PB87-163531

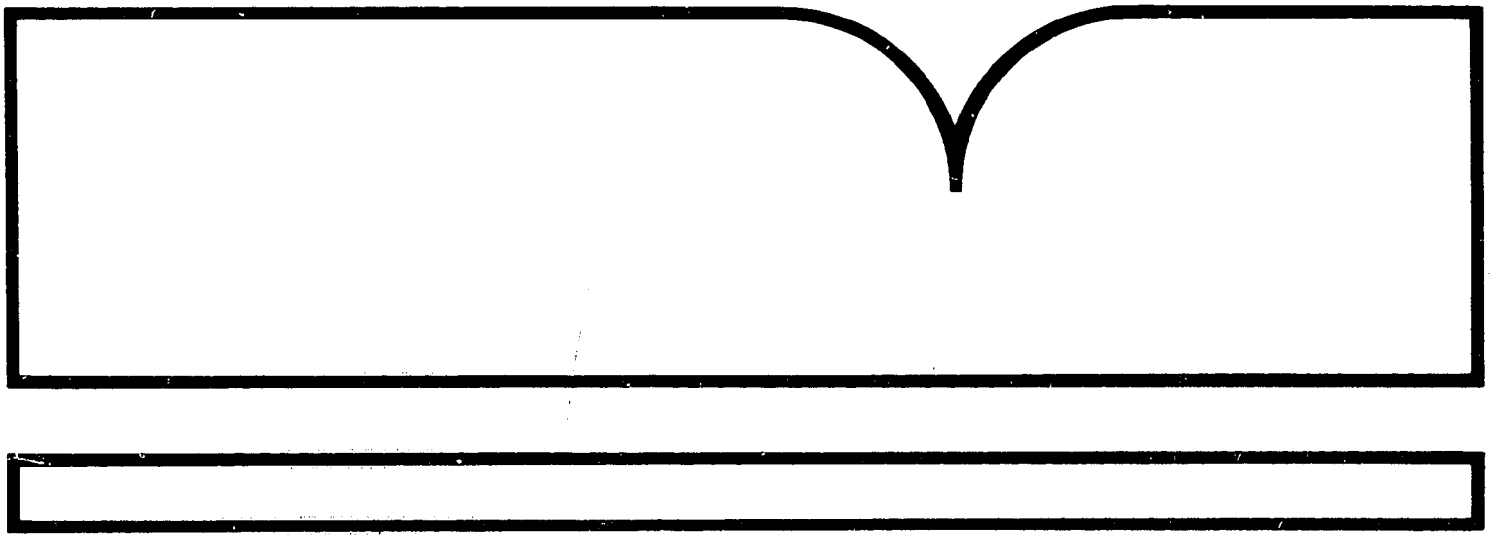
Toxicity Data for Establishing 'Immediately Dangerous to Life or Health' (IDLH) Values

Utah Univ. Research Inst., Salt Lake City

Prepared for

National Inst. for Occupational Safety and Health, Cincinnati, OH

31 Aug 78



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16. Abstract (Limit: 200 words)

Rats and guinea pigs (ten of each) were individually exposed to 30 minute inhalation tests using industrial chemicals to develop Immediately Dangerous to Life or Health (IDLH) values. The no effect (NE) level concentration was defined as that concentration producing none of the following treatment related effects in any animal: death, irreversible pathological lesions, or impaired escape ability during the exposure. The minimal effect (ME) level was defined as that concentration producing one or more of the treatment related effects in one or two animals regardless of sex or species. The frank effect (FE) level was defined as that concentration producing one or more of the treatment related effects in three or more animals regardless of sex or species. Findings for the chemicals tested were as follows: 1,2,3-trichloropropane (96184), NE at 799 parts per million (ppm) and FE at 2,080ppm; monochlorobenzene (108907), NE at 2,990 and FE at 5,850ppm; 1,1,2,2-tetrachloroethane (79345), NE at 576ppm and FE at 5,050ppm; 3-chloropropene (107051), NE at 1,300 and 11,800ppm; N,N-dimethylaniline (121697), NE at 194 and 953ppm; 1,1,2,2-tetrabromoethane (79276), NE at 19.9 and 91.1ppm; dimethoxymethane (109875), NE at 2,810 and 8,540ppm; and phenol (108952), NE at 187ppm and FE at 540ppm.

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FINAL REPORT

Toxicity Data for Establishing "Immediately Dangerous to Life or Health" (IDLH) Values

TR 1510-005

31 August 1978

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NOTICE

This report was prepared as an account of work sponsored by the National Institute for Occupational Safety and Health under Contract CDC-210-76-0143. It is not to be construed as a report, opinion, or recommendation of the National Institute for Occupational Safety and Health.

ABSTRACT

The Utah Biomedical Test Laboratory performed acute (30 minute) whole body inhalation toxicology exposures of animals to test atmospheres generated from each of eight industrial chemicals. The test and control animal groups consisted of 20 animals each, including 10 rats and 10 guinea pigs, with equal numbers of each sex.

Test animals were exposed simultaneously to a given test atmosphere by use of an inhalation chamber equipped with a drawer-type door which allowed rapid entry and exit of all animals concurrently. At the same time, control animals were exposed to air lacking the test compound in a similar, but separate, inhalation chamber.

Samples of the test atmospheres were collected with charcoal or silica gel adsorption tubes or with bubblers for subsequent analysis by flame-ionization detector gas chromatography.

The industrial chemicals evaluated were: 1,2,3-trichloropropane; monochlorobenzene; 1,1,2,2-tetrachloroethane; 3-chloropropene; N,N-dimethylaniline; 1,1,2,2-tetrabromoethane; dimethoxymethane; and phenol.

Exposure of the test animals to the various test atmospheres resulted in manifestations ranging from minor irritation to ataxia, tremors, narcosis, and/or death during, or soon after, exposure. Irreversible pathological lesions were found in organs of some test animals.

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PROJECT SUMMARY

This project entailed the acute (30 minute) whole body inhalation chamber exposure of groups of 10 rats and 10 guinea pigs, each animal individually caged, with equal numbers of both sexes, to various test compound concentrations in air. This work was performed by the Utah Biomedical Test Laboratory (UBTL) under contract to the National Institute for Occupational Safety and Health (NIOSH). The initial exposure concentration for each test compound (estimated IDLH level) was stipulated in the contract. However, subsequent test compound levels were determined on the basis of data collected from the initial exposure level of a given test compound.

This project was a study to determine test compound concentrations which will produce "no effect", a "minimal effect," and a "frank effect" in exposed test animals as defined in the contract. Visual observation of the test animals during the acute exposure and subsequently for 14 days was used to determine gross physiological effects such as: eye and nasal irritation, respiratory difficulties, central nervous system aberrations, incapacitation, and death. The body weight of each animal was monitored and on the 14th day after exposure, all surviving test and control animals were sacrificed. The weights of seven body organs in each test and control animal were determined and histopathological examination was performed on sections from these organs on 3 animals per sex per species for each exposure concentration.

The concentration of each test compound in the inhalation chamber test atmosphere was monitored by appropriate analytical methods at intervals during the exposure as specified in the contract.

After the second concentration exposures for several of the test compounds had been performed, there appeared to be some conflict between the overall goals of the project and the scope of work as specified in the original contract. The scope of work required UBTL to produce the "estimated IDLH level," "a minimal effect level," and a "frank effect level." The original criteria for categorizing either a "minimal" or

"frank" effect level relied mainly on animal deaths during exposure or during the 14 day postexposure period, and on irreversible histopathological tissue damage. UBTL attempted to produce exposure concentrations which would produce the required number of deaths in test animals, but observed that severe irritation or narcosis sometimes occurred without deaths in test animals. Further complications resulted because a "frank effect level" or even a "minimal effect level" for some test compounds appeared to be unattainable because of their relatively low toxicity at concentrations approaching their vapor saturation levels in air. However, no attempts were made to produce a higher level of toxicity by generation of aerosols, because work on the contract was terminated at the time aerosol generation was being contemplated.

After several exposures had been performed at UBTL and the data obtained from those exposures forwarded to NIOSH, the Project Officer revised the criteria for categorizing both "frank" and "minimal" effect levels. These revisions were formalized in a letter to UBTL dated 29 April 1977. The new definitions put more emphasis on the clinical observations during exposure and less emphasis on the total number of animal deaths occurring during, or after, a given exposure. Further discussion with the Project Officer about the criteria for categorizing the effect levels occurred following completion of the animal exposures. These discussions resulted in additional revisions to the criteria by the Project Officer, which were received in a letter to UBTL dated 22 August 1978. These finalized definitions, listed in Table 1, are used to categorize the exposure effects on the animals reported here.

A summary of the exposure effects on the test animals and the effect level categorizations for each experiment is presented in Table 2.

The chemicals which were to be tested are listed in Table 3, along with each chemical formula, molecular weight, NIOSH Toxic Substance List [1] reference number, some relevant toxicological data from the Toxic Substance List, the specified initial exposure concentration, and the threshold limit value. Dimethoxymethane was substituted by the Project Officer for the chlorophenylether compound originally specified in the contract. Also, Aroclor 1254 and phenylhydrazine were not tested in

Table 1

Definition of Effect Levels for Categorizing
Responses of Animals to Acute Inhalation Exposures

Exposure Effect Terminology	Finalized Revised Definition*
No Effect	A "no-effect" concentration produces none of the following treatment-related effects in any animal: 1. Death 2. Irreversible pathological lesions 3. Impaired escape ability during the exposure.
Minimal Effect	A "minimal effect" concentration produces one or more of the following treatment-related effects in one (1) or two (2) animals regardless of sex or species: 1. Death 2. Irreversible pathological lesions 3. Impaired escape ability during the exposure.
Frank Effect	A "frank-effect" concentration produces one or more of the following treatment-related effects in three (3) or more animals regardless of sex or species: 1. Death 2. Irreversible pathological lesions 3. Impaired escape ability during the exposure.

*Letter dated August 22, 1978, to William G. Yates, UBTL, from Trent R. Lewis, NIOSH.

Table 2

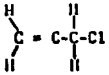
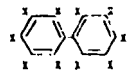
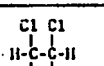
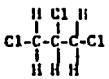
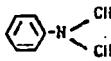
Summary of Exposure Effects on Test Animals and Effect Level Categorization

Test Compound	USTL Exp. #	Estimated IDLH Level (ppm)	Exposure Level (ppm)	OBSERVATIONS DURING EXPOSURE (Numbers indicate severity of symptoms[1])																		CATEGORIZATION CRITERIA[2] (Numbers indicate number of animals)								EFFECT LEVEL CATEGORY[4]
				Initial Hyper-activity		Reduced Activity		Narcosis		Ataxia		Tremors		Irritation: Eye		Eye Closure		Nasal		Oral		Escape Behavior		I: Impaired Escape Ability[3]	II: Deaths Test		Control		III: Irreversible Lesions[4] Test		Control		
				R	G	R	G	R	G	R	G	R	G	R	G	R	G	R	G	R	G	R	G		R	G	R	G	R	G	R	G	
1,2,3-trichloropropane	19-TCP-3	1000	799	+1	+1	+1	0	0	0	0	0	0	0	0	+1	0	+2	0	0	0	+1	0	0	no	0	0	0	0	0	0	0	0	No Effect
	1-TCP-1		2,080	+3	+3	+1	0	0	0	0	+2	0	0	+1	+3	0	+1	0	0	0	0	0	0	yes	1*	0	0	0	0	0	0	0	Frank
	10-TCP-2		5,010	0	0	+1	+1	+3	+3	+1	+1	0	0	+3	+3	0	0	+3	+3	0	0	0	0	yes	2	6	0	0	0	3	0	0	Frank
monochlorobenzene	2-CB-1	2400	2,990	+1	+1	0	0	0	0	0	0	0	0	+1	+3	0	0	0	+3	0	0	0	0	no	0	0	0	0	0	0	0	0	No Effect
	8-CB-2		5,850	+1	+1	+1	+2	+3	+3	+2	0	0	0	+2	+2	0	0	+1	+1	+2	+2	0	0	yes	0	0	0	0	0	0	0	1*	Frank
	16-CB-3		7,970	+1	+1	+3	+3	+4	+4	+3	+3	+2	+2	0	0	0	0	0	0	0	+3	0	0	yes	0	0	0	0	4*	4*	2*	5*	Frank
1,1,2,2-tetrachloroethane	3-TCE-1	150	576	0	0	+1	+1	0	0	0	0	0	0	0	+2	0	0	0	0	0	0	0	0	no	0	0	0	0	0	0	0	0	No Effect
	9-TCE-2		5,050	+1	+1	+4	+4	+4	+4	0	0	0	0	+1	+1	0	0	0	0	0	0	0	0	yes	3	0	0	0	0	0	0	1*	Frank
	15-TCE-3		6,310	0	0	+4	+4	+4	+4	+4	+4	0	0	0	+3	0	0	0	0	0	0	0	0	yes	5	3	0	0	3	1	0	0	Frank
3-chloropropene	4-3CP-1	300	1,300	0	0	0	0	0	0	0	0	0	0	+½	+½	0	0	0	0	0	0	0	0	no	0	0	0	0	0	0	0	0	No Effect
	13-3CP-2		11,800	+1	+1	0	0	0	0	0	+1	0	0	+1	+1	0	0	+1	+1	0	0	0	+1	no	0	2**	0	0	0	0	0	0	No Effect
N,N-dimethylaniline	5-NNDMA-1	100	194	+1	+1	+1	+1	0	0	0	0	0	0	+1	+1	0	+1	0	+1	0	0	0	0	no	0	0	0	0	0	1*	0	0	No Effect
	12-NNDMA-2		953	+2	+2	0	0	0	0	0	0	0	0	+2	+2	0	+2	0	0	0	0	0	0	no	0	0	0	0	0	0	0	0	No Effect
1,1,2,2-tetrabromoethane	6-TBE-1	10	19.9	+1	+2	+2	0	0	0	0	0	0	0	+1	+1	0	0	0	+3	0	+3	0	+1	no	0	0	0	0	0	0	0	0	No Effect
	11-TBE-2		91.1	+2	+2	0	0	0	0	0	0	0	+1	+2	+3	0	+2	+2	+2	0	0	0	0	no	0	0	0	0	0	0	0	2*	No Effect
dimethoxymethane	7-DMM-1	10000	2,810	+1	+1	+1	+1	0	0	0	0	0	0	0	0	0	0	+1	+1	0	0	0	0	no	0	0	0	0	0	0	0	0	No Effect
	14-DMM-2		8,540	+1	+1	0	0	0	0	0	0	0	+1	0	0	0	0	+1	0	0	0	0	0	no	0	0	0	0	4*	0	2*	0	No Effect
phenol	17-P-1	250	187	+1	+1	0	0	0	0	0	0	0	0	0	+1	0	+1	0	0	0	+1	0	0	no	0	0	0	0	3*	2*	4*	2*	No Effect
	18-P-2		540	+1	+1	0	0	0	0	0	0	+4	+4	0	+1	0	+1	+1	0	0	+1	0	0	yes	0	0	0	0	0	0	2*	0	Frank

1 Observations were scored numerically from 0 - +4: 0 = not observed and +4 = profound.
2 The criteria used to classify the exposure levels are defined in Table 1.
3 Impaired escape ability was judged to be present if any of the following were observed at +2 severity or greater: narcosis, ataxia, tremors. (All entries with "yes" involved at least 3 animals of one species. None of the eye closure observed was judged to impair escape ability in and of itself, because animals responded to sharp auditory stimuli unless, for example, they were narcotic.)
4 Three animals per species, sex, and exposure were submitted for histopathology.
* Apparently not exposure related.
** One animal was sacrificed due to a broken leg. The other died 2 days after exposure; the histopathology was inconclusive because of tissue autolysis, therefore, death has been judged not exposure related.

