatilization of methylene chloride from the reactor would be approximately 2×10^7 mg/h. Alternatively, the rate of methylene chloride biodegradation, by an acclimated culture, would be approximately 2.3×10^7 mg/h. In view of the fact that the rate of biodegradation is approximately 12 times greater than the rate of volatilization, it is suggested that biodegradation may be the predominant mechanism determining the fate of methylene chloride in activated sludge systems continuously exposed to the compound.

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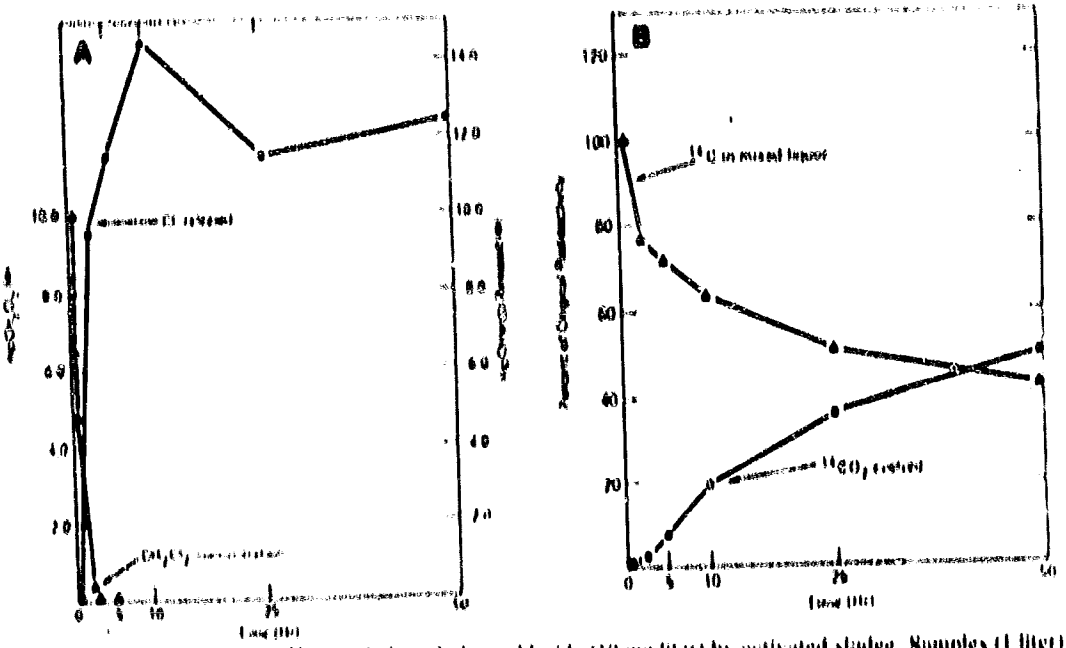

FIG. 1. Biodegradation of ^{14}C-labeled methylene chloride (10 mg liter) by activated sludge. Samples (1 liter) of activated sludge (2.0% of MLSS per liter) were placed into respirometer bottles and supplemented with 10 mg of ^{14}C-labeled methylene chloride (7.6 μCi/mmol). The respirometers were incubated for 50 h at ambient temperature (21°C). Samples of mixed liquor were removed periodically and analyzed for methylene chloride and chloride concentration as described in the text (A). Note that the value for methylene chloride concentration at time zero represents the nominal concentration added to the respirometers (10 mg liter). Radioactivity in both the mixed liquor and in the CO_2 traps was monitored by liquid scintillation counting as described in the text (B). Data points are the average values obtained from the results of duplicate respirometers.

present at low or nonexistent levels in the population before exposure, (ii) selection of new metabolic capabilities as a result of genetic changes, and (iii) an increase in the numbers of organisms capable of a particular transformation (15). Other factors, including the concentration of substrate and the availability of alternate carbon sources, have been reported to influence the acclimation of a microbial population (3). The present study has demonstrated the ability of microorganisms present in municipal activated sludge to acclimate when exposed to methylene chloride concentrations between 1 and 100 mg/liter. The exact mechanisms involved in this process are unknown.

TABLE 1. Effect of methylene chloride on O_2 consumption by activated sludge

Time (h)	Oxygen (mg) consumed for the following CH_2Cl_2 concn[a] in the respirometer			
	0	10	100	1,000
0	0.0	0.0	0.0	0.0
2	16.4	16.7	17.8	15.0
4	41.2	38.8	40.1	33.9
8	78.6	77.9	78.7	74.5
12	111.7	108.7	108.6	103.2
24	201.8	193.3	191.5	186.9

[a] CH_2Cl_2 concentrations are expressed in milligrams per liter.

The mechanism involved in the biodegradation of methylene chloride by activated sludge is as yet unclear. Attempts in this laboratory to demonstrate the presence of an intermediate product have been unsuccessful. Brunner et al. (5) have proposed that a halidohydrolase is responsible for the oxidation of methylene chloride by a *Pseudomonas* species. This reaction has been previously reported by Heppel and Porterfield (7) for the oxidation of methylene chloride by rat liver homogenates. Substitution of a hydroxyl group for one chlorine substituent of methylene chloride would lead to the formation of monochloromethanol. This intermediate would spontaneously decompose to formaldehyde, releasing the second chlorine substituent. The product of the initial reaction, formaldehyde, would be readily consumed during microbial growth, either by incorporation into cellular constituents or by further oxidation to CO_2. Alternatively, Kuhle and Anders (8) have demonstrated that a monooxygenase present in the microsomal fraction of rat liver oxidizes methylene chloride to carbon monoxide via the unsta-

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METHYLENE CHLORIDE	75-09-2

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THE DOSE-DEPENDENT METABOLISM OF ^{14}C-[illegible] FOLLOWING ORAL ADMINISTRATION TO RATS

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ABSTRACT

^{14}C-Methylene chloride ($^{14}CH_2Cl_2$) was administered orally to female Sprague-Dawley rats in doses of 1 and 50 mg/kg. The disposition of radioactivity was followed for 40 hr after dosing. The major metabolites of CH_2Cl_2 were carbon monoxide (CO) and carbon dioxide (CO_2) both of which were found in expired air. The fate of $^{14}CH_2Cl_2$ was dependent upon the administered dose. Rats receiving 1 mg/kg $^{14}CH_2Cl_2$ metabolized 38% of the dose whereas those given 50 mg/kg metabolized only 20% of the administered dose of $^{14}CH_2Cl_2$. Although all tissues examined at 40 hr after dosing contained ^{14}C-activity, the overall tissue retention of ^{14}C was low (<10%) after either dose of $^{14}CH_2Cl_2$. The highest concentrations of ^{14}C-activity were found in the liver. There was no significant accumulation of ^{14}C in the fat. The data indicate that the observed dose-dependency was due to saturation of CH_2Cl_2 metabolism after the 50 mg/kg dose. The implications of these findings and their relationship to CH_2Cl_2 toxicity are discussed.

INTRODUCTION

Methylene chloride (CH_2Cl_2) is widely used in a variety of [illegible] industrial and commercial applications. Because of its low toxicity and high volatility it has become the solvent of choice for several [illegible] products as well as in some food processing applications.

The fate of $^{14}CH_2Cl_2$ following i.p. injection in rats was reported by DiVincenzo and Hamilton (1975). Although these authors established that the major metabolites of $^{14}CH_2Cl_2$ were ^{14}CO and $^{14}CO_2$, the high doses employed in these studies (412-930 mg/kg) precludes the application of these data to considerations of low-level CH_2Cl_2 exposure. More recently Yasair, et al, (1977) reported that the fate of $^{14}CH_2Cl_2$ given orally to mice was dose-dependent, i.e., that high doses of CH_2Cl_2 were not metabolized as extensively as low doses of the solvent.

Several laboratories, including our own, have initiated programs for the safety evaluation of CH_2Cl_2. The studies reported herein represent a portion of our investigations into the pharmacokinetics and metabolism of $^{14}CH_2Cl_2$. The purpose of these studies is to provide an understanding of the fate of CH_2Cl_2 in the body as well as a data base sufficient to aid in the design and interpretation of laboratory investigations related to the hazard of exposure to the solvent.

[illegible]

Animals.

Male Sprague Dawley rats (Spartan Research Animals, Inc., Haslett, Michigan) weighing approximately 250 g were used in this study. The rats were acclimated to the experimental environment (Roth cages) for a minimum of 48 hr prior to dosing. Food (Purina Rat Chow) and water were provided [illegible]

Methylene Chloride.

^{14}C-Methylene chloride ($^{14}CH_2Cl_2$) was obtained from New England Nuclear (lot number 016-297, specific activity 2.25 mCi/mmole). Minimum radiochemical purity of this material was 96.4% when assayed by liquid scintillation counting of the trapped effluent from a gas chromatograph column (see below). The $^{14}CH_2Cl_2$ was diluted to the desired specific activity with cold carrier technical grade methylene chloride (The Dow Chemical Co., Pinquemine, Louisiana, minimum purity 99%).

Experimental Procedure.

$^{14}CH_2Cl_2$ was administered in 1.5 ml distilled water by oral gavage at doses of 1 and 50 mg/kg. Three rats were evaluated at each dose level. The final concentrations of $^{14}CH_2Cl_2$ in the dosing solutions were assayed by gas chromatography and liquid scintillation spectrometry. The specific activities of $^{14}CH_2Cl_2$ in the dosing solutions were 4.20 and 0.13 μCi/mg CH_2Cl_2 for the 1 and 50 mg/kg dose levels respectively.

Immediately after administration of $^{14}CH_2Cl_2$ the rats were placed in individual Roth-type metabolism cages for collection of urine, feces and expired air over the next 48 hours. Expired ^{14}CO, $^{14}CO_2$, urine and feces were collected at 12 hr intervals. The urine receptacles were kept at dry ice temperature to minimize possible losses of volatile urinary excretion

produces. Expired $^{14}CH_2Cl_2$ was collected as [illegible]
5 hr after dosing. Subsequently a single sample for [illegible]
collected for the ensuing 8 hr period to ensure complete collection of
$^{14}CH_2Cl_2$ expired by the animals.

Rats were sacrificed by exsanguination 60 hr after administration of $^{14}CH_2Cl_2$. The carcasses were skinned, tissues excised and all samples were stored frozen.

Breath Analysis.

Collection of radiolabeled volatiles in expired air was facilitated by drawing room air (500 ml/min) through the glass metabolism cages and subsequently through a series of traps for absorption of $^{14}CH_2Cl_2$, $^{14}CO_2$ and ^{14}CO. The air exiting each metabolism cage was passed through an analytical train consisting of: (a) a 1 cm diameter glass tube containing 6 g of activated charcoal (grade 252, Witco Chemical Co. New York, N.Y.) to trap expired $^{14}CH_2Cl_2$; (b) an impinger containing 120 ml of 5M ethanolamine in 2-methoxy-ethanol to trap expired $^{14}CO_2$; (c) a 50 ml impinger containing 45 g Hopcalite catalyst (Mine Safety Appliances, Pittsburgh, Pa.) to convert ^{14}CO to $^{14}CO_2$; (d) an additional 5M ethanolamine trap to collect ^{14}CO oxidized to $^{14}CO_2$. A Drierite® cartridge was placed before the Hopcalite catalyst to absorb moisture which might otherwise adversely affect its catalytic activity.

Measurement of Radioactivity.

A Beckman Mark II or Mark III liquid scintillation spectrometer was used for all radioactivity measurements. External standard-channel ratios were used to determine counting efficiency. Known volumes of the $^{14}CO_2$ traps were counted in a toluene-based scintillation cocktail. The charcoal adsorbent for $^{14}CH_2Cl_2$ was divided into 4 equal aliquots and desorbed for 24 hr in the toluene-based scintillant before counting. Samples of excreta and tissues

were prepared for oxidation in a [illegible] liquid scintillation counting as described previously [illegible].

Gas Chromatography.