Table 3

Listing of 10 Industrial Chemicals to be Tested with Their Chemical Formula, Molecular Weight, Toxic Substance List Reference Number and Selected Toxicology Values from the Toxic Substance List

Name	Chemical Formula	Molecular Weight	Toxic Substance List Reference #	Toxicity Data Inhalation		Initial Exposure Chamber Concentration	Current TLV *
				Rat	Guinea Pig		
1,1,2,2-Tetrabromoethane		167.84	KI 82250	LCLo 1600 ppm 4 hr.		10 ppm	1 ppm
3-Chloropropene		76.53	UC 73500	LCLo 2000 ppm 4 hr.	LC 100 6360 ppm	300 ppm	1 ppm
Monochlorobenzene		112.56	CZ 01750			2400 ppm	75 ppm
Dimethoxymethane	$CH_3OCH_2OCH_3$	76.1	PA87500			10,000 ppm	1000 ppm
Aroclor 1254		Mixture	CF 61250			5 mg/m^3	0.5 mg/m^3
1,1,2,2-Tetrachloroethane		167.84	K 185750	LCLo 1000 ppm 4 hr.		150 ppm	5 ppm
1,2,3- Trichloropropane		147.43	TZ 92750	LCLo 1000 ppm 4 hr.		1000 ppm	50 ppm
N,N-Dimethylaniline		121.09	DX 47250			100 ppm	5 ppm
Phenol		94.12	ST 33250			250 ppm	5 ppm
Phenylhydrazine		108.08	MV 89250			250 ppm	5 ppm

*American Conference of Governmental Industrial Hygienists, "TLV's Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1975," 1975.

this study due to financial limitations. Additional data on the properties of each test compound from various sources are listed in Table 4 [2-9]. The "calculated" mass (mg) and volume (µl) of each test compound necessary to be vaporized per liter of air in order to produce the contract specified concentrations are listed in Table 5. This table also lists the approximate vapor saturation concentration of each test compound in air, which was calculated from vapor pressure data taken from references and listed in Table 4, and assuming a nominal barometric pressure of 640 torr and a nominal chamber temperature of 25°C. Since the temperatures for some of the vapor pressure data are higher than those used in the exposure atmospheres, the maximum concentration estimates were somewhat high.

Table 4.

TEST COMPOUND DATA FOR IDLH INHALATION TOXICITY PROJECT

TEST COMPOUND	UBTL ABREV.	M.W.	DENSITY	B.P. (°C)	VAP. PR. (Torr @°C)	VAP. D	FL. LIM. (%)	Auto. Ig. (°C)	FL.PT. (°C)
monochlorobenzene	CB	112.56	1.113	131.7	10 @ 22.2	3.88	1.3-7.1	638	29
1,1,2,2-tetrachloroethane	TCE	167.86	1.600	146.3	6	5.8	3.2-12.6	---	--
1,2,3-trichloropropane	TCP	147.44	1.389	156.2	4	5.0	---	304	82
1,1,2,2-tetrabromoethane	TBE	345.70	2.964	243.5 Dec.	~0.1 @ 20	---	---	---	--
3-chloropropene	3CP	76.53	0.938	44.6	368	2.64	3.3-11.2	392	-32
N,N-dimethylaniline	NNDMA	121.09	0.956	193.1	~ 1@ 30	4.17	---	371	63
phenol	P	94.12	1.072	181.9	1 @ 40	3.24	1.5-	715	79
phenylhydrazine	PH	108.08	1.098	243.5 Dec.	1 @ 72	3.7	---	174**	89
dimethoxymethane	DMM	76.1	0.864	42.3	330 @ 20	2.63	1.6-17.6	237	-18
AROCHLOR 1254	A1254	204-377	1.44	340-375	<1	---	---	---	176

**Phenylhydrazine may ignite at lower temperatures depending upon the contacting surface.

Table 5.

Calculated Test Compound Concentrations at Specified ppm and at Vapor Saturation in Air

TEST COMPOUND	UBTL ABREV.	INITIAL CONCENTRATION: (ppm@*)	(mg/l@*)	(ul/l@*)	EST. MAX. CONCENTRATION: (ppm@**)	(mg/l@**)	(ul/ l@**)	RATIO: MC/IC***	T.L.V. (ppm)
monochlorobenzene	CB	2400	9.304	8.359	15625	54.4	48.9	6.51	75
1,1,2,2-tetrachloroethane	TCE	150	0.867	0.542	9315	54.2	33.9	62.5	5
1,2,3-trichloropropane	TCP	1000	5.077	3.656	6250	31.7	22.8	6.25	50
1,1,2,2-tetrabromoethane	TBE	10	0.119	0.0402	∿156	∿1.9	∿0.64	15.6	1
3-chloropropene	3CP	300	0.791	0.843	575000	1516	1616	1916	1
N,N-dimethylaniline	NNDMA	100	0.417	0.436	∿1563	∿6.51	∿6.80	∿15.6	55
phenol	P	250	0.810	(0.756)	<1563	<5.06	(<4.72)	<6.25	5
phenylhydrazine	PH	250	0.931	0.848	<1563	<5.82	<5.30	<6.25	5
dimethoxymethane	DMM	10000	36.209	30.334	515600	1352	1965	51.6	1000
AROCHLOR 1254	A1254	5mg/m^3	5ul/1	3.4ul/1	----	----	----	----	0.05mg/m^3

*Calculations based on assumed conditions of 25°C and 640 Torr.
**Maximum concentrations estimated from reported (see Table 4) vapor pressure at approximately 20-72 °C.
***Ratio: MC/IC = Maximum Concentration/Initial Concentration

INTRODUCTION

The UBTL agreed to perform inhalation toxicity tests in animals of ten industrial compounds for NIOSH under Contract CDC-210-76-0143.

Background

The industrial chemicals tested at UBTL were reported to be immediately hazardous to a worker exposed to sufficiently high concentrations in air. This hazardous air concentration of a particular chemical is known as the "Immediately Dangerous to Life or Health" or "IDLH" level. This level is defined as the highest known concentration of the chemical which does not cause impairment of the ability to escape, nor irreversible adverse health effects, nor subsequent death, for up to 30 minutes of exposure. The logic behind this regulation is that if a worker were exposed to air concentrations up to the IDLH, for instance after failure of a respirator, he would have up to 30 minutes to escape. If a person were, however, required to work in an environment in which the ambient air concentration exceeded the IDLH level, it would be manditory that he wear a respirator which had been tested and approved as "highly reliable."

Objective

The purpose of the animal experimentation was to generate heretofore unavailable acute inhalation toxicology data in laboratory animals on ten industrial chemicals. The resultant data will be useful in setting, or verifying, IDLH values for the compounds tested.

GENERAL METHODS

This section contains general methods relevant to the project as a whole. The specific methods for each test compound are listed in the Specific Methods and Results Sections.

Procurement of Test Compounds

The contract required that the test compounds used to generate the inhalation atmospheres be of "commercial technical grade." UBTL contacted various chemical manufacturers directly concerning the procurement of test compounds which they manufactured [10].

The manufacturers from which we received the technical grade test compounds are listed in Table 6. We acknowledge the cooperation of those companies in supplying test compounds and relevant product information.

Design and Operation of Inhalation Chambers

UBTL constructed two inhalation exposure chambers in 1976, and used them in performing the acute inhalation exposure studies for this project. These chambers were patterned after those developed by R. G. Hinners of the National Center for Air Pollution Control in Cincinnati, Ohio [11,12].

These chambers (see Figures 1, 2, 3, 6, 7) are constructed of stainless steel and glass, are essentially cubical in shape with conical top and bottom sections, with dimensions of approximately 1.0 m (3.3 ft.) on a side, and contain a total volume of approximately 1260 liters (45 cubic feet). One chamber is equipped with a sliding-drawer system for rapid, concurrent insertion or removal of individually caged animals (10 rats and 10 guinea pigs). This chamber was used to expose the test animals to various test compound atmospheres. A second exposure chamber of similar construction was used to expose control animals to an atmosphere lacking the test compound. Visibility of the test animals is afforded by windows on all four sides of the exposure chambers.

Gate valves on the bottom cones of the chambers facilitate the removal of residual test compound and animal waste material. The chambers are cleaned by scrubbing with hot water and detergent.

Table 6.

Sources of Test Compounds for IDLH Study

1,1,2,2-Tetrachloroethane	Matheson, Coleman & Bell Norwood, Ohio
Aroclor 1254 (Chlorodiphenyl - 54% Cl)	Monsanto St. Louis, Missouri
N,N-Dimethylaniline	E.I. Dupont deNemours Wilmington, Delaware
Phenylhydrazine	Pfaltz & Bauer, Inc. Flushing, New York
1,1,2,2-Tetrabromoethane (Acetylene tetrabromide)	Dow Midland, Michigan
1,2,3-Trichloropropane	Dow Midland, Michigan
Phenol, U.S.P., Liquified*	J.T. Baker Chemical Company Phillipsburg, New Jersey
Phenol, U.S.P.	Union Carbide Corporation Texas City, Texas
3-Chloropropene (Allyl chloride)	Dow Midland, Michigan
Chlorobenzene	Dow Midland, Michigan
Dimethoxymethane	Celanese Chemical Corp. Bishop, Texas

*This material was used to generate test atmosphere for animal exposure.

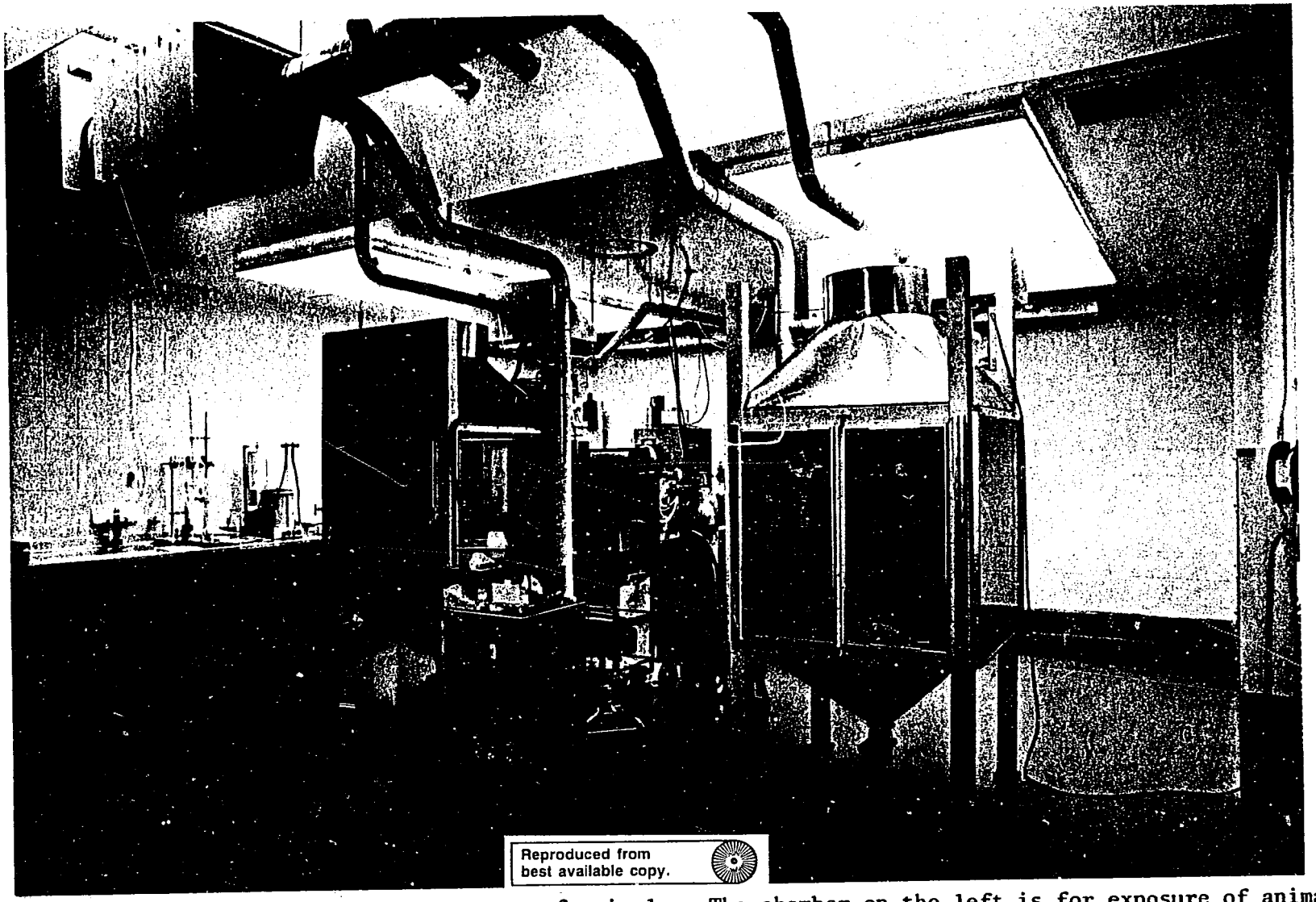


Figure 1: UBTL chambers for exposure of animals. The chamber on the left is for exposure of animals to compounds while the chamber on the right is for exposure of control animals.