Determination of radiochemical purity of $^{14}CH_2Cl_2$ was performed on two different columns in a Hewlett Packard Model 5750 gas chromatograph with thermal conductivity detection. Conditions for these analysis were as follows: System 1: column, 6' x 1/8" OD stainless steel packed with 80/100 mesh Porapak QS; injector and detector temperature, 200°C; column temperature, 80°C; carrier gas, helium at 40 ml/min. System 2: column, 6' x 1/8" OD stainless steel packed with 100/120 mesh Chromosorb 102; injector and detector temperature, 220°C; column temperature 210°C; carrier gas, helium at 30 ml/min. The carrier gas effluent from the detector was bubbled through traps of toluene-based scintillation cocktail which were changed at regularly timed intervals. ^{14}C-activity was assayed by liquid scintillation counting. Radiochemical purity of $^{14}CH_2Cl_2$ was expressed as the percentage of total ^{14}C-activity injected on the column which was associated with the methylene chloride peak.

$^{14}CH_2Cl_2$ in the dosing solutions was assayed on a Varian Model 3440 gas chromatograph using H_2 flame ionization detection. Column and instrument conditions for this analysis were essentially the same as described for System 1 above.

Pharmacokinetic Analysis.

Concentration-time data obtained for excretion of ^{14}C-activity in expired air or urine of each individual animal were characterized by a two-compartment open model. The best pharmacokinetic parameter estimates were obtained by a least squares linear regression curve stripping program on a Hewlett Packard

[illegible] calculator. The curves [illegible] reconstructions of the elimination curves [illegible] intercepts arrived from the individual [illegible].

The disposition of ^{14}C-activity in rats 48 hr after oral administration of $^{14}CH_2Cl_2$ is shown in Table 1. $^{14}CH_2Cl_2$ was found only in exhaled air during these experiments. The major metabolites of $^{14}CH_2Cl_2$ were ^{14}CO and $^{14}CO_2$ also present in expired air. Urine, feces and stomach were devoid of volatile radioactivity. Thus the ^{14}C-activity in these samples was attributed to biotransformation products of $^{14}CH_2Cl_2$.

Rats given the 1 mg/kg dose metabolized a greater percentage of the administered dose than those given 50 mg/kg $^{14}CH_2Cl_2$. This more complete metabolism of the administered 1 mg/kg dose was characterized by increased percentages of the dose recovered as $^{14}CO_2$, ^{14}CO, urinary ^{14}C-activity, and as ^{14}C-metabolites retained in the carcass. In contrast, rats given 50 mg/kg $^{14}CH_2Cl_2$ exhaled approximately 72% of the dose as unchanged $^{14}CH_2Cl_2$, resulting in proportionately less metabolism of the administered dose.

Figure 1 shows the time course for the pulmonary elimination of $^{14}CH_2Cl_2$ during the first five hours after intubation. Elimination of $^{14}CH_2Cl_2$ in the expired air of rats receiving the 1 mg/kg dose was characterized by a bi-exponential decay curve which was resolved into two first order processes. The mean (±S.D.) rate constants of these first-order processes were 3.34±0.67 hr^{-1} ($t_{1/2}$ = 0.21 hr) and 0.93±0.17 hr^{-1} ($t_{1/2}$ = 0.76 hr) for the initial and terminal portions of curve, respectively.

The pulmonary excretion of $^{14}CH_2Cl_2$ by rats given the 50 mg/kg dose approximated zero-order kinetics for the first hour after administration of CH_2Cl_2. However as $^{14}CH_2Cl_2$ in expired air declined, the elimination of the unchanged solvent could be described by the same kinetic parameters which characterized pulmonary elimination of $^{14}CH_2Cl_2$ following the 1 mg/kg dose.

[illegible] shown in Figure 2. Since ^{14}CO excretion is [illegible] additional data on this, the data clearly [illegible] $^{14}CH_2Cl_2$ metabolized a significantly greater fraction of [illegible] than did those given 50 mg/kg $^{14}CH_2Cl_2$. The time course [illegible] expired air following administration of a [illegible] mg/kg $^{14}CH_2Cl_2$ [illegible] biphasic exponential decay curve which was resolved into two first-order processes. The apparent first-order rate constants were 0.37±0.03 hr^{-1} and 0.08±0.01 hr^{-1} (Mean S.D., n=3) for the initial and terminal portions of the curve, respectively. These rate constants corresponded to mean half lives of 1.8 and 8.7 hours. Similar kinetic analysis of the data obtained following administration of the 50 mg/kg dose of $^{14}CH_2Cl_2$ was hampered by the variability in the ^{14}CO excretion during the latter part of the experiment (24-48 hours). However the initial portion of the excretion curve was amenable to characterization by a single first-order elimination process. The mean (±S.D.) rate constant for ^{14}CO excretion by the three animals in this experiment was 0.26±0.02 hr^{-1}, or a half life of approximately 2.7 hr, similar to that obtained for the initial portion of the ^{14}CO excretion curve from the rats given 1 mg/kg $^{14}CH_2Cl_2$.

Figure 3 shows the time course for the elimination of $^{14}CO_2$ in expired air. Once again, the rats given 1 mg/kg $^{14}CH_2Cl_2$ clearly metabolized a greater percentage of the administered dose to $^{14}CO_2$ throughout the entire 48 hr collection period. The biphasic elimination curves for $^{14}CO_2$ excretion shown in Figure 3 were essentially parallel. The data from the 1 mg/kg group gave a mean (±S.D.) first-order rate constant of 0.27±0.02 hr^{-1} ($t_{1/2}$, 2.6 hr) and 0.04±0.010 hr^{-1} ($t_{1/2}$, 17.3 hr) for the initial and terminal phases of

the curves, respectively. For the 50 mg/kg [illegible] 0.10 hr^{-1} ($t_{1/2}$, 1.4 hr) and 0.035±0.001 hr^{-1} ($t_{1/2}$, 23.1 hr).

The [illegible] for urinary excretion of ^{14}C-activity following [illegible] administration is shown in Figure 4. Rats given 1 mg/kg $^{14}CH_2Cl_2$ excreted a greater percentage of the administered dose of $^{14}CH_2Cl_2$ in the urine. The rates of ^{14}C-elimination in the urine following either dose of $^{14}CH_2Cl_2$ were not significantly different. The biphasic elimination of urinary ^{14}C-activity after 1 mg/kg $^{14}CH_2Cl_2$ was resolved into two first-order processes with mean (±S.D.) rate constants of 0.21±0.03 hr^{-1} ($t_{1/2}$, 3.3 hr) and 0.03±0.01 hr^{-1} ($t_{1/2}$, 23.1 hr) for the initial and terminal portions of the curves, respectively. For the 50 mg/kg dose group these values were 0.14±0.01 hr^{-1} ($t_{1/2}$, 5 hr) and 0.03±0.01 hr^{-1} ($t_{1/2}$, 23.1 hr).

The tissue distribution of ^{14}C-activity 48 hr following administration of $^{14}CH_2Cl_2$ is shown in Table 2. The highest concentrations of ^{14}C-activity were found in liver, kidney and lung. All remaining tissues contained less ^{14}C-activity per gram than the concentrations observed in either blood or the remaining carcass. The lowest concentration of ^{14}C-activity was observed in the fat.

DISCUSSION

The results of this study indicate that the [illegible] is clearly dose-dependent. The metabolic and excretory patterns [illegible] qualitatively similar after administration of either the 1 or 50 mg/kg dose of the solvent. Moreover, the rates of elimination of $^{14}CO_2$ and metabolites of $^{14}CH_2Cl_2$ were essentially unaffected by dose despite a [illegible] fold difference of excretion of $^{14}CH_2Cl_2$ metabolites in the total [illegible] of the dose metabolized. Therefore the dose-dependent fate of $^{14}CH_2Cl_2$ appears to be due to a saturation of one or more metabolic pathways for the solvent.

Dose-dependent metabolism of CH_2Cl_2 is also apparent from a comparison of the relative percentages of the major metabolites, $^{14}CO_2$ and ^{14}CO, recovered. After the 1 mg/kg dose of $^{14}CH_2Cl_2$, the ratio of $^{14}CO_2$ to ^{14}CO recovered was 1.15. However, after the 50 mg/kg dose, this ratio decreased to 0.5 indicating that the production of these metabolites is likely governed by two different metabolic pathways. Since the percentage of the dose recovered as either $^{14}CO_2$ or $^{14}CO_2$ decreased as the dose of $^{14}CH_2Cl_2$ was increased to 50 mg/kg, both metabolic pathways exhibit dose-dependency. This may have been due to saturation of metabolism or to limited accessibility of the CH_2Cl_2 to the actual site of metabolism following oral administration. Although the available data are inadequate to describe the exact mechanism by which metabolism of $^{14}CH_2Cl_2$ is limited in this situation, the net result of either of the above alternatives would be the same, i.e. a decrease in the percentage of the dose metabolized with increasing doses of CH_2Cl_2.

The observed differences in pulmonary excretion of $^{14}CH_2Cl_2$ after administration of the 50 mg/kg dose may also be accounted for in terms of

the saturation of metabolism of CH_2Cl_2. The [illegible] elimination of $^{14}CH_2Cl_2$ during the first hour after dosing, [illegible] to greater concentrations of the solvent available for pulmonary elimination resulting from its limited biotransformation. This interpretation of the data is consistent with the finding that following the initial [illegible] phase, the rate constants for pulmonary elimination were virtually identical to those observed after the 1 mg/kg dose.

The metabolism of $^{14}CH_2Cl_2$ to ^{14}CO and $^{14}CO_2$ has been described previously (DiVincenzo and Hamilton, 1975). Further studies (Anders et al, 1977), on the enzyme systems involved in the biotransformation of CH_2Cl_2 demonstrated that metabolism of CH_2Cl_2 to CO is localized in hepatic microsomal fractions and requires both NADPH and molecular oxygen for optimal activity. Additional evidence for the dose-dependent metabolism of CH_2Cl_2 to CO in vivo has been reported by Kubic, et al (1974) and Hogan, et al (1976). In both studies saturation of the CO pathway was evident in the lack of a dose-related response of blood carboxyhemoglobin levels with increasing doses of CH_2Cl_2.

Heppel and Porterfield (1948), reported that CH_2Cl_2 was dehalogenated in rat tissue slices to formaldehyde (CH_2O), hydrogen and chloride ions. This pathway has been investigated in detail by Ahmed and Anders (1976). CH_2O was not a major metabolite of $^{14}CH_2Cl_2$ in this study or in that of DiVincenzo and Hamilton (1975). However, Healy (1964) has reported that $^{14}CH_2O$ is primarily metabolized in the rat to $^{14}CO_2$. Thus it is conceivable that the production of $^{14}CO_2$ observed in this study may be the end result of the biotransformation of CH_2Cl_2 to CH_2O.

In view of the nature of the major biotransformation reactions for CH_2Cl_2 discussed above, it is evident that appreciable potential exists for

the incorporation of CH_2Cl_2 derived [illegible] endogenous substrates involved in normal [illegible] Thus, it would be expected that each of the [illegible] 40 hr after administration of $^{14}CH_2Cl_2$ may be derived [illegible] In addition, studies by Anders et al (1977) on the [illegible] conversion of $^{14}CH_2Cl_2$ to $^{14}CH_2O$ and $^{14}CO_2$ have suggested that this transformation pathway yields intermediates which are capable of alkylating various tissue nucleophiles. Thus, tissue alkylation by $^{14}CH_2Cl_2$ [illegible] may offer an additional potential contribution to the observed residues of ^{14}C-activity.

The relationship between the metabolism of CH_2Cl_2 and the toxicity of the solvent is not fully understood. Although definitive evidence for saturation of the primary metabolic pathways for CH_2Cl_2 has been presented, there appears to be little indication in the available data for a dose-rate effect on either metabolic detoxification or on metabolic pathways with potentially toxic end products. However, the rapid pulmonary elimination of unchanged CH_2Cl_2 following oral administration suggests that the parent molecule is not directly responsible for systemic toxicity under such circumstances. Since the available data indicate a longer biological half-life for the metabolites of CH_2Cl_2 and more potential for their accumulation upon repeated exposure, it is more likely that some component of CH_2Cl_2 metabolism is associated with toxicity.