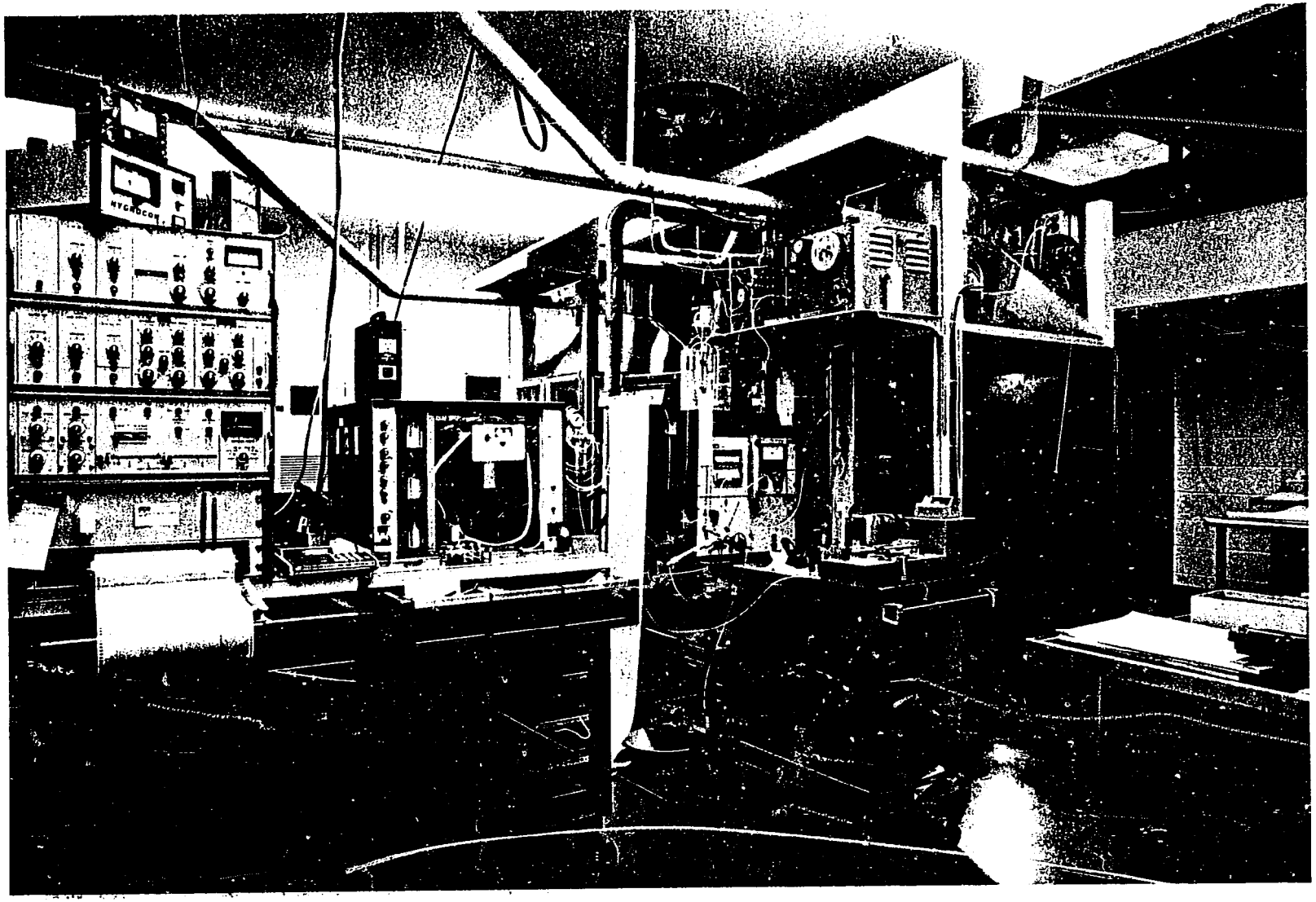
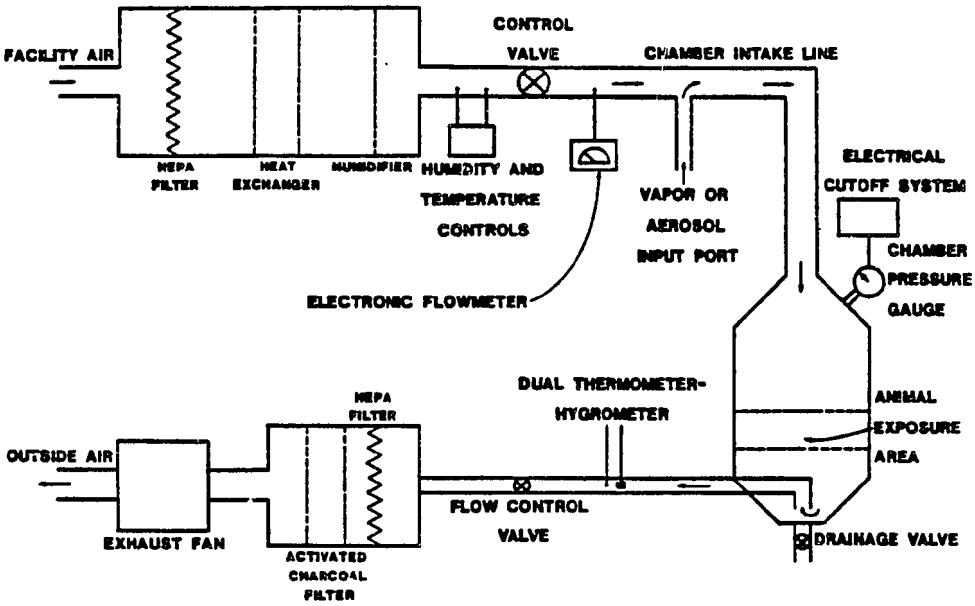


Figure 2: A view of UBTL gas chromatograph with on line gas sampling valve, liquid vaporizer and exposure chambers.



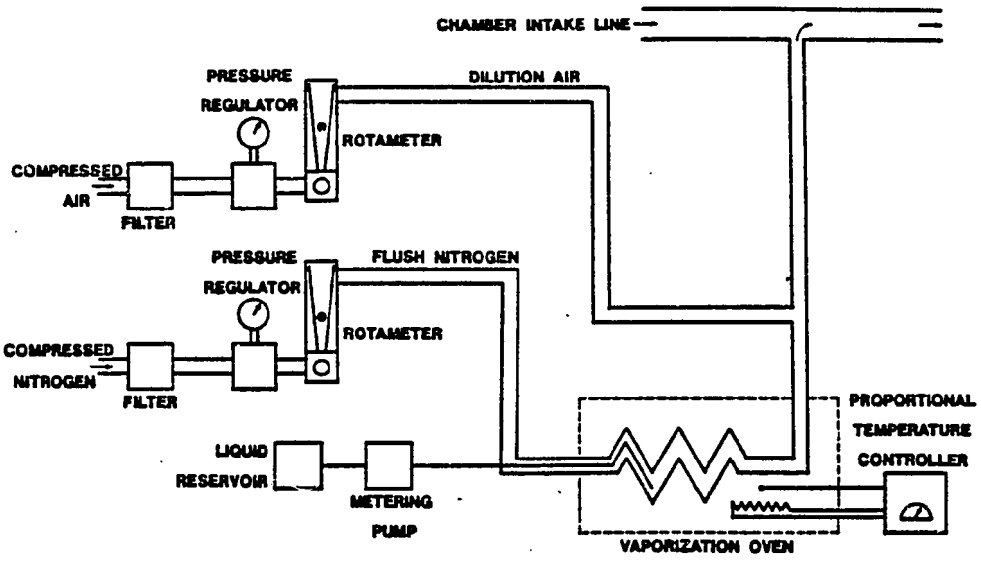


Figure 3: Schematic Of Exposure Chamber System (Above) And Liquid Vaporization System (Below).

Prior to entering the chambers, the air supply passes through an absolute filter and then through an air conditioning system which controls the temperature over a range from 13-30°C ± 3°C and the relative humidity (RH) over a range from 20-80% ± 5% RH. The animal exposures in this study were conducted at temperatures of 25-30°C and relative humidity of 20-30%. Air flow is adjustable over a moderate range (200-900 liters/minute) and in this study was usually supplied at 600 liters/minute, a rate which provides 29 chamber volume changes per hour. At this flow rate, the test atmosphere concentration is restored to 90% of the original nominal level within about 5 minutes after the chamber drawer is opened and closed.

An exhaust fan draws air through the inhalation chambers and into a filtering system consisting of an absolute particle filter (HEPA) and an activated charcoal bed filter (Westate Carbon) before exhausting to the outside. A negative pressure of approximately 8 to 19 mm of water is maintained within the chamber and exhaust system in order to reduce the possibility of contaminating the work area from system leaks.

Generation of Test Compound Atmospheres

After reviewing general references [13-16] and periodical literature [17-25] dealing with test atmosphere generation and analysis, UBTL designed and built a small prototype thermal vapor generator. Pilot tests using acetone as the liquid test compound proved satisfactory. Therefore, a larger capacity vapor generator of similar design was constructed for use in the animal inhalation experiments.

The vapor generator system (see Figures 2 and 3) consisted of a liquid metering pump which injected the liquid test compound into Teflon-lined stainless steel tubing (Alltech Associates, Catalog # 3142) which was wound around a temperature controlled aluminum cylinder. The injected liquid was rapidly vaporized and flushed from the stainless steel tubing with a low flow of nitrogen into a dilution stream of compressed air. This vapor generator output was then dispersed into the inhalation chamber intake duct for final dilution and mixing to produce the final test atmosphere for the chamber.

Two metering pumps of different flow capacity were necessary in order to adequately bracket the anticipated liquid test compound flow range from near 0 to over 50 ml/min (Fluid Metering, Inc., Models #RP1SY/CSC and #RP1G400/CSC). Optimum performance of these pumps required that they be mounted vertically, and that they be initially primed at atmospheric pressure before the output pressure was increased above atmospheric pressure. A pressure gauge (0-100 psig) was installed on the output side of the pump to monitor the liquid output pressure and the vaporization tubing pressure. All connections in contact with the liquid test compound were made with stainless steel fittings (Swagelok) and Teflon tubing was used throughout to carry the liquid (Alltech Associates Catalog #3134, Altex Catalog #200-31).

The tubing vaporization chamber was constructed with two, 15-foot lengths of Teflon-lined stainless steel tubing (1/8" OD x 0.083" ID) wound side by side around a 3 1/4" OD x 6" aluminum cylinder. Tee fittings were connected to the input ends of each stainless steel tube section. The output ends of both stainless steel tube sections were connected to the same tee, thus combining their vapor output. Each of the two tees on the input ends of the stainless steel tubes was connected to a nitrogen source and to small-bore Teflon tubing (0.8 mm ID x 1.5 mm OD). These small-bore Teflon tubes were inserted through the tees into the stainless steel tubes to a length of about 14 inches to insure that the liquid test compound would be delivered to a portion of stainless steel tubing which was in contact with the heated aluminum cylinder. The Teflon tubes were small enough in outside diameter to readily allow nitrogen to pass around them in the tees and stainless steel tubing.

Temperature control of the stainless steel vaporization tubing was provided by a proportional controller (RFL Industries, Model #70-115) with thermal sensors (RFL Industries, Model #27687-5 and #27687-7). These sensors, along with two cartridge heaters (Mitty Watt 600 and 300 watt sizes) were mounted inside the 3 1/4" OD x 6" aluminum cylinder, around which the Teflon-lined stainless steel tubing was wound. A thermally activated cutout switch (Fenwall #17202-0) was also mounted

within the aluminum cylinder as a thermal protective device. The aluminum cylinder with its internal components and external Teflon-lined stainless steel tubing was mounted in a 23 cm x 15 cm x 12 cm aluminum box and insulated with glass wool. The stem of a dial thermometer (McGatlin Instrument Company) was inserted from outside the box into the core of the aluminum cylinder as a means of monitoring the vaporization tubing temperature.

The vaporization tubing temperature was selected by adjustment of an external potentiometer connected to the proportional controller system. After a few minutes, steady-state temperature was established and little temperature variation was observed, thereafter.

The flows of nitrogen and compressed air were regulated by rotameters equipped with gas metering valves. The nominal nitrogen flow was 2 liters per minute and the nominal compressed air flow was 50 liters per minute.

The vaporizer output was mixed with the inhalation chamber intake duct air by use of an aluminum dispersion nozzle placed in the chamber intake duct. The nozzle consisted of an aluminum disc with several small-bore holes drilled radially into its center which was plumbed with an elbow fitting to allow it to be centered radially inside the intake air duct.

Monitoring of the inhalation chamber intake air flow was performed by the use of an air mass velocity meter (Sierra Instruments, #441) with its sensing probe placed in the inhalation chamber intake duct upstream from the vapor generator dispersion nozzle. The air flow in the duct was calculated by multiplying the air mass velocity reading by the ratio of the density of air under standard conditions (21.1°C, 760 torr) to nominal ambient UBTL conditions (25°C, 640 torr) and by the cross-sectional area of the intake duct. Then, by addition of the gas flow from the vapor generator, the total air flow into the chamber was calculated.

Inhalation chamber pressure was indicated by a dial pressure gauge (Dwyer, "Photohelic" #3302; 0 to ± 1 in H_2O) equipped with set point contacts connected to an alarm and an automatic electrical supply cutoff

relay system. If the chamber pressure varied outside preselected limits, then the electrical supply to the vapor generator would be cut off and a bell alarm would sound.

Liquid test compound mass flow was determined by placing the test compound container on a top loading balance (Mettler, #P1210N) and measuring the weight loss over a known time interval.

The concentration range of each test compound that could be produced was limited by the physical and chemical properties of that test compound and the operational characteristics of the UBTL vapor generation system.