If CH_2Cl_2 metabolism is indeed associated with toxicity of the solvent then the observed dose-dependent or saturable character of this metabolism has a direct impact on the design and interpretation of toxicity studies of CH_2Cl_2 when administered orally. In such studies it becomes desirable to distinguish between the administered dose of CH_2Cl_2 and the "effective dose,"

[illegible] CH_2Cl_2 [illegible] and the effective dose of the solvent [illegible] Furthermore, an extrapolation of metabolism of CH_2Cl_2 [illegible] in the magnitude of the effective dose between [illegible] increase in the administered dose of the solvent [illegible] pharmacokinetic data is critical in the extrapolation of exposure to CH_2Cl_2 for toxicity studies to good dose-response information to [illegible] Finally, the observation of dose-dependent pharmacokinetics for CH_2Cl_2 [illegible] the linear extrapolation of toxicity data from high doses to predict the hazard of exposure to low doses of the solvent following oral administration.

[illegible]

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TABLE [illegible]

[illegible] OF ^{14}C-[illegible]

SINGLE ORAL DOSE IN [illegible]

	[illegible]	[illegible]
Expired CH_2Cl_2	12.33±2.40	72.09±1.33
CO_2	35.01±1.47	6.33±0.40
CO	30.92±2.90	11.07±1.27
Urine	4.52±0.09	1.96±0.08
Feces	0.93±0.03	0.25±0.04
Carcass	5.84±0.42	2.40±0.41
Skin	1.56±0.09	1.15±0.11
Cage Wash	0.53±0.07	0.08±0.02
Recovery	91.64±3.59	96.13±1.76

Liver	0.40±0.07[1]	[illegible]
Kidney	0.19±0.03	[illegible]
Lung	0.09±0.02	[illegible]
Brain	0.041±0.11	[illegible]
Epididymal Fat	0.022±0.004	0.33±0.01
Skeletal Muscle	0.076±0.039	[illegible]
Testes	0.010±0.004	0.22±0.10
Whole Blood	0.057±0.006	1.41±0.33
Remaining Carcass	0.051±0.003	0.99±0.10

[1]Values are $\bar{X} \pm$ S.D., N = 3

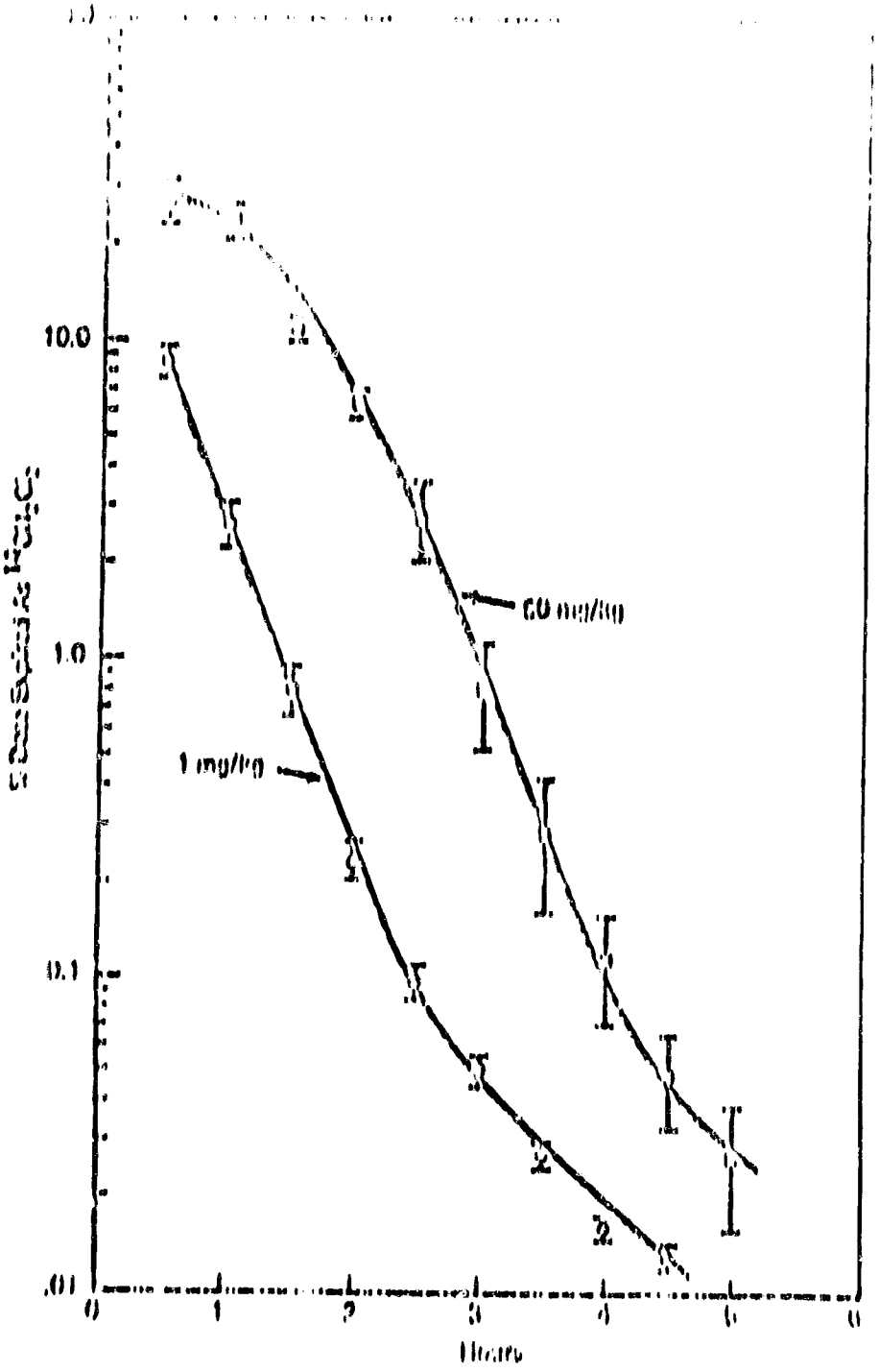
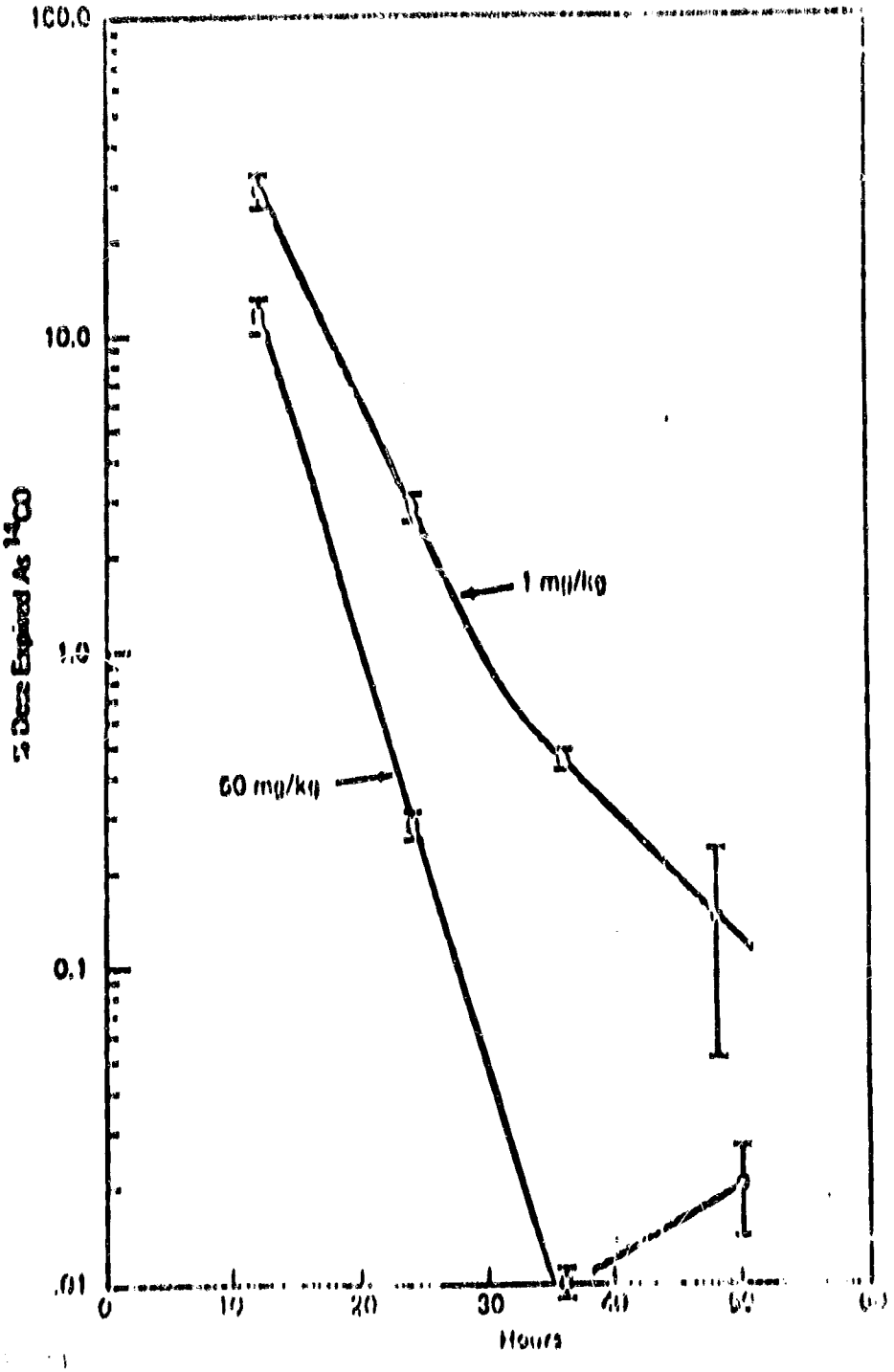