The rate of vaporization of a liquid test compound is dependent on the area of the contacting surface, the temperature of the contacting surface, the heat capacity of the liquid, and the flow of flush gas. For each test compound, there was a maximum concentration that could be produced, above which unvaporized liquid test compound would be present in the vaporizer output line. However, even with this limitation, this system did allow the production of test compound levels ranging from 20 to 11,800 ppm, depending upon the compound.

A further limitation on the maximum test compound concentration that was generated was safety. It was mutually agreed by NIOSH and UBTL that generation of test compounds at concentrations within their known explosive limits would present undue hazard and should not be conducted.*

In order to determine how uniformly the test atmosphere was distributed within the inhalation chamber, a test atmosphere of acetone was generated and fed into the chamber. The distribution of acetone concentration with respect to position sampled in the inhalation chamber was determined using a gas sampling valve system with a Teflon sampling line. Four positions inside the chamber were sampled by use of a sampling line connected to a movable L-shaped metal rod. The rod was positioned such that the sampling tubing was centered between the back and front of the chambers. By turning the rod, each of the positions listed in Table 7 was sampled.

The distribution of acetone concentration within the inhalation chamber appeared to be quite uniform. Also, the acetone concentration

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*Letter dated April 26, 1977, addressed to A. U. Daniels, Ph.D., UBTL, from Trent R. Lewis, Ph.D., NIOSH.

Table 7

Examination of Acetone Vapor Concentration Dependence Upon Position Sampled in UBTL Inhalation Chamber (Gas Sampling Valve Injection - FID/GC Analysis)

Position in Chamber	GSV Loop #1 Peak Height (mm)	GSV Loop #2 Peak Height (mm)
Bottom Center		
Replicates	8	8
Mean	76.4	81.4
SD	2.9	0.5
CV	4%	1%
Top Center		
Replicates	9	9
Mean	75.0	81.6
SD	7.9	1.5
CV	11%	2%
Left Side Center		
Replicates	5	5
Mean	74.6	79.2
SD	4.8	0.5
CV	6%	1%
Right Side Center		
Replicates	3	3
Mean	78.3	81.7
SD	0.6	0.6
CV	1%	1%
Cummulative Sampling Response All Positions		
Replicates	25	25
Mean	75.8	81.0
SD	5.37	1.34
CV	7.09%	1.65%

remained relatively constant over a period of several hours. These data imply that a relatively uniform atmosphere can be produced in the chamber for relatively volatile test compounds.

Monitoring of Test Atmospheres

Initially, a gas sampling valve (GSV) flame ionization detector gas chromatography (FID-GC) system was used to monitor and calibrate the inhalation chamber test atmosphere concentrations while adsorption sampling tubes were used to confirm those test atmosphere concentrations at a later time by gas chromatographic analysis. However, the gas-sampling-valve-determined concentrations were consistently much lower than those obtained from the analysis of adsorption tube samples taken directly from inside the inhalation chamber during exposures.

Thorough examination of the gas sampling valve system did not disclose any significant leaks which would account for the lower apparent test atmosphere concentrations measured by the GSV-FID-GC system. Modification of the GSV system to reduce potential surface adsorption and/or absorption effects by installation of heated glass sampling tubing and heating of the gas sampling valve about 100°C above ambient temperature did not eliminate the measurement differences. Therefore, since the adsorption tube samples could be taken directly inside the chamber, they were assumed to be the more accurate and thus were used along with subsequent FID-GC analysis in subsequent experiments to calibrate and verify the inhalation chamber concentrations [14,33-51].

During a site visit at UBTL, Bill Moorman of NIOSH offered to lend UBTL a HNU #201 continuous gas monitor as an aid in monitoring the exposure chamber test atmosphere concentrations. UBTL accepted his offer and after arrival of the HNU #201 at UBTL, a low volume calibration vapor generator was constructed for use in calibration of the monitor. The low volume calibration vapor generator consisted of a rotameter equipped with a gas metering valve, a syringe pump (Harvard, #901) with a gas/liquid tight syringe (Hamilton #1001), a section of glass tubing (Pyrex 6 mm O.D.) as a vaporization chamber, and a sampling manifold [13, 14,34] (see Figure 4). The rotameter was calibrated with a spirometer

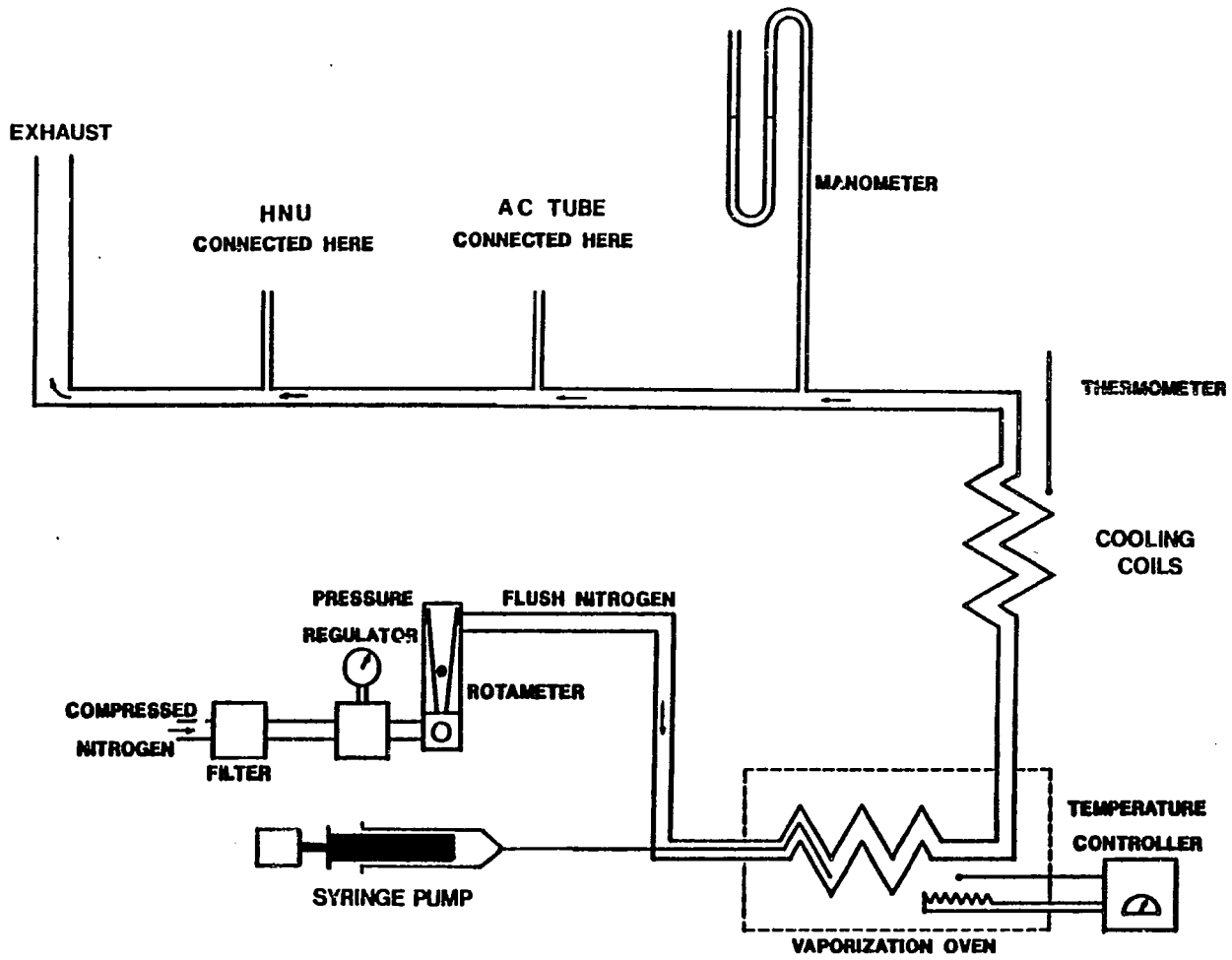

Figure 4: Low Capacity Vapor Generation System For Calibration Of HNU Monitor.

and an electric timer for a flow range from 1.84 to 32.6 liters per minute. The syringe pump was calibrated to deliver liquid test compound flows of 0.503, 1.02, 2.19, 5.04, 10.10, 20.73, and 52.89 µl per minute. By selecting the appropriate flows of nitrogen and test compound, it was possible to generate "nominally known" test compound atmospheres near those desired for calibration of the HNU #201 monitor [52]. The portion of the glass tubing where liquid was injected was heated as necessary with an electrical resistance wire that was wound around the outside of the glass tubing. Evaporation of the test compound could be observed through the glass tubing in the vaporization area and the absence of visible condensation in the cooling coil portion of the glass tubing implied that complete vaporization of the input liquid test compound had taken place. This calibration vapor atmosphere was then fed into a sampling manifold with connections for concurrent sampling by adsorption tubes, the HNU #201 organic vapor monitor, and a manometer. The excess calibration vapor atmosphere was vented into the inhalation chamber exhaust system.

Sampling of the calibration vapor generator output with charcoal adsorption tubes and subsequent FID-GC analysis produced results within 2% of those expected for 3-chloropropene and N,N-dimethylaniline, while results for 1,1,2,2-tetrabromoethane were within 10% of those expected. However, when the HNU #201 monitor which had been calibrated with the same test atmosphere was used to calibrate the inhalation chamber test atmosphere, the concentration measured by the monitor did not agree closely with that measured by the use of adsorption tubes with subsequent FID-GC analysis.

In order to reduce chamber calibration problems, an air velocity meter was installed to determine chamber intake flow, and a top loading scale was used to measure the mass flow of liquid test compound. By using the chamber air flow and liquid test compound flow, a nominal test compound atmosphere concentration can be calculated for "ideal" conditions. However, "ideal" conditions such as the following do not always prevail:

complete evaporation of all test compound injected into the vaporization tubing, no condensation of vaporized test compound in mixing area, no adsorption or absorption of test compound to exposed surfaces, and complete, instantaneous mixing of test compound with intake air. Thus, after the first few experiments, the conditions expected to produce a given concentration were established and adsorption tube samples were taken and analyzed by FID-GC. When necessary, adjustments in the vaporization conditions were made prior to animal exposure to insure that the test compound concentration was near that desired.

In some cases, a rat and a guinea pig were placed in the chamber concurrently with those preliminary test atmosphere samplings in order to determine what effects the given test atmosphere would have on the exposure group of animals.

The HNU #201 continuous gas monitor was used to monitor changes in test atmosphere concentration before and during the animal exposures. However, the HNU #201 was not a very satisfactory monitoring device for a few compounds due to its lack of sensitivity for the compound or due to a prolonged response to residual test compound, making it insensitive to rapid concentration changes.

Sampling and Analysis of Test Compound Atmosphere Samples

Sampling of test compound atmospheres for subsequent gas chromatographic or spectrometric analysis was performed by passing a "known" volume of test compound atmosphere through adsorption tubes (SKC, Inc., #226-01) or through fritted glass bubblers (Wheaton, #709283) [33,51].

The volume of test compound atmosphere sampled by adsorption tubes or bubblers was controlled by use of a vacuum sampling system consisting of a rotameter equipped with a gas metering valve (Brooks), a manometer, a combination activated charcoal/silica gel filter, a vacuum line pressure regulator (Porter), and a laboratory vacuum line system (see Figure 5). The vacuum sampling system was calibrated for a given rotameter setting by using a soap film flow meter and an electric timer [13,33,36]. During actual test atmosphere sampling, the rotameter was adjusted to the same setting used during its calibration for each

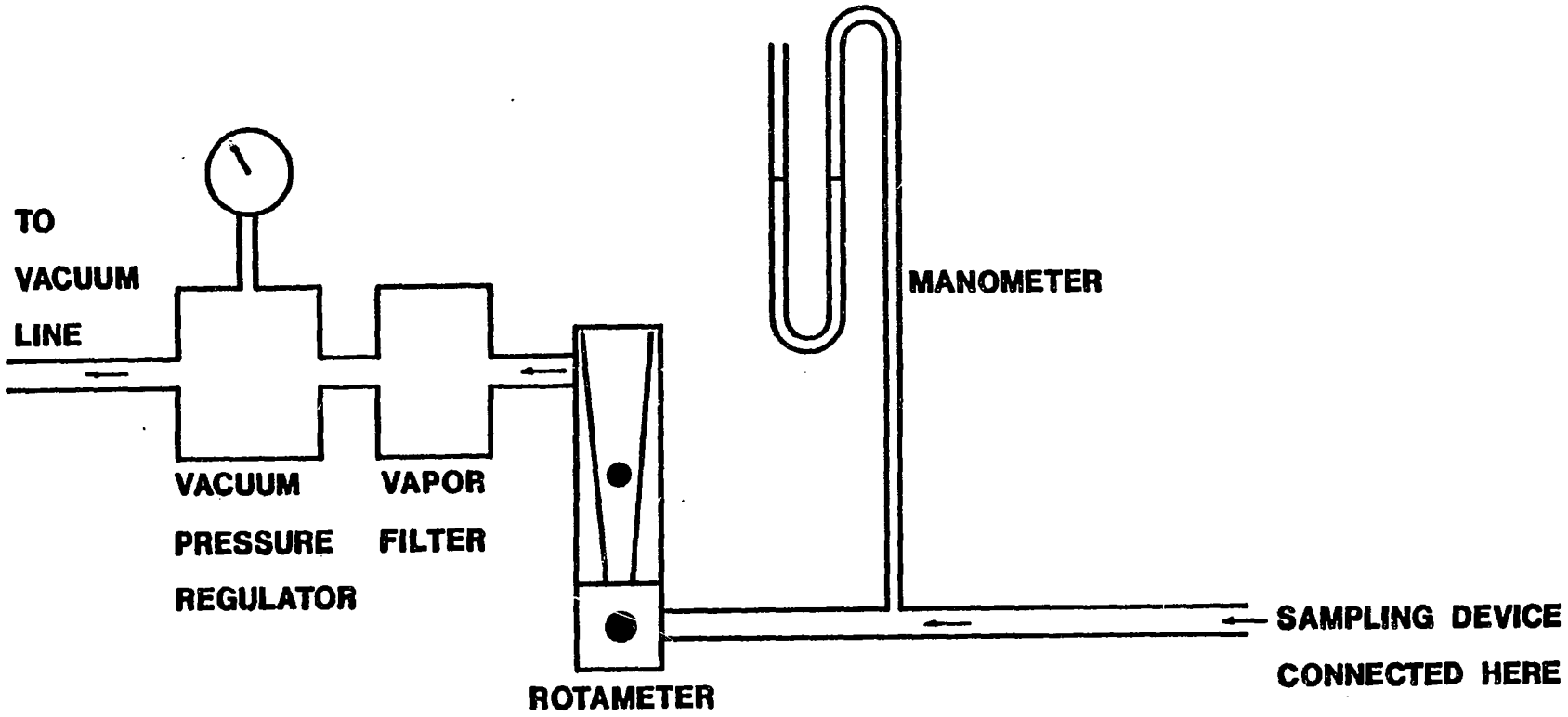

Figure 5: Vacuum Sampling System.

adsorption tube or bubbler used in sampling. The collection interval, the exposure at initiation of each sample, and the pressure drop across the sampling device were recorded at the time of sampling. Later, multiplication of the sampling interval (minute) times the sampling flow (liters/minute) produced the total volume (liters) of test compound atmosphere sampled. The sampling flow for each bubbler was determined individually to check for possible large variations among the bubblers used.

Immediately prior to animal exposure, 2-3 "pre-exposure" adsorption tube or bubbler samples were taken. After animals had been placed in the chamber, 6-8 "exposure" adsorption tube or bubbler samples were taken during the exposure for subsequent chemical analysis. To take a sample, an adsorption tube was scored at both ends with a file and both ends were broken off. The backup section end of the adsorption tube was then attached to PVC sampling tubing connected to the vacuum sampling system described previously. The adsorption tube and connecting tubing were inserted into the inhalation chamber at a point just above the test animal area for atmospheric sampling. The sample interval timer was started as soon as the adsorption tube was inserted through the sampling port in the rear of the inhalation chamber. Upon withdrawal of the adsorption tube from the inhalation chamber, the interval timer was disengaged, the sampling time was recorded, and the adsorption tube was sealed with polyethylene caps and labeled.

The desorption efficiency of a given test compound from an adsorbent material was determined by adding three different levels of known amounts of test compound bracketing the expected levels to duplicate 100 mg samples of activated charcoal in the glass tubes sealed at one end. After addition of the test compound, the tubes containing the charcoal were sealed with polyethylene caps and stored at least 24 hours before analysis, along with the test atmosphere samples. The same procedure was also used for silica gel adsorption tube desorption determinations.

For sample desorption, the primary and backup adsorption tube sections were placed in separate vials (Wheaton, #223713). One or two ml of desorption solvent (often containing an internal standard compound) was accurately added to the vial with a pipette (Kimax, Class A), and

the vial was sealed with a Teflon-lined cap (Wheaton, #224212). The samples were allowed to stand at room temperature for at least 1/2 hour with occasional shaking before GC analysis [35,37,39,40-51].

Phenol samples were collected in bubblers and subsequently transferred to volumetric flasks with distilled water rinses. Then, proper reagents were added to the volumetric flask samples and dilution to appropriate final volume was done with distilled water [33,36,47,48].

Calibration standards for the liquid test compounds were prepared by using microsyringes to measure volumes of each 98% purity test compound, and then diluting the test compound in the same volume of desorption solvent (with internal standard when used) as was used for the unknown samples. Usually, the same volumes of test compound were used to prepare the calibration standards as were added to the activated charcoal samples in the desorption efficiency determinations.

The calibration standards for phenol analysis were prepared by weighing solid phenol in a 100 ml volumetric flask. These weighed aliquots were then diluted with appropriate solvent to produce a stock solution which was further diluted to prepare working calibration standards.

Since all samples in a given experiment were desorbed in the same volume and handled similarly, it was convenient to express calibration standard concentrations in terms of mg of test compound present per sample vial (mg/vial).

Multiple injections of each calibration standard were made into the gas chromatograph (Hewlett-Packard #5750B equipped with FID), and the resultant recorder peak height (mm) responses for both the test compound and the internal standard were measured with a clear plastic ruler. The mean peak height or peak height ratio (when internal standard was used) value for each test compound standard was plotted against the amount of test compound added per vial (mg/vial) to check the linearity of the response.

Analysis of atmospheric samples was accomplished by injection of multiple aliquots of each solvent desorbed sample solution into the gas chromatograph in the same manner as was done with the calibration standards.

Next, all gas chromatographic responses to calibration standards along with their respective concentrations (mg/vial) were fed into a printing desk calculator (Hewlett-Packard 91), and a linear regression program was executed. Then, the mean peak height or peak height ratio GC response for each test atmosphere sample or desorption efficiency sample was entered into the calculator and a linear estimate of the amount of test compound per vial was obtained. Using the mean desorption efficiency value, the gas chromatographic analysis value for a sample, and total atmosphere volume sampled, the concentration of test compound (mg/l) in each sample of the inhalation chamber atmosphere was calculated. The individual sample values, the mean exposure values, and the standard deviation for each exposure are shown in the SPECIFIC METHODS AND RESULTS section of this report.

Procurement and Handling of Test Animals

UBTL procured Hartley albino guinea pigs from Buckberg Lab Animals, Tomkins Cove, New York and Sprague-Dawley rats from Talonic Farms, German Town, New York. All animals were shipped by air carrier and checked upon arrival for normality before being appropriately caged. All incoming animals were isolated in a room separate from the exposed animals for a period of two weeks to check for latent health problems and to condition them to the UBTL environment.

The animal cages were cleaned and bedding was changed twice weekly. All animals were caged separately after exposure. Both food and water were available to all animals continually except during the inhalation exposure.

Extra animals were procured in each shipment to allow for animal deaths and to increase the probability of having all animals within the weight range limits specified. The upper and lower weight range limits specified in the contract were expanded by ten grams after consultation with the Project Officer.

Exposure of Animals to Test Compound Atmospheres

Four, multicompartment stainless steel wire mesh cages, having ten separate compartments each, were used to house the animals during an

inhalation exposure. Twenty animals were test animals and twenty were controls. The animals exposed to a given atmosphere consisted of five male and five female rats placed alternately in one multicompartment cage, and five male and five female guinea pigs also placed alternately in a different multicompartment cage. The test chamber was equipped with a sliding drawer system which allowed all 20 test animals to be concurrently placed in the inhalation chamber (see Figures 6 and 7). A similar group of control animals was placed in an inhalation chamber having airflow, but without test compound atmosphere.

After the animals had been placed in the exposure chambers, they were observed continuously during the 30 minute exposure interval and observation notes were recorded on data sheets.

At the end of the 30 minute exposure interval, the animals were immediately removed from the chambers and then transferred to an animal holding room where they were observed and weighed before being placed in individual cages for the post-exposure observation period (see Figure 8)

The day of animal exposure was denoted as Day #1, and the day of animal necropsy was denoted as Day #15. This denotation was the same as that used in the contract.

Weighing and Necropsy of Animals

In order to have an approximately even distribution of test animals between test and control groups with respect to weight, the following procedure, designed by John A. Burkart, Ph.D., UBTL Biostatistician, was used on the day prior to animal exposure. Ten animals of a given species and sex were weighed (example, 10 male rats). Next, the body weights were ranked in pairs by decreasing weight. One animal of each pair was randomly selected to serve as a control, and the other member of the pair served as a test animal. Thus, each test animal had a corresponding control animal of similar weight. The process was repeated until all four groups of animals had been randomized with respect to weight. At this time, each test animal was assigned a number which corresponded to

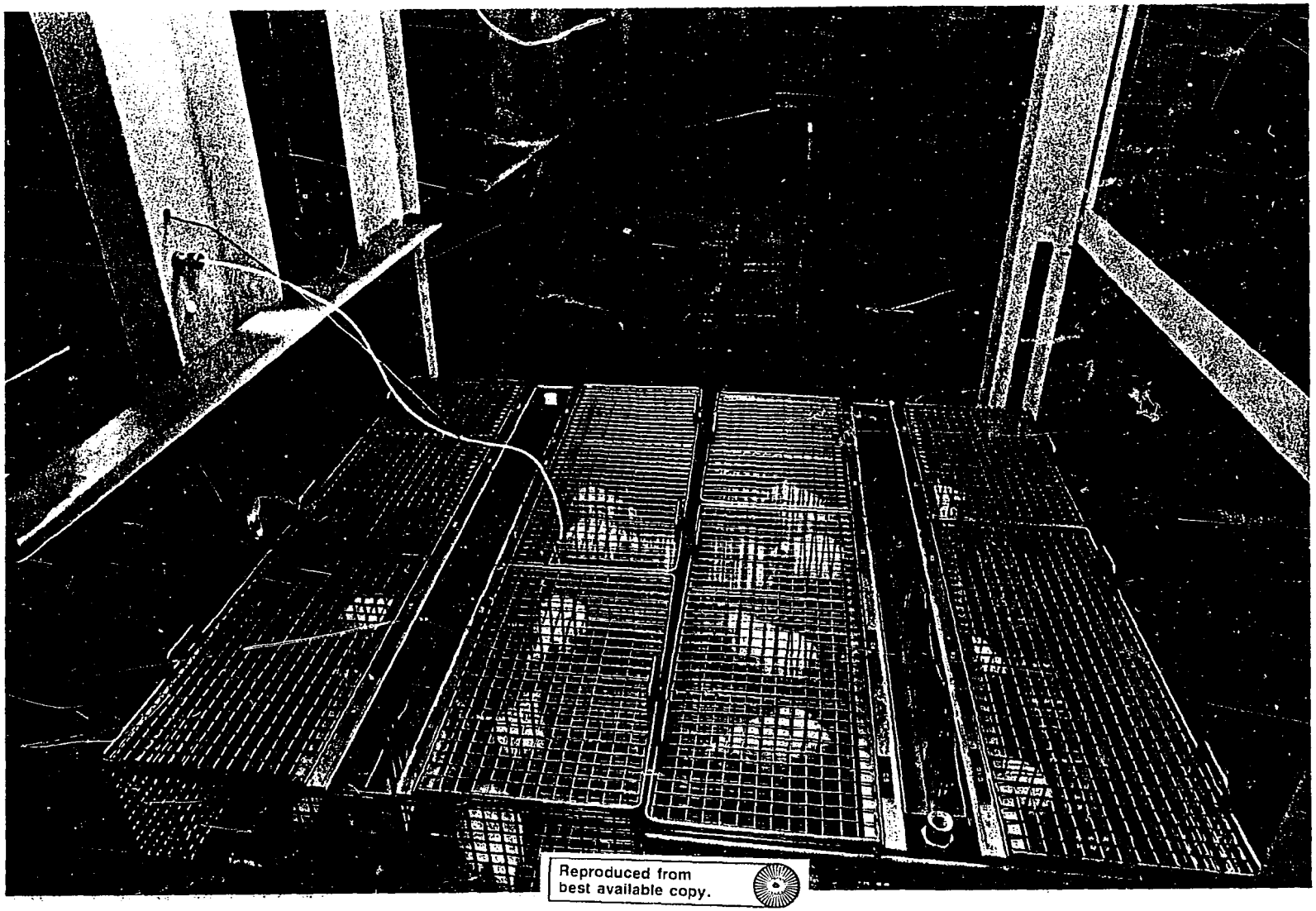

Figure 6: UBTL exposure chamber with drawer in "closed" position.

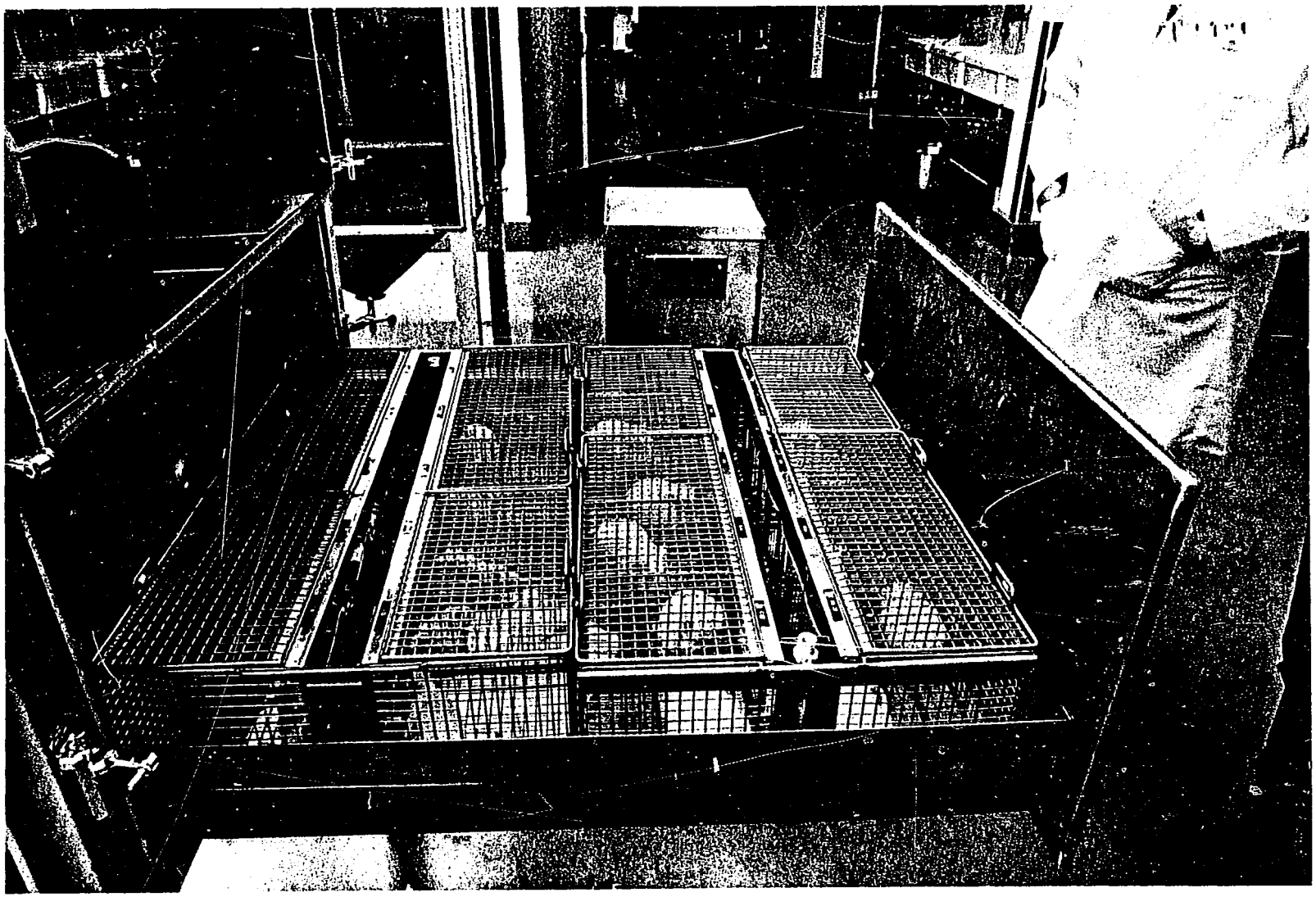

Figure 7: UBTL exposure chamber with drawer in "open" position and individually caged animals.