10.0
1.0
0.1
.01
1 mg/kg
Hours

Figure 3



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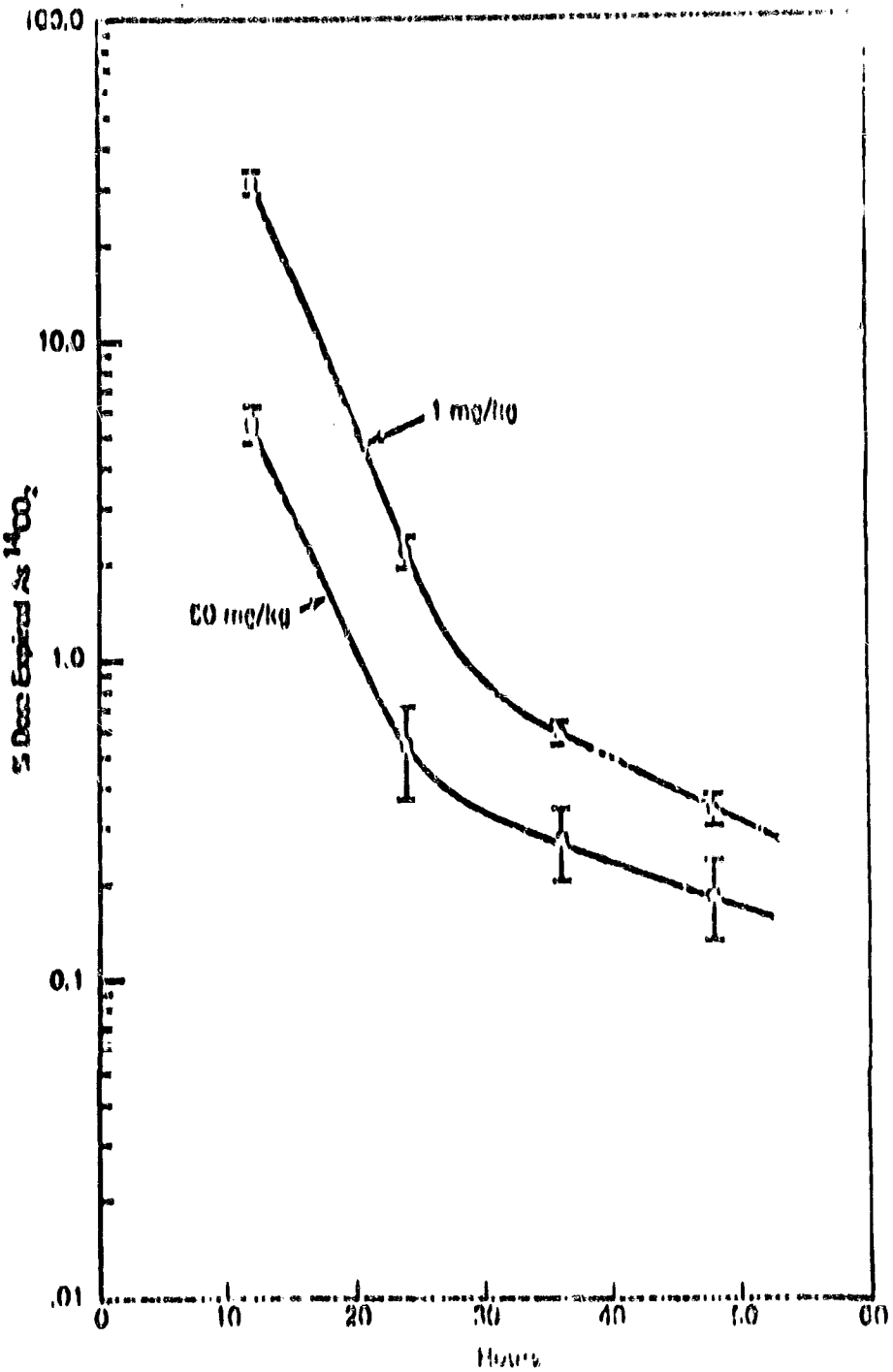
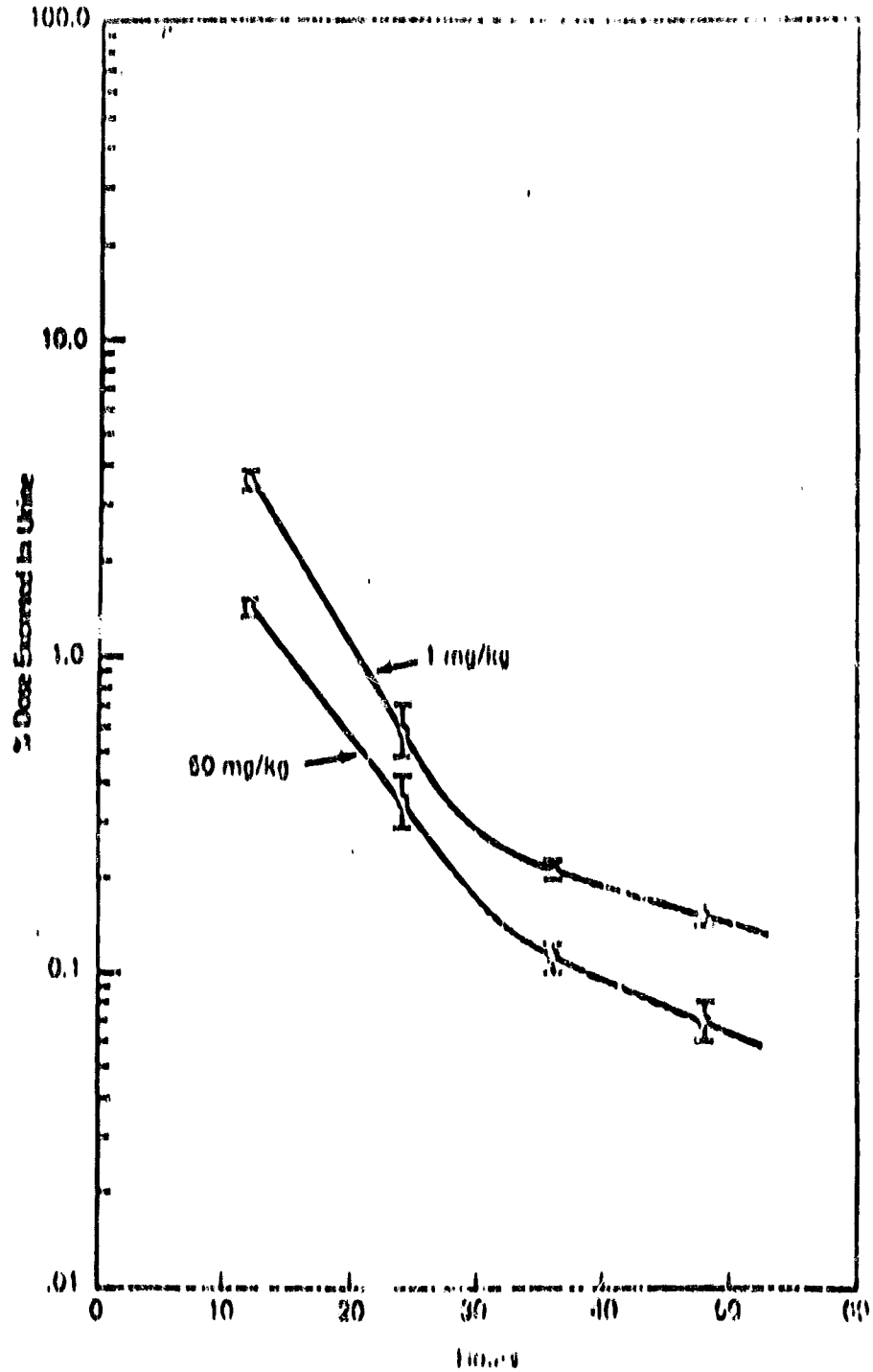

100.0
10.0
1.0
0.1
.01
% Dose Expired As $^{14}CO_2$
1 mg/kg
CO mg/kg
0
10
20
30
40
50
60
Hours

000022

Figure 4


100.0
10.0
1.0
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.01
% Dose Excreted in Urine
1 mg/kg
80 mg/kg
0
10
20
30
40
50
60
Hours

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PULOMONARY PHYSIOLOGY AND INHALATION DOSIMETRY IN RATS: DEVELOPMENT OF A METHOD WITH TWO EXAMPLES

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ABSTRACT

Methods were developed to quantitate the net uptake of an inhaled material in rats under apparent steady state conditions, and to simultaneously measure respiratory frequency, tidal volume, and minute volume. During steady state, if metabolism is the only significant means of elimination, the net uptake rate of an inhaled material will equal its rate of metabolism. The rates of metabolism of methyl chloride (MeCl) in 50 and 1000 ppm exposed rats were 0.20 and 3.3 nM/min/g; the rates of metabolism of methylene chloride ($MeCl_2$) in 50 and 1500 ppm exposed rats were 0.57 and 2.8 nM/min/g. The uptake values obtained for both solvents were consistent with pharmacokinetic and metabolism data that was previously obtained in our laboratory. A pharmacokinetic model incorporating the metabolic rate at steady state, blood concentration vs time, and respiratory minute volume was used to describe the fate of inhaled MeCl in F344 rats, and to estimate the inhaled "effective" dose in 50 and 1000 ppm 6 hr-exposed rats (3.8 and 67 mg/kg respectively). The approach used in these studies appears to be a useful method for the evaluation of metabolic rates and for inhalation dosimetry.

INTRODUCTION

Pharmacokinetic data have been used extensively in evaluating exposure concentration-response relationships of inhaled materials. One parameter that is not routinely measured in toxicity studies is the uptake of parent compound. This is a potentially useful means for assessing the rate of metabolism and, in conjunction with pharmacokinetic data, the cumulative net uptake during exposure (effective dose). At steady state, the rate of uptake is equal to the rate of metabolism plus non-pulmonary excretion of parent compound (i.e., by definition input must equal output at steady state). If there is no significant non-pulmonary excretion of parent compound, steady state uptake rate must equal the metabolism rate. Two potentially important applications of these determinations are the comparisons between routes of administration made possible by knowing the inhaled dose, and the assessment of possible exposure concentration dependent (or saturation) kinetics. The latter phenomenon has been shown to profoundly influence the relationship between administered dose and toxicity (Ramsey and Gehring, 1980).

Inhaled materials can cause changes in pulmonary physiological parameters. Alarie (1973) has reviewed the phenomenon of sensory irritation by airborne chemicals. Irritants, according to Alarie, produce a protective reflex designed to reduce exposure of the respiratory tract. A chronic inhalation toxicity study of formaldehyde provides an example in which the relative sensitivity of mice and rats to nasal carcinoma appears to be related to the irritant reflex producing potential in the two species (Chang, et al., 1981). Mice reduce respiratory frequency and minute volume in response to formaldehyde to a greater extent than rats, and mice also have a smaller incidence of formaldehyde induced nasal carcinomas.

Likens and Maudsely (1979) have reviewed methods that have been used for respiratory measurements in small laboratory animals. One method was volume displacement plethysmography. Barrows (1980) used this method in a system that utilized an animal restraining tube with a plethysmograph tube that slipped over the restraining tube when measurements were made. An advantage of this procedure is that the animal is unlikely to become overheated while in the ventilated restraining tube. Long periods within a small closed plethysmograph potentially cause animal heat stress which can have marked effects on respiration.

We developed an apparatus to utilize whole body plethysmography in conjunction with a low dead volume, head only, dynamic inhalation exposure apparatus. This system allowed inhalation exposure of a rat to a known concentration of a material, and simultaneous measurement of vapor uptake and ventilatory functions.

Our system was tested by performing repeated respiratory physiological measurements in rodents to assess the optimal time(s) for sampling and to determine the precision and repeatability of the procedure. Steady state uptake of methyl chloride (MeCl) and methylene chloride ($MeCl_2$) were determined, and ventilatory function measurements were made during exposure. These chemicals were selected because blood concentration data from exposed rats were already available for comparisons (Landry, et al., 1981; McKenna, et. al., 1979). Methyl chloride provided an example of chemical that exhibits linear kinetics from 50 to 1000 ppm exposure in rats (Landry et al., 1981) and $MeCl_2$ was an example of a chemical displaying saturable kinetics in rats exposed to 50 and 1500 ppm (McKenna et al., 1979).

MATERIALS AND METHODS

A. Test Materials and Analysis

Methyl chloride was obtained from Matheson Gas Products (Joliet, IL). Purity was specified to be >99.5%; this was confirmed by GC analysis with a flame ionization detector. Technical grade $MeCl_2$ was obtained from Dow Chemical USA. The stabilizer and principle impurities identified (those above 10 ppm) in the test material were cyclohexane (a stabilizer, 383 ppm), trans-1,2-dichloroethylene (100 ppm), vinylidene chloride (42 ppm), and water (22 ppm).

Test atmospheres were analyzed with a Varian 2400 gas chromatograph (Varian Instrument Co., Sunnyvale, CA). A six-port valve with a 1 ml sampling loop was used to load samples on the columns (6' x 1/8"; 60/80 mesh Tenax GC for MeCl and Poropak QS for $MeCl_2$), and peak height response of the flame ionization detector was used to quantitate MeCl and $MeCl_2$ concentrations. Standard curves were made prior to exposures by preparing a series of known concentrations of MeCl or $MeCl_2$ in 100 ℓ gas sampling bags made of Saran resin.

B. Animals

Male Fischer 344 rats (300 to 250 g when exposed) were obtained from the Charles River Breeding Laboratory. Rats were acclimated to the laboratory for at least one week prior to exposure. Animals were provided water and certified Purina Rodent Chow ad libitum (except during exposure) and were housed and exposed in rooms designed to maintain ~22°C, 40 to 60% relative humidity, and a 12 hour photocycle. Body weights were recorded prior to plethysmography measurements or inhalation exposures. Animals were assigned to test groups so that differences in mean body weights and variance would be minimized.

C. Exposure Apparatus

This apparatus (Figure 1a) employed a wire mesh restraining tube to support the body of the rat. The rat's head was exposed in an acrylic plastic chamber. The conical-shaped exposure chamber was designed to minimize dead volume and rebreathing of test atmosphere. A rubber diaphragm was placed around the rat's neck to seal the exposure chamber from the plethysmograph chamber. A dual exhaust manifold prevented the rat from inadvertently blocking the chamber exhaust.

The test atmosphere was pumped from a gas sampling bag containing the desired concentration of test material. An FMI pump (Fluid Metering Inc., Oyster Bay, NY) was used to produce a 300 ml/min chamber airflow, as measured with a soap bubble flowmeter. Since the FMI positive displacement pump could cause displacement of the rubber diaphragm seal around the rat's neck, a 4 ℓ ballast reservoir was placed in the airstream to reduce significant pressure fluctuations due to the pump. The head-only exposure apparatus was tested for possible adsorption of test material by passing known concentrations of MeCl and $MeCl_2$ through the device without a rat. No uptake of either material was detected.

D. Plethysmography

An aluminum tube (Figure 1b) was placed over the rat restraining tube 1 to 2 minutes prior to making ventilatory function measurements. The aluminum tube had a brass port (7 mm i.d.) that allowed connection with a Fleisch pneumotachograph (Gould Inc., Oxnard, CA). A pressure transducer (Grass PT5A; Grass Instrument Co., Quincy, MA) created an electrical signal proportionate to the pressure produced by airflow in the pneumotachograph. The signal from the pressure transducer was processed by a Beckman Dynograph strain gage coupler and amplifier (Beckman Instrument Inc., Schiller Park, IL). The amplifier output was connected to an LLL 8080 microcomputer with an analogue to digital convertor. The digitalized airflow data were used to calculate respiratory frequency, tidal volume, and minute volume (RMV).

E. Vapor Uptake Measurements

Air entering and leaving the exposure chamber (provided at 300 ml/min) was sampled at 12 ml/min via 1/8" Teflon tubing. A stainless steel 3-way valve (Whitey Co., Cleveland, OH) was used to select the sampling line.

The concentrations entering the chamber were normally measured in duplicate before and after measuring the test material concentration in the air leaving the chamber (also measured in duplicate). This procedure maximized the sensitivity to small differences between intake concentration and exhaust concentration.

The uptake of test material was calculated according to equation (1):

$$\mu M/min = (a-b)c \div 24\ \ell/M \qquad (1)$$

where a equals concentration entering chamber (ppm, i.e. μM(test material)/M(air)), b equals concentration leaving chamber (ppm), and c equals chamber airflow (0.3 ℓ/min).

The ratio of expired to inspired concentrations of test material (R) was also calculated:

$$R = 1-[(1-b/a)(c/RMV)] \qquad (2)$$

where RMV is the respiratory minute volume in ℓ/min.

Vapor uptake was measured at 1.5 and 2 hours (MeCl) and at 2.5 and 3 hrs ($MeCl_2$). These times corresponded to an apparent steady state of parent compound in blood for inhaled MeCl (Landry, et al., 1981) and $MeCl_2$ (McKenna, et al., 1979). At the end of the $MeCl_2$ exposures, air from the chamber exhaust was collected for ~10 min in a gas sampling bag and the carbon monoxide concentration was measured with an Ecolyzer (Energetics Science, Inc., Elmsford, NY). The instrument was calibrated with a known concentration of CO and checked for interferences with chamber exhaust air from a nonexposed rat.

RESULTS

A. Plethysmograph Optimization

Calibration of the airflow measurement device (consisting of the pneumotachograph, pressure transducer, amplifier, and microcomputer) was routinely checked by pumping air from a syringe through the pneumotachograph. The microcomputer then calculated the volume of the air displaced by the syringe by integrating the airflow through the pneumotachograph. This system was also calibrated with a rat carcass inside the plethysmograph, and a piston displacement pump (FMI model RP G-150) connected to the plethysmograph to simulate breathing. The volume of air calculated by the microcomputer was 15% lower than that indicated by the calibration check with the syringe. This difference was incorporated into the calculations as a correction factor.

Because uptake measurements are dependent on quantitating the difference in concentration entering and leaving the exposure chamber, the minimum adequate airflow for normal respiration was desired to maximize sensitivity. Alterations in respiratory frequency and RMV reflect the adequacy of the air supply. Airflows of 200, 300, and 500 ml/min were then evaluated with 5 rats/group. Each rat was placed in the restraining device and measurements were made at 5, 10, 15 and 20 min (Table 1). A two-way repeated measures ANOVA of these data indicated an airflow-related effect on RMV, and a time-related effect on variance of respiratory parameters. RMV values in rats provided 200 ml/min were considerably greater than those of rats provided 300 or 500 ml/min (Table 1). However, the difference in RMV between the groups provided 300 ml/min or 500 ml/min was not statistically significant ($p < 0.05$). Rats provided 300 (or 500) ml/min airflow (without test material) initially struggled within the restraining device, and had elevated respiratory frequency and RMV (Figure 2). After ~10 min, the respiratory parameters appeared to have decreased in magnitude, and were somewhat stabilized. Since 300 ml/min was approximately 1.5 to 2x the rat's RMV, and it resulted in similar respiratory physiology values to rats provided 500 ml/min; 300 ml/min was selected as a minimum adequate airflow.

B. Uptake and Respiratory Measurements

Methyl chloride uptake rate and the ratio of MeCl concentrations in expired and inspired air are provided in Table 2. Measurements made at 1.5 and 2.0 hr of exposure revealed no detectable change in uptake rate. The uptake rate was 16.5 fold greater in rats exposed to 1000 ppm versus rats exposed to 50 ppm. This ratio was of borderline statistical significance ($0.05 < p < 0.1$, t test) when tested for a difference from the ratio of 20 as predicted by a linear model. Respiratory frequency, tidal volume, and RMV were measured concomitantly with uptake (Table 3). Mean tidal volume and RMV were significantly less (t test, $p < 0.05$) in rats exposed to 1000 vs 30 ppm MeCl.

Methylene chloride uptake rate, ratios of inspired to expired air concentrations, and expired air CO concentrations are provided in Table 4. Measurements made at 2.5 and 3.0 hr of exposure revealed no detectable change in uptake. Neither the $MeCl_2$ uptake rate nor the end exposure expired air CO concentrations were proportionate to exposure concentration. There was only a 5 fold difference in uptake rate and 3 fold difference in expired air CO concentration despite a 30 fold difference in exposure concentration. There were statistically significant differences in mean respiratory frequency, tidal volume, and RMV between rats exposed to 1500 versus 50 ppm $MeCl_2$. The mean RMV was 28% less in 1500 ppm exposed rats.

DISCUSSION

An important feature of the exposure device/plethysmograph combination is that simultaneous measurement of uptake (i.e. metabolism) and respiratory parameters is possible. Exposure concentration dependent reduction of RMV will reduce the rate at which a test atmosphere is inspired. This effect will reduce uptake of the material, if uptake is limited by ventilation.

A pharmacokinetic model describing the fate of inspired MeCl allowed a quantitative analysis of the interrelationships of MeCl exposure concentration, RMV, uptake, and blood MeCl concentration. (Blood MeCl concentrations were measured in male Fischer 344 rats under similar exposure conditions by Landry, et al., 1981). In this pharmacokinetic model (Figure 3)[1], MeCl inspiration rate was equal to chamber concentration times RMV. Blood concentration and steady-state uptake data were used to obtain the least squares estimate of rate constants for expiration and metabolism of MeCl and intercompartment exchange, and the volume of the central compartment (V1). The values obtained for these parameters are provided in Table 3. The simulations of blood concentration data are provided in Figure 4. After data from the 1000 ppm exposure were fit, estimates were made for the rate of expiration and metabolism of MeCl during and after a 50 ppm exposure. The RMV measured during the 50 ppm exposure was incorporated into these estimates. Attempts to obtain reasonable fits with other combinations of V1 and rate constants were not successful.

This model showed that although RMV was diminished by 20% in 1000 ppm exposed rats relative to 50 ppm exposed rats, the relative decrease in MeCl inspiration did not entirely account for the apparently non-linear uptake rates. (The mean uptake rate in 1000 ppm exposed rats was 16.5 fold, not 20 fold greater than in 50 ppm exposed rats.) Rate constants for both metabolism (k_m) and expiration (k_{1E}) were affected by exposure concentration (Table 5). This suggests that, <u>at steady state</u>, MeCl uptake rate was not strictly ventilation limited.

Methyl chloride inspiration rate (chamber concentration times RMV), metabolism rate (k_m times the amount of MeCl in compartment 1 according

[1]The computer program is provided in Appendix A.

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to the model), and expiration rate (k_{1E} times the amount of MeCl in CL) are shown in Figure 5. The expiration rate is depicted in Figure 5 to illustrate that, at steady state, expiration plus metabolism equals MeCl inspiration. An analogous depiction of the fate of inhaled carbon disulfide (CS_2) in humans was made by Damus (1967), who plotted concentration of inspired and expired CS_2. Division of MeCl inspiration and expiration rates (Figure 5) by RMV gives concentration of MeCl in inspired and expired air. The integration of rates of MeCl inspiration, metabolism and expiration yielded values for the amount of MeCl that was inspired, metabolized during and after exposure, or expired unchanged. These amounts are illustrated in Figure 5.

The amount of MeCl inspired during exposure (areas B plus M plus E in Figure 5) may be considered to be the administered dose of MeCl. The effective dose is the amount of MeCl that remains in the body at the end of exposure (B) plus the amount that has been metabolized (M). The pharmacokinetic model used to describe MeCl allowed an estimate of the effective dose to be made for rats exposed to 50 or 1000 ppm MeCl (Table 5). The effective dose resulting from exposing 226 g F344 rats to 50 or 1000 ppm were estimated to be 3.8 and 67 mg/kg, and were nearly proportionate (67/3.8=17.6) to the exposure concentrations. The small difference from the ratio of exposure concentrations (1000/50=20) apparently reflects slightly less MeCl inspiration and metabolism (relative to exposure concentration) in the 1000 ppm exposed rats. As indicated in Figure 5, most of the effective dose consists of MeCl metabolized during exposure, rather than retained as parent compound.

Methylene chloride metabolism was saturable in male Sprague-Dawley rats: apparent steady state plasma $MeCl_2$ concentrations were disproportionately greater in 1500 versus 50 ppm exposed rats, and post-exposure excretion of $MeCl_2$, CO_2, and CO were consistent with a marked degree of exposure concentration dependent metabolism in animals exposed to 1500 vs 50 ppm (McKenna et al., 1979). Although the values provided by McKenna are not directly comparable to our uptake measurements, the demonstration of exposure concentration dependent metabolism by different methods helps to confirm the value of the uptake and CO expiration measurements.

The marked exposure-concentration dependence of $MeCl_2$ uptake cannot be accounted for by the 28% decrease in mean R/V of 1500 versus 50 ppm exposed rats. The uptake is largely related to saturable $MeCl_2$ metabolism, and not simply a physiological response to inhaled $MeCl_2$.

Andersen, et al. (1980) and Filser and Bolt (1981) have described methods that utilize the recirculation of test atmosphere in a closed system. These methods have been used to measured uptake of a number of materials, and are somewhat similar to the methods described in this paper. However, our dynamic exposure apparatus measures uptake at steady state, and respiratory physiology parameters can be evaluated during exposure with the whole body plethysmograph. Other differences include the possible interaction of desiccants and adsorbents with test material in the recirculating system, and the potential differences in measurement sensitivity of the two systems.

Andersen (1980) measured uptake in male Fischer 344 rats exposed to MeCl in a closed recirculating system. Based on Andersen's equation, the metabolic rate in 50 and 1000 ppm exposed rats would be 0.17 and 1.8 nM/min/g. There appears to be reasonable agreement in estimated rates of metabolism (our values were 0.20 and 3.3 nM/min/g) despite the differences between the methods.

Gahalan et al. (1982) have demonstrated the utility of uptake measurements to determine metabolic rates in humans. These authors measure RMV and anesthetic concentration in inspired and expired air to estimate clearance and metabolism of isoflurane and halothane. These data allowed determination of V_{max} and K_m for halothane which was related to the clinical use of this anesthetic.

The measurement of uptake under dynamic exposure conditions is suitable for a variety of chemicals that are rapidly metabolized (so that the difference between inspired and expired concentrations is quantifiable), and that attain steady-state in a reasonable period of time. Elimination of parent compound must either be predominantly via exhalation, or other routes of elimination must be quantitated. For example, urinary excretion of parent compound might be erroneously interpreted as metabolism.

Important features of gas uptake measurements make it a useful tool. Precise measurement of vapors or gases in a test atmosphere is normally much easier than other assessments of metabolism rates (eg radiotracer disposition studies or analysis of parent compound in blood). Also, uptake (and dose) have not been commonly measured in inhalation studies. When sufficient pharmacokinetic information is

available, gas uptake measurements allow the evaluation of dose in relation to exposure concentration utilising measured respiratory minute volume and uptake.