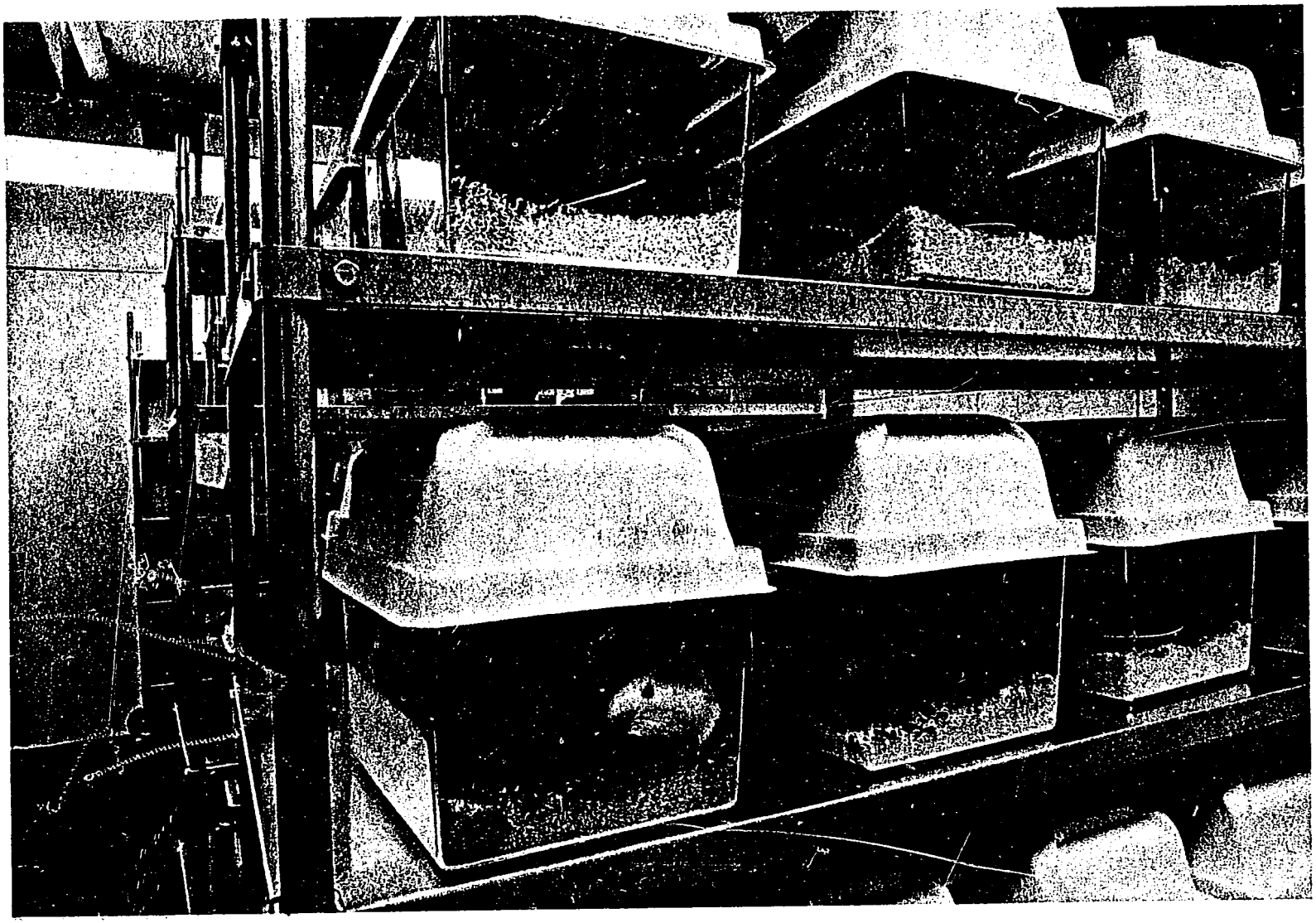

Figure 8: One of UBTL's rodent holding rooms.

the inhalation chamber multicompartment cage section in which it would be exposed. This number also served as an identification number for a given animal throughout the remaining data gathering processes, as shown below:

Coding:	(Expt. #)	(Species)	(Usage)	(Animal Cage # in Group)	(Sex)
Example:	1	R	C	1	M
Explanation:	1st Expt.	Rat	Control	Rat #1 of Control Group	Male

All animals were weighed on the day prior to their inhalation chamber exposure. Then, they were all weighed immediately after the exposure, and again 2 days, 5 days, 9 days, and 14 days after exposure.

All animals were observed daily for signs of toxicity, and those animals found dead were placed in a refrigerator until necropsy could be performed.

On the day of necropsy, a given animal was sacrificed with an overdose of ethyl ether. Then, the animal was weighed, dissected, and examined grossly for pathological lesions. The following whole organs were removed, trimmed, weighed, and those to be sent out for histopathology were placed in 10% neutral buffered formalin: kidneys, adrenals, gonads, lungs, heart, liver, and brain. The paired organs were weighed together rather than separately. Also, gonads refer to both the testes and the epididymides in males and both ovaries and the uterus in females.

To increase the uniformity of the organ trimming and weighing procedures, one person performed all the necropsies. However, this necropsy technician had other responsibilities and was unable to necropsy all 40 animals in a single day. Therefore, the rats were necropsied on the 14th day after exposure, and the guinea pigs on the 15th day after exposure. The animals necropsied on the 15th day after exposure were also weighed again on the 15th day so that the organ-to-body-weight ratios would be determined by body weight at necropsy.

Since animals were occasionally incorrectly sexed prior to exposure, it was necessary to make a note of the sex determined at time of necropsy and change the original sex notation when necessary.

Histopathology of Test Animal Organ Specimens

At necropsy, the organs were removed, weighed, and placed in 10% neutral buffered formalin. After the initial experiments, whole organs, except for the liver, were submitted for histopathological preparation.

The histopathological preparation was done by a certified histologist at Intermountain Laboratories, Midvale, Utah; and the prepared slides were read by Kent R. Van Kampen, DVM, Ph.D, Veterinary Pathologist. Dr. Van Kampen has had several years of experience in evaluating histopathological lesions in rodents and he is board certified by the American College of Veterinary Pathologists. Dr. Van Kampen indicated on the histopathology report those lesions that he judged to be "irreversible."

Statistical Evaluation of Body Weight and Organ Weight Data

After all the body weight, organ weight, and animal death data had been collected for a given experiment, the data were analyzed by John A. Burkart, Ph.D., UBTL Biostatistician. Dr. Burkart and his assistant (Richard Voss) made comparisons of organ-to-body weight ratios (at the time of necropsy) between control and test animal groups, using Student's t-test. In addition, weight changes during the post-exposure interval were computed by subtracting pre-exposure weight from each post-exposure weight on specific days, and those changes were analyzed using an analysis of variance for repeated measures design. Statistically significant differences between test and control animal data were indicated on the first page of each report prepared by Dr. Burkart while the actual data were summarized in subsequent tables (see SPECIFIC METHODS AND RESULTS section).

Project Schedule

Due to unanticipated delays in the installation of inhalation chamber facilities and in the procurement of test compound samples, exposures did not follow the originally-planned schedule. Also, there was a longer-than-expected turn-around time necessary for histopathology data. As a consequence of this latter delay, histopathological evaluation was not used as an end point to determine the second exposure level.

During the first exposure of each test compound, we attempted to produce a concentration near the IDLH values estimated by NIOSH; but in some cases, actual exposure concentrations were either higher or lower for reasons explained in the previous sections of this report. The second exposure concentration level for a given compound was based on the effects observed in test animals at the initial exposure concentration and on the toxicity data available from previous studies made by other investigators. The original exposure level catagorization criteria were used as a guide in selecting a second exposure level until the time modifications had been received from NIOSH.

SPECIFIC METHODS AND RESULTS

INTRODUCTION

A large number of experiment-specific and compound-specific methods and results are associated with this study. A summary of the overall experimental results was presented in Table 2, and the general methodology was discussed in the previous section. We have decided that the detailed, specific information for each of the eight compounds is most usefully presented in a separate section for a given compound. Therefore, eight sections of "SPECIFIC METHODS AND RESULTS" follow. Each section is subdivided into two or three subsections, depending on the number of exposures. The specific information for each exposure is presented in the following order:

* INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS
* INHALATION CHAMBER ATMOSPHERE ANALYSIS REPORT
* CLINICAL OBSERVATIONS
* STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES AND ORGAN-TO-BODY WEIGHT RATIOS
* HISTOPATHOLOGICAL EVALUATION OF ORGAN SPECIMENS FOR IRREVERSIBLE LESIONS

We have attempted to present the data in a manner which will be useful to NIOSH in continuing the study to establish IDLH values for each test compound.

SPECIFIC METHODS AND RESULTS

1,2,3-trichloropropane

Experiment #19-TCP-3 (799 ppm)

Experiment #1-TCP-1 (2080 ppm)

Experiment #10-TCP-2 (5010 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,2,3-trichloropropane Exp.#: 19-TCP-3 Date: 5/11/77

Chamber Facility Parameters

Room Temperature: 24.5 °C Chamber Temperature: 27.5 °C Barom. Pressure: 640 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 25 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 176 °C Setting: 400 Sensor: 2 Heater Power: 600 watts

Pump Setting: 1.20 Liquid Pressure: 7 psig Liquid Flow: 1.9 ml/min ~2.7 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.918 l/min Sampling Interval: 3.00 min

Total Sample Volume: 2.75 l Rotameter Setting: 110 mm Pres. Drop: <20 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: mono-chlorobenzene Concentration: 300 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 215 °C

Analytical Column: 1/8" x 6' S.S. 5% Carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 150 °C

Attenuation: 32 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 60 ml/min Detector Gasses: H_2 25 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 2.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.4 min Internal Standard: 0.8 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,2,3-trichloropropane Exp.#: 19-TCP-3 Date: 5/11/77

Ambient Temperature: 27.5 °C Barometric Pressure: 740 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-8.0	3.96	780
2	-4.0	4.01	792

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	+0.8	3.75	739
4	4.0	4.00	789
5	9.0	4.12	813
6	12.5	4.06	800
7	16.2	4.20	829
8	19.5	4.13	815
9	22.5	4.04	797
10	26.5	4.07	803
	MEAN:	4.05	799
	S.D.:	0.135	26.5
	C.V.:	0.0333	0.0333

Clinical Observations*
19-TCP-3
1,2,3 - trichloropropane (799 ppm)

Rats: Rats showed initial hyperactivity but this subsided after five minutes. Some animals were observed to be squinting at five minutes and at ten minutes, some depressed behavior was noticed. This depression, however, was minimal in that all animals were quite responsive to external auditory stimuli.

Guinea Pigs: Increased initial activity was also noted in the guinea pigs with slight lacrymation. At five minutes, some salivation was noted. At twenty minutes, most animals had their eyes closed, but were still mobile and responsive to auditory stimuli.

General Comments:

Little effect was observed in test animals during exposure.

Scores:**

	Hyperactivity	Reduced Activity	Lacrymation	Salivation	Eye Closure
Rats	+1	+1	0	0	0
Guinea Pigs	+1	0	+1	+1	+2

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss RJV

Statistical Evaluation of IDLH Experiment 19-TCP-3: 1,2,3-Trichloropropane (799 ppm)

7 June 1977

77M263

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

For each of the four animal groups, heart-to-body weight ratios were lower in the test group than in the control group; the difference was statistically significant for female rats and male guinea pigs.

Weight Changes

All groups showed significant increases in weight over the course of the experiment. For both male and female rats, the control group showed larger weight increases except for the last day of the experiment; on day 15, the test group showed larger increases.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

Attachments

77M263 Table 1

Experiment No. 19-TCP-3 : 1,2,3-Trichloropropane (799 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	277	6.25	47.51	8.05	3.82	4.34	.22	14.81
		Test	5	281	6.23	50.46	7.56	3.80	5.47	.21	14.73
		t-value		.41	-.03	1.32	-.82	-.05	2.10	-1.39	-.10
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	195	7.38	41.59	7.07	3.95	6.19	.31	5.24
		Test	5	198	7.16	44.74	7.57	3.43	6.16	.31	5.33
		t-value		.42	-.42	1.12	1.23	-4.14	-.06	-.56	.14
		significance		N.S.	N.S.	N.S.	N.S.	$p<.01$	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	292	7.50	54.59	9.27	4.12	10.23	.44	4.37
		Test	5	286	7.58	51.00	9.05	3.50	9.39	.49	4.19
		t-value		-.31	.44	-.55	-.60	-2.38	-1.29	1.45	-.32
		significance		N.S.	N.S.	N.S.	N.S.	$p<.05$	N.S.	N.S.	N.S.
	Female	Control	5	269	7.18	49.28	9.17	4.49	9.52	.51	4.02
		Test	5	281	7.69	51.42	8.67	3.71	8.89	.49	3.67
		t-value		.96	.55	.58	-1.36	-1.82	-1.04	-.52	-.28
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, $\alpha=.05$.

77M263 Table 2a.

EXP. NO. 19-TCP-3: 1,2,3-TRICHLOROPROPANE (799 PPM) ON MALE RATS

ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	669.78	669.78	3.7673	N.S.
SUBJECTS IN GROUPS	8	1422.3	177.79		
TIMES	4	28754.	7188.5	75.976	p<.001
TIMES X GROUPS	4	1378.7	344.68	3.6430	p<.05
TIMES X SUBJECTS IN GROUPS	32	3027.7	94.615		
TOTAL	49	35252.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	-4.600	19.200	32.600	46.200	57.400	30.160
TEST	2	-8.000	1.600	16.600	35.400	68.600	22.840
TOTAL		-6.300	10.400	24.600	40.800	63.000	26.500

77M263 Table 2b.

EXP. NO. 19-TCP-3: 1,2,3-TRICHLOROPROPANE (799 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	95.220	95.220	.87744	N.S.
SUBJECTS IN GROUPS	8	868.16	108.52		
TIMES	4	2424.3	606.07	60.186	p<.001
TIMES X GROUPS	4	247.08	61.770	6.1341	p<.001
TIMES X SUBJECTS IN GROUPS	32	322.24	10.070		
TOTAL	49	3957.0			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	-9.200	1.400	3.800	4.000	11.800	2.360
TEST	2	-7.200	-7.400	-3.200	2.200	13.600	-.400
TOTAL		-8.200	-3.000	.300	3.100	12.700	.980

77M263 Table 2c.

EXP. NO. 19-TCP-3: 1,2,3-TRICHLOROPROPANE (799 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	216.33	216.33	.39479	N.S.
SUBJECTS IN GROUPS	8	4383.7	547.96		
TIMES	4	38488.	9622.1	119.46	p<.001
TIMES X GROUPS	4	298.08	74.520	.92516	N.S.
TIMES X SUBJECTS IN GROUPS	32	2577.5	80.547		
TOTAL	49	45964.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	5.600	25.200	49.200	73.800	73.600	45.480
TEST	2	1.800	17.800	41.800	66.400	78.800	41.320
TOTAL		3.700	21.500	45.500	70.100	76.200	43.400

EXP. NO. 19-TCP-3: 1,2,3-TRICHLOROPROPANE (799 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANT
GROUPS	1	524.89	524.89	2.6236	N.S.
SUBJECTS IN GROUPS	8	1600.4	200.05		
TIMES	4	23464.	5866.1	53.835	p<.001
TIMES X GROUPS	4	343.31	85.828	.78768	N.S.
TIMES X SUBJECTS IN GROUPS	32	3486.8	108.96		
TOTAL	49	29420.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	3.800	20.400	39.200	46.400	58.600	33.600
TEST	2	4.600	21.800	44.600	60.800	69.000	40.160
TOTAL		4.200	21.100	41.900	53.600	63.800	36.920

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 19-TCP-3

Chemical: 1,2,3-trichloropropane (799 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
-	-	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,2,3-trichloropropane Exp.#: 1-TCP-1 Date: 12/22/76

Chamber Facility Parameters

Room Temperature: 24.0 °C Chamber Temperature: 26.2 °C Barom. Pressure: 646 Torr

Chamber Pressure: -13 mm H_2O Chamber Intake Air Supply Relative Humidity - %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: ~600 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 165 °C Setting: 275 Sensor: 2 Heater Power: 600 watts

Pump Setting: 1.25 Liquid Pressure: 20 psig Liquid Flow: ~2.0 ml/min ~2.8 g/min

Nitrogen Flow: 3.10 l/min Comp. Air Flow: 71.3 l/min Total Gas Flow: 74.4 l/min

Comments:

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.816 l/min Sampling Interval: 5.00 min

Total Sample Volume: 4.08 l Rotameter Setting: 99 mm Pres. Drop: <25 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 2.00 ml

Internal Standard Used: none Concentration: - ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6' S.S. 5% Carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 150 °C

Attenuation: 128 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 75 ml/min Detector Gasses: H_2 25 ml/min Air 380 ml/min

% CH_4/A ml/min

Injection Volume: 3.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 3.0 min Internal Standard: - min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,2,3-trichloropropane Exp.#: 1-TCP-1 Date: 12/22/76

Ambient Temperature: 26.2 °C Barometric Pressure: 646.1 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments: Sample #6 was omitted due to sampling problems.

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-28.0	11.4	2060
2	-22.0	11.5	2080

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.2	10.7	1930
4	8.0	11.1	2010
5	13.4	12.9	2330
6	-	-	-
7	25.0	11.4	2060
	MEAN:	11.5	2080
	S.D.:	0.96	174
	C.V.:	0.083	0.083

Clinical Observations*
1-TCP-1
1,2,3 - trichloropropane (2080 ppm)

Rats: Hyperactivity was evident upon placing the animals in the chamber. At 10 minutes, some lacrymation was noted. Immobility was evident in all animals after 30 minutes of exposure. This syndrome was preceded by ocular edema.

Guinea Pigs: Hyperactivity was pronounced upon initial exposure. Eyes were closed and lacrymation pronounced. By 15 minutes, ataxia was noted and nodding was seen in most animals.

General Comments:

Compound produced ataxia in most guinea pigs. Effects were less evident in rats.

Scores:**

	Hyperactivity	Squinting	Lacrymation	Ataxia	Eye Closure	Reduced Activity
Rats	(Early)+3	+1	+1	0	0	+1
Guinea Pigs	(Early)+3	+3	+3	+2	+1	0

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X		X	X
1-2				
3-9		X		
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

to Dan Daniels
from John A. Burkart / Richard J. Voss
subject Statistical Evaluation of IDLH Experiment 1-TCP-1: 1,2,3-Trichloropropane (2080 PPM)
date 16 February 1977
refer to 77M083

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

One male rat in the test group died and is not included in these analyses.

Organ-to-Body Weight Ratios

Lung-to-body weight ratios were lower in the test groups than in the control groups for male and female rats and guinea pigs, but only for female guinea pigs was the difference statistically significant. Female guinea pigs also showed a significantly lower adrenal ratio in the test group as compared to the control group.

Weight Changes

All groups showed significant increases in weight over the course of exposure. Male rats in the control group showed significantly larger weight changes than male rats in the test group. Male guinea pigs had higher post-exposure weights in the control group than in the test group.

Mortality

One male rat in the test group died one day after exposure.

xc: N. Price
A. F. Toronto
J. H. Nelson

77M083 Table 1

Experiment No. 1-TCP-1 : 1,2,3-Trichloropropane (2080 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	282	6.54	45.06	8.24	3.54	6.17	.21	14.99
		Test	4[a]	273	6.07	48.16	8.13	3.30	5.87	.22	13.64
		t-value		-1.22	-1.04	1.75	-0.27	-1.94	-1.06	0.72	-1.92
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	210	7.15	39.73	7.64	3.78	7.91	.28	3.44
		Test	5	211	6.66	40.90	7.40	3.57	7.51	.27	3.39
		t-value		0.13	-1.96	0.43	-0.38	-0.92	-0.81	-0.62	-0.25
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	4	334	8.47	60.91	9.06	3.59	10.09	.44	3.77
		Test	6	290	7.44	57.38	9.46	3.77	10.95	.41	3.87
		t-value		-2.18	-1.13	-0.60	1.78	0.81	1.69	-0.59	0.28
		significance		p<.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	6	288	8.60	52.11	9.82	3.48	11.66	.46	4.53
		Test	4	282	7.47	53.05	9.56	3.91	11.06	.38	4.29
		t-value		-0.32	-2.86	0.21	-0.50	1.59	-0.86	-2.34	-0.40
		significance		N.S.	p<.05	N.S.	N.S.	N.S.	N.S.	p<.05	N.S.

N.S.- Not statistically significant, α=.05.

[a] - Data not included for one animal which died.

77M083 TABLE 2a

EXPERIMENT 1-TCP-1: 1,2,3-TRICHLOROPROPANE (2080 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGES FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1874.9	1874.9	5.7880	p< .05
SUBJECTS IN GROUPS	7	2267.5	323.93		
TIMES	4	30170.	7542.4	22.747	p< .001
TIMES X GROUPS	4	2641.8	660.45	1.9910	N.S.
TIMES X SUBJECTS IN GROUPS	28	9284.2	331.58		
TOTAL	44	46230.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY14	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-3.000	20.400	36.200	55.400	58.200	33.440
TEST	2	-12.000	-4.500	6.500	39.750	72.500	20.450
TOTAL		-7.000	9.333	23.000	48.444	64.556	27.667

77M083 TABLE 2b

EXP NO 1-TCP-1: 1,2,3-TRICHLOROPROPANE (2080 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGES FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	62.720	62.720	.23600	N.S.
SUBJECTS IN GROUPS	8	2119.0	264.87		
TIMES	4	5498.7	1374.7	26.081	p< .001
TIMES X GROUPS	4	326.28	81.570	1.5476	N.S.
TIMES X SUBJECTS IN GROUPS	32	1686.6	52.707		
TOTAL	49	9693.3			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-9.000	-5.600	7.000	9.200	13.400	3.000
TEST	2	-13.000	-13.200	.200	15.000	14.800	.760
TOTAL		-11.000	-9.400	3.600	12.100	14.100	1.880

77M083 TABLE 2c

EXP 1-TCP-1: 1,2,3 TRICHLOROPROPANE (2080 PPM) (10)
ANALYSIS OF WEIGHT CHANGES FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	5958.6	5958.6	2.1828	N.S.
SUBJECTS IN GROUPS	8	21838.	2729.8		
TIMES	4	47686.	11921.	22.258	p< .001
TIMES X GROUPS	4	2390.9	597.72	1.1160	N.S.
TIMES X SUBJECTS IN GROUPS	32	17139.	535.60		
TOTAL	49	95013.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	1.000	17.250	44.000	82.750	90.750	47.150
TEST	2	-2.500	-.667	14.667	37.500	75.333	24.867
TOTAL		-1.100	6.500	26.400	55.600	81.500	33.780

77M083 TABLE 2d

EXP 1-TCP-1: 1,2,3-TRICHLOROPROPANE (2080 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGES FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	91.859	91.059	.23577E-01	N.S.
SUBJECTS IN GROUPS	8	31169.	3896.2		
TIMES	4	36306.	9076.5	15.165	p< .001
TIMES X GROUPS	4	1234.4	308.61	.51562	N.S.
TIMES X SUBJECTS IN GROUPS	32	19153.	598.52		
TOTAL	49	87954.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	3.333	10.667	-2.833	51.500	69.500	26.433
TEST	2	4.000	9.750	10.000	41.250	73.000	29.200
TOTAL		3.600	10.300	5.500	47.400	70.900	27.540

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 1-TCP-1

Chemical: 1,2,3-trichloropropane (2080 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
1RT9M	1 day	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,2,3-trichloropropane Exp.#: 10-TCP-2 Date: 3/9/77

Chamber Facility Parameters

Room Temperature: 23.3 °C Chamber Temperature: 28.0 °C Barom. Pressure: 637 Torr

Chamber Pressure: -8 mm H_2O Chamber Intake Air Supply Relative Humidity 30 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 176 °C Setting: 400 Sensor: 2 Heater Power: 600 watts

Pump Setting: 6.00 Liquid Pressure: 20 psig Liquid Flow: 13.0 ml/min 17.9 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.171 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.513 l Rotameter Setting: 30 mm Pres. Drop: <35 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: mono-chlorobenzene Concentration: 400 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6' S.S. 5% Carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 150 °C

Attenuation: 16 Range: 10^3 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 75 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 2.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.6 min Internal Standard: 0.8 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,2,3-trichloropropane Exp.#: 10-TCP-2 Date: 3/9/77

Ambient Temperature: 28.0 °C Barometric Pressure: 63.7 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments: Condensation of test compound occurred on walls of inhalation chamber.

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-15.0	25.6	5030
2	-10.0	26.1	5130

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	23.1	4550
4	5.0	26.7	5250
5	10.0	26.4	5180
6	14.0	24.3	4780
7	18.0	25.1	4940
8	23.0	26.9	5280
9	25.0	25.8	5080
	MEAN:	25.5	5010
	S.D.:	1.39	269
	C.V.:	0.054	0.054

Clinical Observations*
10-TCP-2
1,2,3 - trichloropropane (5010 ppm)

Rats: All rats showed acute nasal irritation when first exposed in the chamber. Most animals were lacrymating by five minutes and showed decreased activity and ataxia by 10 minutes. By 15 minutes, all animals were depressed and some showed occasional convulsive movements. At the end of the exposure, all rats appeared to be narcotic and remained so until several minutes after they were removed from the chamber. No deaths occurred during the experiment, however, 2 rats died within 3 days after exposure.

Guinea Pigs: The observations for the guinea pigs were the same as those noted for the rats. Also, 6 guinea pigs died within 3 days after the exposure.

General Comments:

A definite irritating effect was noted initially and a narcotic effect was apparent in all animals at 25 minutes. Although no animals died during exposure, 8 animals died subsequently.

Scores:**

	Eye and Nasal Irritation	Reduced Activity	Narcosis	Ataxia
Rats	+3	+1	+3	+1
Guinea Pigs	+3	+1	+3	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0			X	X
1-2				
3-9				
10	X	X		

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss

Statistical Evaluation of IDLH Experiment 10-TCP-2: 1,2,3-Trichloropropane (5010 ppm)

3 May 1977

77M207

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Of the test group animals, two male rats, four male guinea pigs, and two female guinea pigs died following exposure and were not included in these analyses.

Organ-to-Body Weight Ratios

When compared to the control group, the female guinea pig test group was found to have significantly lower body weights at autopsy as well as significantly higher lung and kidney organ-to-body weight ratios.

The male rat test group had significantly higher adrenal weight ratios when compared to the control.

Weight Changes

All groups showed significant weight increases over the course of exposure. For rats (both male and female), the final weight change at autopsy was approximately the same for test and control groups, although the control group increased weight significantly faster in the early days after exposure day and the test group increased weight significantly faster in the latter days. For guinea pigs (both male and female), the control group showed significantly larger weight increases throughout the experiment.

Mortality

Combining males and females, 2 out of 10 rats in the test group died (not statistically significant), and 6 out of 10 guinea pigs in the test group died (significant at the .05 level, comparing test and control groups using Fisher's exact test).