The device and methods described here provide a useful means of assessing apparent steady-state metabolism of an inhaled vapor and respiratory physiological parameters (frequency, tidal volume, and minute volume). These determinations were applied to MeCl and $MeCl_2$, and resulted in values for metabolic rates that were consistent with what is currently known. Methyl chloride metabolism was predominantly linear, and $MeCl_2$ was markedly non-linear over the concentrations tested. Incorporation of uptake values into a pharmacokinetic model allowed an estimation of dose in rats exposed to 50 or 1000 ppm MeCl.

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LIST OF FIGURES

Figure 1 — Head-only exposure apparatus (Figure 1a), including wire mesh restraining tube, conical exposure chambers, and stand. Exposure apparatus with plethysmograph tube in place (Figure 1b). The vent on top of the plethysmograph is connected to a pneumotachograph.

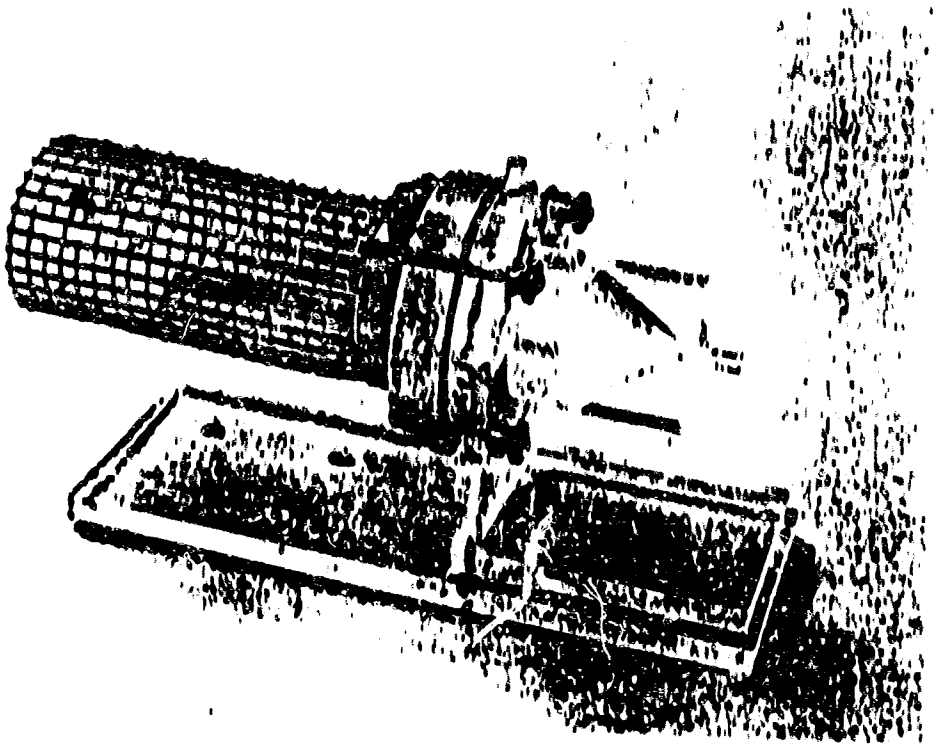
Figure 2 — Respiratory frequency (Figure 2a) and RMV (Figure 2b) in 5 rats provided 300 ml/min of chamber airflow each (Values are X±SD).

Figure 3 — Pharmacokinetic model for inhaled MeCl. Input = chamber concentration (CC) times RMV. First order rate constants include k_{1E} (exhalation of MeCl), k_m (metabolism), and k_{12} and k_{21} (transport constants between compartments). Compartment 1 includes blood.

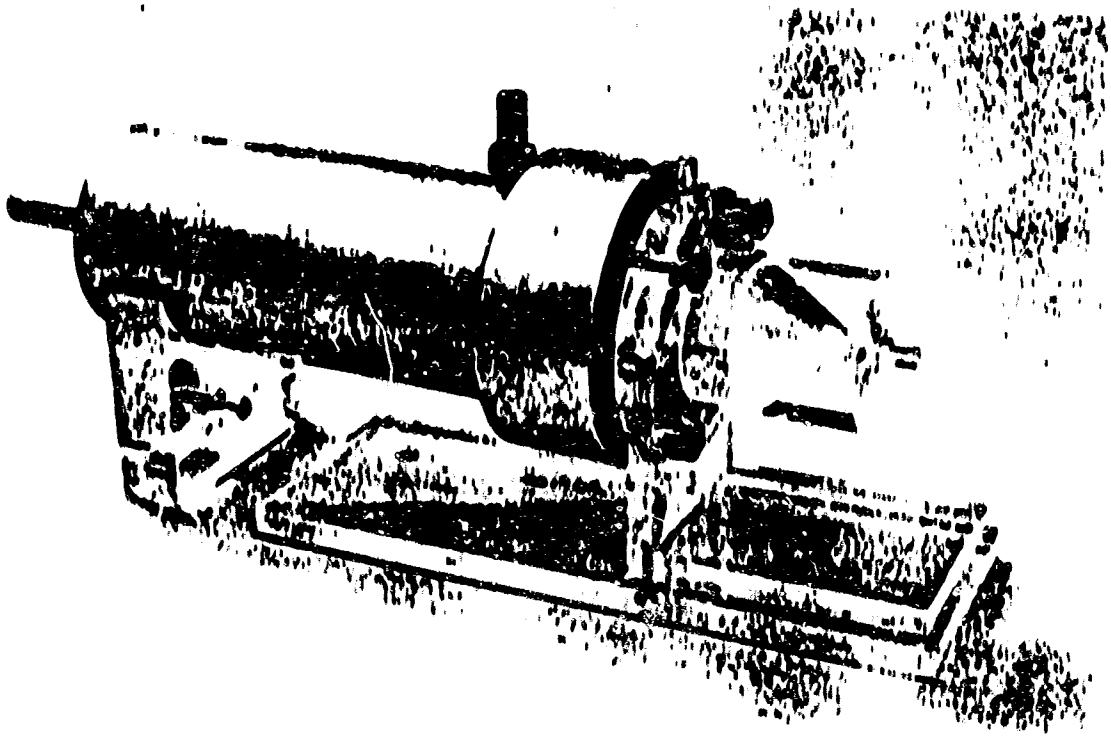
Figure 4 — Blood MeCl concentrations in rats during and after a 360 min MeCl exposure to 50 ppm (■) or 1000 ppm (●). Data points are from individual rats from groups of 4 per exposure concentration (data from Landry, et al., 1981). The lines drawn through the data points correspond to the pharmacokinetic model in Figure 3.

Figure 5 — Fate of inhaled MeCl in rats exposed to 50 ppm or 1000 ppm. The lines represent MeCl inspiration rate (- - - -), metabolism rate (————), and expiration rate (—— ——). The shaded area corresponds to the dose of inhaled MeCl. These lines are derived from a computer model that utilized chamber concentration and RMV to calculate MeCl inspiration, MeCl uptake in 50 (■) and 1000 ppm (●) exposed rats measured in duplicate 6 times/exposure concentration, and blood MeCl concentrations (as shown in Figure 4). Area B is equivalent to the amount of MeCl remaining in the body as parent compound when the exposure ends. Area M is equivalent to the amount of MeCl metabolized during exposure. Area E is the amount of MeCl that is expired during the exposure. The amount of MeCl inspired equals the sum of B, M, and E. When the exposure is terminated the MeCl in the body (B) can be expired as parent compound (Ep) or metabolized (Mp). The effective dose (B+M) is the area that is shaded grey.

FIGURE 1



a



b

FIGURE 2a

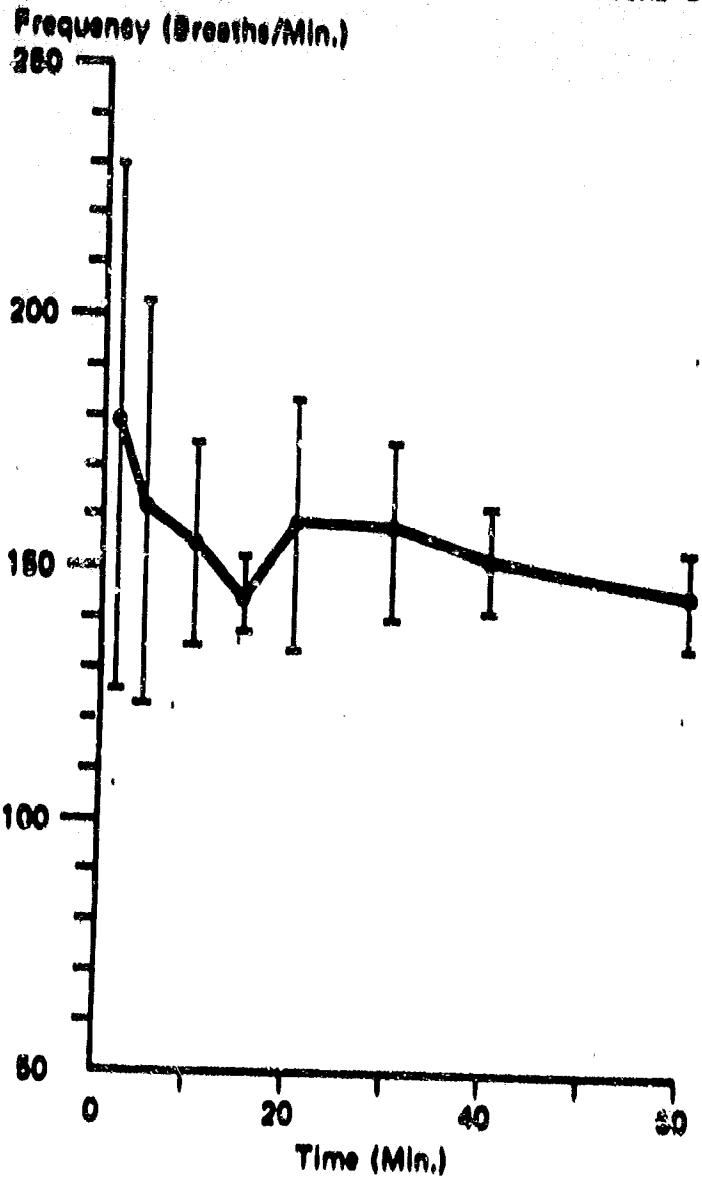


FIGURE 2b

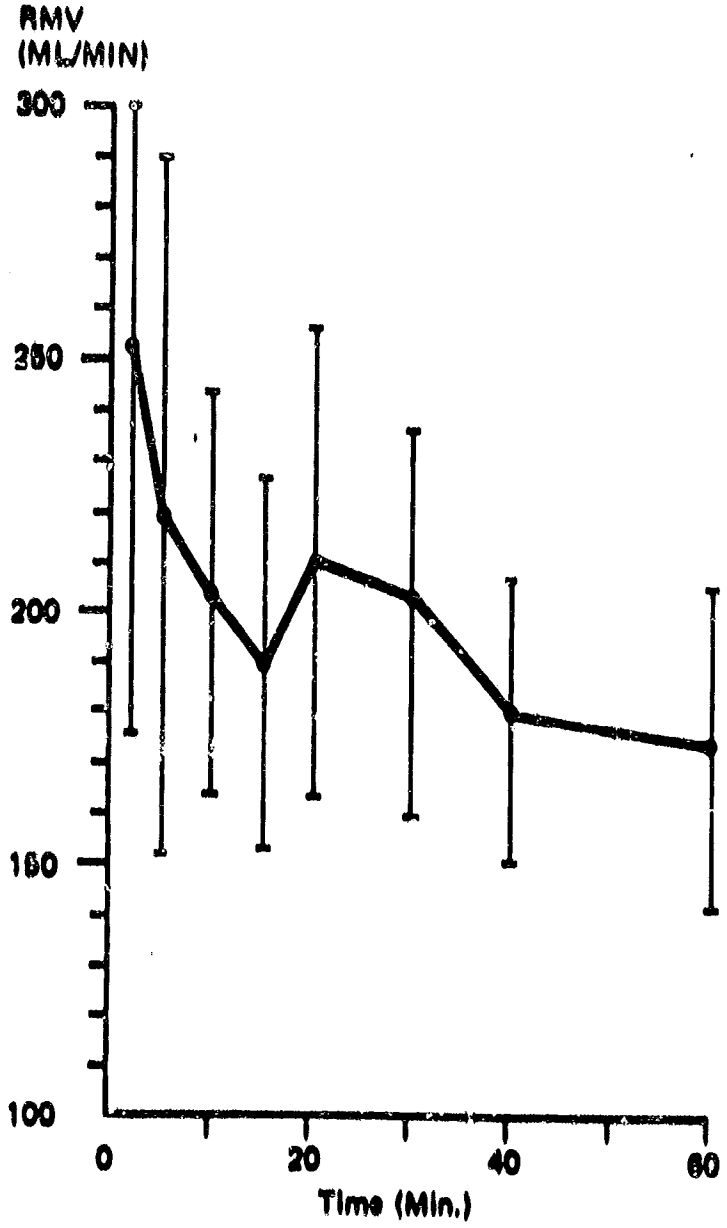


FIGURE 3

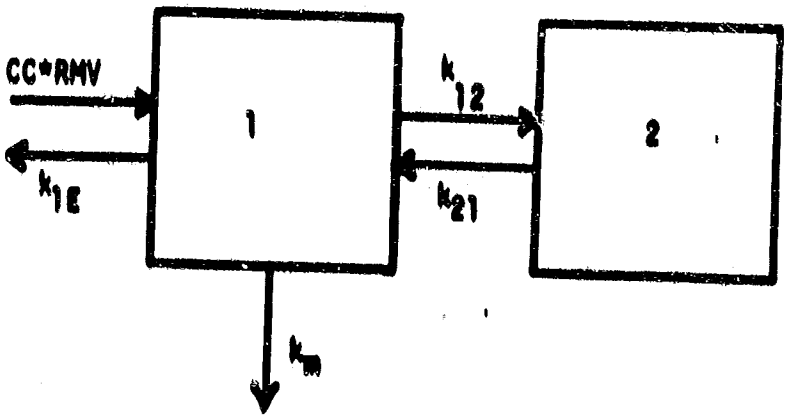


FIGURE 4

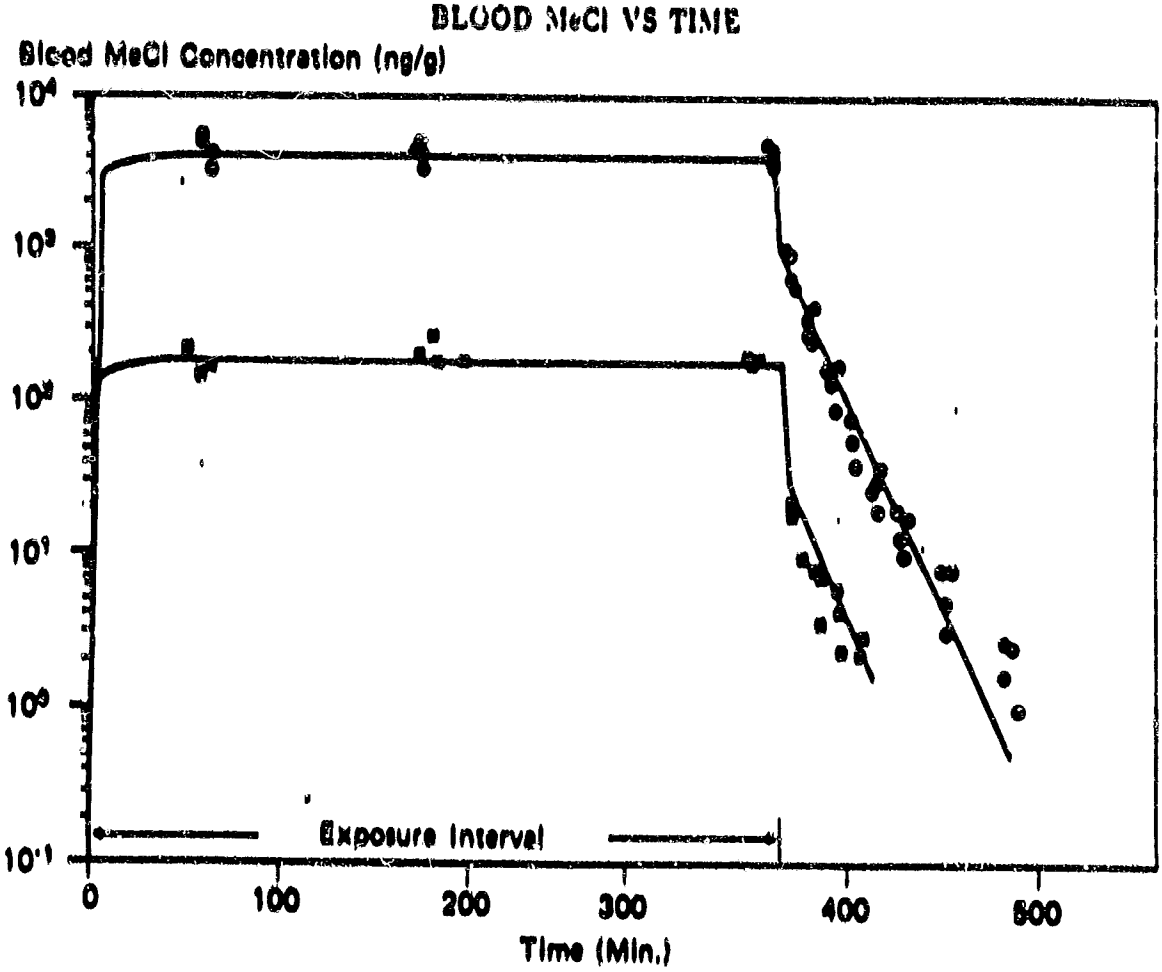

BLOOD MeCl VS TIME
Blood MeCl Concentration (ng/g)
10⁴
10³
10²
10¹
10⁰
10⁻¹
Exposure Interval
0
100
200
300
400
500
Time (Min.)

FIGURE 5

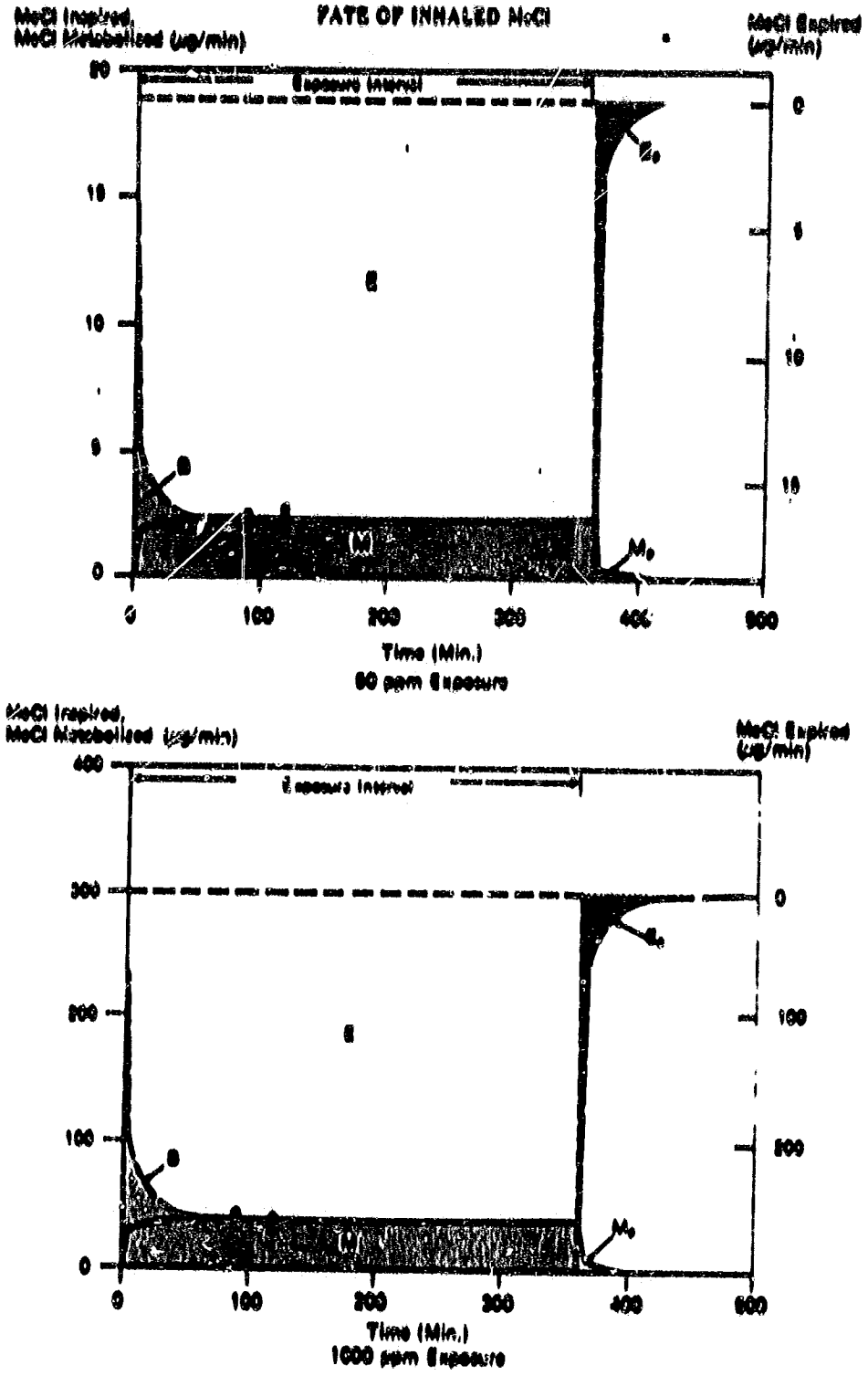

FATE OF INHALED MeCl
MeCl Inspired, MeCl Metabolized (µg/min)
MeCl Expired (µg/min)
Exposure Interval
E
B
M
E_0
M_0
Time (Min.)
50 ppm Exposure
MeCl Inspired, MeCl Metabolized (µg/min)
MeCl Expired (µg/min)
Exposure Interval
E
B
M
E_0
M_0
Time (Min.)
1000 ppm Exposure

TABLE 1

Respiratory Frequency and Minute Volume in Rats Provided 200, 300, or 500 ml/min Chamber Airflow

Sampling Time (min):	Respiratory Frequency (breaths/min) 5	10	15	20	MVV (ml/min) 5	10	15	20
Chamber Airflow (ml/min)								
200	156	159	167	156	265	255	268	250
	22	20	33	40	49	36	32	65
300	156	163	170	156	196	201	204	193
	24	37	35	25	52	39	49	15
300	163	155	145	160	221	203	190	210
	40	20	7	24	69	40	38	46
500	155	123	152	122	231	184	174	174
	29	13	37	19	66	27	39	26

Values are the mean (±SD) of 5 rats.

220002

TABLE 2

MeCl Uptake and Proportion of MeCl in Expired Air

[MeCl] (ppm)	MeCl Uptake ml/min/rat at 1.5 hr	2 hr	ml/min/g[a]	conc. expired/conc. inspired
50	48.5	52.5	0.22	0.84
50	43.9	38.9	0.19	0.86
50	40.9	43.2	0.19	0.88
$\bar{X}$±SD	44.7 ± 5.0		0.20 ± 0.02	0.86 ± 0.02
1000	774	774	3.55	0.85
1000	649	624	2.67	0.87
1000	809	786	3.58	0.86
$\bar{X}$±SD	736 ± 79		3.27 ± 0.51	0.86 ± 0.01

(Uptake values are provided as ml/min per rat and per gram body weight.)

[a]Mean of values obtained at 1.5 and 2 hr.

000024

TABLE 3

Respiratory Parameters in Rats Exposed to MnCl or $MnCl_2$

	MnCl		$MnCl_2$	
	50 ppm	1000 ppm	50 ppm	1000 ppm
Respiratory Frequency (breaths/min)	122±13	135±34	121±20	166±25
Tidal Volume (μl)	1.5±0.1	1.1±0.3	1.4±0.1	0.8±0.1
MMV (ml/min)	179±18	143±13	173±30	126±17

Values are $\bar{X} \pm SD$, n = 3 rats with duplicate measurements

00002

TABLE 4

$MeCl_2$ Uptake and CO Expiration

$[MeCl_2]$ (ppm)	$MeCl_2$ Uptake ml/min/rat at 2.5 hr	3 hr	ml/min/g[a]	conc. expired/conc. inspired	Expired Air [CO] ppm
50	133	131	0.55	0.65	5.3
50	121	111	0.52	0.66	3.1
50	138	139	0.63	0.61	6.0
$\bar{X}$±SD	129 ±11		0.56±0.05	0.64±0.03	4.8 ±1.5
1500	606	789	3.05	0.91	14.3
1500	771	645	3.12	0.90	15.3
1500	436	583	2.23	0.95	10.6
$\bar{X}$±SD	625 ±141		2.80±0.50	0.92±0.03	13.6 ±2.5

[a]Mean of values obtained at 2.5 and 3 hr.

020000

TABLE 5

Pharmacokinetic Parameters Describing the Fate of Inhaled MeCl in Rats

MeCl Exposure Concentration (ppm)	Steady-State Blood [MeCl] (mg/g)	MeCl Uptake Rate[a] (ml/min/g)	k_{1} (min^{-1})	k_{12} (min^{-1})	Effective dose for 6 hr exposure (mg/kg)
50	173	0.20	0.19	1.38	3.8
1000	3920	3.3	0.14	0.96	67

[a]Rats in both exposure groups averaged 226 g, values are ml MeCl/min/g of rat.

Values in this table (except exposure concentration) were obtained from the computer optimization.

000021

APPENDIX A1

DACSL Program for MeCl Pharmacokinetics

(1000 ppm Exposure)

```
00000100 PROGRAM = 'CM2A.ACSL'
00000200           'THREE COMPARTMENT'
00000300           'UPTAKE MEASURED'
00000400           'MECL BLOOD DATA FOR RATS'
00000500       INITIAL
00000600         CONSTANT POINTS    =  90.
00000700         CONSTANT TSTOP     = 490.
00000800         CONSTANT RMV       = 143.
00000900         CONSTANT K12       =   0.4443
00001000         CONSTANT K21       =   0.08885
00001100         CONSTANT KM        =   0.1393
00001200         CONSTANT K1E       =   0.9418
00001300         CONSTANT V1        =  69.54
00001400         CONSTANT CC        =2100.0
00001500           CINT  =  TSTOP / POINTS
00001600       END$'OF INITIAL'
00001700       DYNAMIC
00001800         AKO = CC * RMV * (1. - STEP (359.9))
00001900         CE  =(K1E*A1)/RMV
00002000        DOSE = A1+A2+AM
00002010        D2=MASSIN-AE
00002100       TMASS = A1 + AE + A2 + AM
00002110       RE=(CC * RMV) - DAEDT
00002200      DERIVATIVE
00002300          ALGORITHM IALG=2
00002400          C1    = A1/V1
00002500          DA1DT = AKO - (K12 + KM + K1E) * A1 + K21 * A2
00002600          DA2DT = K12 * A1 - K21 * A2
00002700          DAEDT = K1E * A1
00002810          DAMDT = KM * A1
00002820          MASSIN = INTEG(AKO,0.0)
00002900            A1   =   INTEG(DA1DT,0.0)
00003000            A2   =   INTEG(DA2DT,0.0)
00003100            AE   =   INTEG(DAEDT,0.0)
00003200            AM   =   INTEG(DAMDT,0.0)
00003300        'DEFINE TERMINATION CONDITION'
00003400       TERMT( T .GE. TSTOP )
00003500     END$'OF DERIVATIVE'
00003600     END$'OF DYNAMIC'
00003700     TERMINAL
00003800     END$'OF TERMINAL'
00003900 END$'OF PROGRAM'
```

APPENDIX A2

DACSL Program for MeCl Pharmacokinetics

(50 ppm Exposure)

```
00000100 PROGRAM - 'GM4A.ACSL.'
00000200           'THREE COMPARTMENT'
00000300           'UPTAKE MEASURED'
00000400           'MECL BLOOD DATA FOR RATS'
00000500       INITIAL
00000600         CONSTANT POINTS    =  90.
00000700         CONSTANT TSTOP     = 410.
00000800         CONSTANT RMV       = 179.
00000900         CONSTANT K12       =   0.4463
00001000         CONSTANT K21       =   0.08003
00001100         CONSTANT KM        =   0.1050
00001200         CONSTANT K1E       =   1.3750
00001300         CONSTANT V1        =  69.54
00001400         CONSTANT CC        = 105.
00001500           CINT  =  TSTOP / POINTS
00001600       END$'OF INITIAL'
00001700       DYNAMIC
00001800         AKO = CC * RMV * (1. - STEP (359.9))
00001900         CE  =(K1E*A1)/RMV
00002000        DOSE = A1+A2+AM
00002100       TMASS = A1 + AE + A2 + AM
00002110          RE = (CC * RMV) - DAEDT
00002200      DERIVATIVE
00002300           ALGORITHM IALG=2
00002400           C1     = A1/V1
00002500           DA1DT = AKO - (K12 + KM + K1E) * A1 + K21 * A2
00002600           DA2DT = K12 * A1 - K21 * A2
00002700           DAEDT = K1E * A1
00002800           DAMDT = KM * A1
00002810           MASSIN = INTEG(AKO,0.0)
00002900             A1   =   INTEG(DA1DT,0.0)
00003000             A2   =   INTEG(DA2DT,0.0)
00003100             AE   =   INTEG(DAEDT,0.0)
00003200             AM   =   INTEG(DAMDT,0.0)
00003300         'DEFINE TERMINATION CONDITION'
00003400       TERMT( T .GE. TSTOP )
00003500     END$'OF DERIVATIVE'
00003600     END$'OF DYNAMIC'
00003700     TERMINAL
00003800     END$'OF TERMINAL'
00003900 END$'OF PROGRAM'
```

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Dear Sir or Madam:

We are submitting the two attached lists of Health and Safety studies as required by the referenced rule.

The first list, marked D650, is a list of studies in progress as of the date of this submission.

Please note that the study entitled "Acrylamide: A Two-Year Drinking Water Chronic Toxicitity - Onogenicity Study in CDF Fischer 344 Rats" is submitted on behalf of the following companies who are participating in the study:

Dow Chemical
American Cyanamid
Exxon
Nalco

The second list, marked D652, contains references to unpublished studies of which we are aware but do not have a copy.

Very truly yours,

R. L. Hagerman
Regulatory Specialist
Regulatory and Legislative Issues
Health and Environmental Sciences
1803 Building
(517)636-6855

rt

Copy 4 of 8

Dow Chemical Company
D-650
Studies in Progress as of Date of Submission

1. A Mortality Study of Men Engaged in the Manufacture of Organic Dyes.
   Purpose: Examine the mortality experience of men assigned to production areas in which aromatic amines were present as raw materials or chemical intermediates.
   Data: Mortality data
   Progress: Report submitted for publication
   Date of Completion: Unknown - when accepted for publication
   CAS Nos: 62533

2. In Vitro Reactivity Study of Alkylene Oxides
   Purpose: See title
   Data: Relative kinetic data
   Progress: Started Dec. 1, 1982
   Date of Completion: Report due 1984
   CAS Nos: 106887, 75218, 75569

3. Range-Finding Study of Methylchloroform Formulation XN 50120
   Purpose: See title
   Data: Mammalian toxicity
   Progress: Report being written
   Date of Completion: Jan. '83
   CAS Nos. 71556

4. Propylene Dichloride: Acute and Two-week Inhalation Study
   Purpose: See title
   Data: Toxicity in Rats, Mice and Rabbits
   Progress: Report being written
   Date of Completion: Jan '83
   CAS Nos: 78875

5. Methyl Chloride: Inhalation Toxicity in Female C57BL6 Mice Continuously or Intermittently Exposed for 12 Days
   Purpose: See title
   Data: See title
   Progress: Report to be written
   Date of Completion: First Quarter '83
   CAS Nos. 74873

6. Methylene Chloride: A Comparison of the Pharmacokinetics and Macromolecular Interactions of Inhaled ^{14}C-Methylene Chloride in Hamsters and Rats as Related to Toxicity
Purpose: Evaluate species differences in the fate of inhaled methylene chloride
Data: See title
Progress: Pathology in progress
Date of Completion: Sept. '84
CAS Nos: 79061

7. Acrylamide: A Two Year Drinking Water Chronic Toxicity - Onogenicity Study in DCF Fischer 344 Rats
Purpose: See title
Data: See title
Progress: Pathology in progress
Date of Completion: September '84
CAS Nos: 79061

8. Acrylamide: Pharmacokinetics in the Rat
Purpose: See title
Data: See title
Progress: Report to be written
Date of Completion: First quarter '83
CAS Nos: 79061

9. Acute Range-finding - Diglycidylether of Biphenol A
Purpose: See title
Data: See title
Progress: Report being written
Date of Completion: First quarter '83
CAS Nos: 1675543

10. Characterisation of Butylene Oxide Blood Levels, Macromolecular Binding and Hepatic Nonprotein Sulfhydryl Levels After Inhalation Exposure in Rats
Purpose: See title
Data: See title
Progress: Report being written
Date of Completion: First quarter '83
CAS Nos: 26249207

11. 1,1,1-Trichloroethane: Two-year Inhalation in Rats and Mice
Purpose: See title
Data:
Progress: Awaiting pathology
Date of Completion: June, '83
CAS Nos: 71556

12. Reduction Dechlorination of Chlorinated Methanes, Ethanes, & Ethylenes in Aqueous Solutions
    Purpose: Investigate non-biological, reductive dehalogenation of a series of chlorinated hydrocarbons in aqueous solution
    Data: Laboratory analytical data on concentration of chemicals in solution as a function of conditions
    Progress: Underway
    Date of Completion: Mid-1983
    CAS Nos: 75092, 71556

13. A Case-Control Study of Renal Cancer Mortality at a Texas Chemical Plant.
    Purpose: To determine if a possible etiologic agent for human renal cancer can be found related to occupation.
    Data: Mortality and past presumptive exposures to chemicals produced or used in the chemical plant.
    Progress: Preliminary report nearing completion
    Date of Completion: Early 1983

14. A Case-Control Study of Leukemia and Occupational Exposure to Ethylene Oxide.
    Purpose: To determine if past presumptive exposures to ethylene oxide are associated with leukemia.
    Data: Mortality and past presumptive exposures to ethylene oxide.
    Progress: Work histories on cases and controls being collected.
    Date of Completion: Early 1983.

15. A Cross-Sectional Study of Employees with Potential Workplace Exposure to Ethylene Oxide.
    Purpose: To determine whether those with potential exposure to ethylene oxide had a higher prevalence of abnormalities of the hematopoietic, hepatic, renal or reproductive systems than did an unexposed control group.
    Data: Medical surveillance and presumptive occupational exposure
    Progress: Preliminary report in process of review and revision
    Date of Completion: Early 1983

16. Methyl Chloride Mortality Study.
    Purpose To evaluate the mortality patterns of employees with past potential exposure to methyl chloride.
    Data: Mortality and past potential occupational exposure to methyl chloride.
    [illegible]: Collecting work history and exposure data.
    [illegible] of Completion: Mid-1983

17. 1,1,1-Trichloroethane

Purpose: Define the time course of 1,1,1-trichloroethane in human volunteers following a single six-hour inhalation exposure to 350 and 35 ppm.

Data: Human pharmacokinetic study

Progress: In progress

Date of Completion: February 1983

CAS Nos: 71556

18. Acrylamide

Purpose: Evaluate vibratory sensitivity in an exposed population and controls.

Data: Field survey

Progress: Preliminary data collected.

Date of completion: Unknown; Pending development and validation of methodology.

CAS Nos: 79061

Dow Chemical Company
D-652
References to Unpublished Studies by Others

1. Studies of the visual system in chronic acrylamide poisoned monkeys. Lapham, L.W., et. al.
   Dr. L. W. Lapham, University of Rochester Medical Center, Department of Pathology, Division of Neuropathology, Rochester, NY, 14642.

2. The integration of multiple measures in behavioral toxicology. Merison, W.H., et. al.
   Dr. W. H. Merison, University of Rochester Medical Center, Division of Toxicology, Rochester, NY, 14642