XC: N. Price
A. F. Toronto
J. H. Nelson

gj

77M207 Table 1

Experiment No. 10-TCP-2 ; 1,2,3-Trichloropropane (5010 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	313	5.74	53.09	8.20	3.50	4.69	.19	13.16
		Test	3[a]	308	5.61	49.13	8.73	3.77	5.04	.22	14.24
		t-value		-.41	-.38	-2.42	1.43	1.92	1.11	2.69	1.74
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	$p<.05$	N.S.
	Female	Control	5	212	6.96	47.39	7.62	4.06	6.78	.30	4.83
		Test	5	215	6.98	47.67	7.57	3.61	6.75	.28	4.38
		t-value		.30	.06	.11	-.16	-1.05	-.11	-.96	-1.06
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	315	7.56	58.80	9.43	4.63	8.99	.42	5.27
		Test	1[b]	298	7.72	59.06	10.07	4.60	10.30	.47	4.33
		t-value	*	*	*	*	*	*	*	*	*
		significance									
	Female	Control	5	309	7.62	53.45	9.07	4.17	8.63	.44	4.90
		Test	3[c]	263	9.04	60.15	11.59	4.96	10.44	.50	3.49
		t-value		-3.48	2.87	1.04	3.43	1.56	1.93	2.00	-2.01
		significance		$p<.05$	$p<.05$	N.S.	$p<.05$	N.S.	N.S.	N.S.	N.S.

* - t-test not applicable

N.S.- Not statistically significant, $\alpha=.05$.

a - Two animals died before scheduled autopsy and were not included in analysis.

b - Four animals died before scheduled autopsy and were not included in analysis.

c - Two animals died before scheduled autopsy and were not included in analysis.

77M207 Table 2a

EXP. NO. 10-TCP-2: 1,2,3-TRICHLOROPROPANE (5010 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	124.21	124.21	.19888	N.S.
SUBJECTS IN GROUPS	6	3747.4	624.56		
TIMES	4	45601.	11400.	129.03	
TIMES X GROUPS	4	1858.0	464.51	5.2573	p<.001
TIMES X SUBJECTS IN GROUPS	24	2120.5	88.355		p<.01
TOTAL	39	53451.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-21.800	6.000	31.000	56.400	78.600	30.040
TEST	2	.333	-10.333	15.667	48.333	78.000	26.400
TOTAL		-13.500	-.125	25.250	53.375	78.375	28.675

77M207 Table 2b

EXP. NO. 10-TCP-2: 1,2,3-TRICHLOROPROPANE (5010 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	462.08	462.08	1.9366	N.S.
SUBJECTS IN GROUPS	8	1908.8	238.60		
TIMES	4	12631.	3157.9	73.099	p<.001
TIMES X GROUPS	4	1605.7	401.43	9.2924	p<.001
TIMES X SUBJECTS IN GROUPS	32	1382.4	43.200		
TOTAL	49	17990.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-19.000	1.400	12.400	14.200	23.800	6.560
TEST	2	-16.400	-23.400	-1.200	18.600	24.800	.480
TOTAL		-17.700	-11.000	5.600	16.400	24.300	3.520

EXP. NO. 10-TCP-2: 1,2,3-TRICHLOROPROPANE (5010 PPM) ON MALE GUINEA PIGS
ANALYSIS OOF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICA
GROUPS	1	3670.4	3670.4	16.817	p≤.05
SUBJECTS IN GROUPS	4	873.04	218.26		
TIMES	4	41018.	10254.	228.84	p<.001
TIMES X GROUPS	4	767.90	191.97	4.2842	p<.05
TIMES X SUBJECTS IN GROUPS	16	716.96	44.810		
TOTAL	29	47046.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY10	DAY15	TOTAL
		1	2	3	4	5	
CONTROL	1	.000	22.000	45.600	75.800	106.000	49.880
TEST	2	-6.000	-17.000	.000	50.000	74.000	20.200
TOTAL		-1.000	15.500	30.000	71.500	100.667	44.933

EXP. NO. 10-TCP-2: 1,2,3-TRICHLOROPROPANE (5010 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	11137.	11137.	19.894	p<.01
SUBJECTS IN GROUPS	6	3358.9	559.82		
TIMES	4	37463.	9365.8	93.700	p<.001
TIMES X GROUPS	4	2865.7	716.43	7.1674	p<.001
TIMES X SUBJECTS IN GROUPS	24	2398.9	99.956		
TOTAL	39	57224.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-5.200	10.800	36.400	62.600	96.400	40.200
TEST	2	-8.667	-17.000	-5.667	15.667	44.333	5.733
TOTAL		-6.500	.375	20.625	45.000	76.875	27.275

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 10-TCP-2

Chemical: 1,2,3-trichloropropane (5010 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
-	-	Irreversible:	none
Test			
10GT1M	1 day	Irreversible:	Adrenal cortico-medullary necrosis
10GT10F	2 days	"	"
10GT2F	3 days	"	"
10GT5M	Exposure day	Reversible:	Adrenal medullary hemorrhage and necrosis
10GT7M	Exposure day	"	Adrenal medullary necrosis
10GT9M	Exposure day	"	Adrenal medullary necrosis
10RT3M	Exposure day	"	Renal tubular necrosis
10RT5M	2 days	"	Possible congestion and necrosis of liver but animal somewhat autolyzed

SPECIFIC METHODS AND RESULTS

monochlorobenzene

Experiment #2-CB-1 (2990 ppm)

Experiment #8-CB-2 (5850 ppm)

Experiment #16-CB-3 (7970 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: monochlorobenzene Exp.#: 2-CB-1 Date: 1/5/77

Chamber Facility Parameters

Room Temperature: 23.3 °C Chamber Temperature: 26.4 °C Barom. Pressure: 638 Torr

Chamber Pressure: -13 mm H_2O Chamber Intake Air Supply Relative Humidity - %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: ≈600 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 143 °C Setting: 800 Sensor: 1 Heater Power: 600 watts

Pump Setting: 1.20 Liquid Pressure: 14 psig Liquid Flow: 1.5 ml/min 1.7 g/min

Nitrogen Flow: 3.1 l/min Comp. Air Flow: 71.3 l/min Total Gas Flow: 74.4 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: Adsorption Tubes Collection Medium: Charcoal

Sampling Flow: 0.482 l/min Sampling Interval: 4.00 min

Total Sample Volume: 1.93 l Rotameter Setting: 62 mm Pres. Drop: < 12 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 3.00 ml

Internal Standard Used: 1,1,2,2 - tetrachloroethane Concentration: 12.000 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6 ft S.S. 5% Carbowax 20M-TPA 80/100 Mesh Chromosorb W-AW @ 100 °C

Attenuation: 8 Range: 10^3 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 60 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.2 min Internal Standard: 3.2 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: monochlorobenzene Exp.#: 2-CB-1 Date: 1/5/77

Ambient Temperature: 26.4 °C Barometric Pressure: 638 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-16.0	10.5	2740
2	-11.6	13.6	3540

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	+ 1.2	11.3	2930
4	5.5	11.3	2930
5	13.4	11.7	3041
6	19.7	11.4	2976
7	24.0	11.7	3040
8	28.6	11.7	3040
MEAN:		11.5	2990
S.D.:		0.204	53.0
C.V.:		0.0177	0.0177

Clinical Observations*
2-CB-1
monochlorobenzene (2990 ppm)

Rats: Upon exposure to the test material, animals appeared hyperactive. At 10 minutes, a few showed squinting and lacrymation. Abnormal behavior was apparently temporary because recovery from squinting and lacrymation was immediate when removed from the chamber.

Guinea Pigs: Guinea pigs were hyperactive and nose wiping was evident immediately. As the exposure progressed, some animals showed squinting and an occasional animal would wipe his nose. By the end of 30 minutes of exposure, all guinea pigs showed a degree of lacrymation.

General Comments:

Slight eye and nasal irritation was the only observable effect during exposure.

Scores:**

	Hyperactivity	Lacrymation	Squinting
Rats	+1 (initially)	+1	+1
Guinea Pigs	+1 (initially)	+3 with nose wiping	+2

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

Dan Daniels

John A. Burkart / Richard J. Voss

Statistical Evaluation of IDLH Experiment 2-CB-1: Monochlorobenzene (2990 PPM)

16 February 1977

77M084

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by substracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

The test female rat group showed a significantly higher liver-to-body weight ratio than did the control group.

Weight Changes

All groups showed significant increases in weight over the course of the exposure. Female rats showed significantly greater weight increase in the control group than in the test group.

xc: N. Price
A. F. Toronto
J. H. Nelson

77M084 Table 1

Experiment No. 2-CB-1 : Monochlorobenzene (2990 PPM)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	287	5.79	48.91	8.00	3.46	6.18	.21	14.61
		Test	5	271	6.10	50.51	7.93	3.31	6.29	.23	14.84
		t-value		-1.49	1.14	0.52	-0.13	-1.22	0.21	1.66	0.39
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	201	6.82	40.92	7.50	3.57	7.76	.29	3.49
		Test	5	194	7.41	46.17	7.27	3.79	8.11	.29	3.60
		t-value		1.08	1.45	3.61	-0.95	0.99	0.89	0.15	0.27
		significance		N.S.	N.S.	p<.01	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	312	8.48	58.82	10.07	4.05	10.46	.43	4.40
		Test	5	292	8.00	58.68	10.32	3.92	10.59	.46	3.90
		t-value		-1.40	-0.62	-0.02	0.29	-0.40	0.26	1.58	-1.56
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	294	8.28	51.25	9.56	3.69	9.84	.44	4.60
		Test	5	295	7.88	52.77	9.49	3.57	10.54	.45	6.18
		t-value		0.03	-0.67	0.50	-0.23	-0.56	1.51	0.41	1.77
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α=.05.

77M084 TABLE 2a

EXP. NO. 2-CB-1: MONOCHLOROBENZENE (2990 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	3061.0	3061.0	1.5495	N.S.
SUBJECTS IN GROUPS	7	13832.	1976.0		
TIMES	4	18445.	4611.3	21.424	p < .001
TIMES X GROUPS	4	1485.0	371.24	1.7248	N.S.
TIMES X SUBJECTS IN GROUPS	28	6026.6	215.24		
TOTAL	44	42851.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-10.200	3.000	13.800	12.600	45.800	13.000
TEST	2	-6.750	15.250	29.750	50.750	59.000	29.600
TOTAL		-8.667	8.444	20.809	29.556	51.667	20.378

77M084 TABLE 2b

EXP. NO. 2-CB-1: MONOCHLOROBENZENE (2990 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	6846.3	6846.3	3.7241	N.S.
SUBJECTS IN GROUPS	9	16545.	1838.3		
TIMES	4	7935.1	1983.8	15.478	p< .001
TIMES X GROUPS	4	1649.7	412.43	3.2180	p< .05
TIMES X SUBJECTS IN GROUPS	36	4614.0	128.17		
TOTAL	54	37590.			

MEANS

	TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
	1	2	3	4	5	TOTAL
GROUPS:						
CONTROL 1	-.000	13.400	25.600	36.600	50.400	25.040
TEST 2	-10.500	.000	6.667	5.667	11.333	2.633
TOTAL	-6.091	6.091	15.273	19.727	29.091	12.818

77M084 TABLE 2c

EXP. NO. 2-CB-1: MONOCHLOROBENZENE (2990 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	176.73	176.73	.24029	N.S.
SUBJECTS IN GROUPS	8	5884.2	735.52		
TIMES	4	59077.	14969.	39.895	p< .001
TIMES X GROUPS	4	2531.1	632.77	1.6864	N.S.
TIMES X SUBJECTS IN GROUPS	32	12007.	375.22		
TOTAL	49	80476.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-5.400	21.200	35.400	74.800	101.000	45.400
TEST	2	-1.400	41.000	15.000	66.200	87.400	41.640
TOTAL		-3.400	31.100	25.200	70.500	94.200	43.520

77M084 TABLE 2d

EXP. NO. 2-CB-1: MONOCHLOROBENZENE (2990 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	512.00	512.00	1.2347	N.S.
SUBJECTS IN GROUPS	8	3317.5	414.69		
TIMES	4	61277.	15319.	191.54	p< .001
TIMES X GROUPS	4	208.62	52.156	.65214	N.S.
TIMES X SUBJECTS IN GROUPS	32	2559.3	79.977		
TOTAL	49	67874.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-1.200	22.200	35.200	77.600	97.400	46.240
TEST	2	-4.400	17.800	31.000	63.200	91.600	39.840
TOTAL		-2.800	20.000	33.100	70.400	94.500	43.040

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 2-CB-1

Chemical: monochlorobenzene (2990 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
-	-	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: monochlorobenzene Exp.#: 8-CB-2 Date: 2/23/77

Chamber Facility Parameters

Room Temperature: 23.3 °C Chamber Temperature: 27.1 °C Barom. Pressure: 640 Torr

Chamber Pressure: -9 mm H_2O Chamber Intake Air Supply Relative Humidity 27 %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: ≈600 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 204 °C Setting: 700 Sensor: 2 Heater Power: 600 watts

Pump Setting: 7.00 Liquid Pressure: 20 psig Liquid Flow: - ml/min - g/min

Nitrogen Flow: 1.4 l/min Comp. Air Flow: 20.6 l/min Total Gas Flow: 22.0 l/min

Comments:

Test Atmosphere Sampling Parameters

Collection Method: Adsorption Tubes Collection Medium: charcoal

Sampling Flow: 0.284 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.853 l Rotameter Setting: 40 mm Pres. Drop: Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: 1,1,2,2 - tetrachloroethane Concentration: 12,000 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6 ft S.S. 5% Carbowax 20M-TPA 80/100 Mesh Chromosorb W-AW @ 140° °C

Attenuation: 32 Range: 10^3 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 60 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 0.8 min Internal Standard: 1.8 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: monochlorobenzene Exp.#: 8-CB-2 Date: 2/23/77

Ambient Temperature: 27.1 °C Barometric Pressure: 640 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments: Some problem with metering pump priming caused greater than usual variation in concentration.

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-90	19.8	5090
2	-40	20.4	5230
3	-5	13.8	3530

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
4	1.0	15.4	3950
5	4.5	17.0	4370
6	10.0	21.0	5390
7	13.5	23.0	5910
8	17.5	26.0	6680
9	21.0	28.5	7320
10	24.7	28.4	7300
	MEAN:	22.8	5850
	S.D.:	5.26	1350
	C.V.:	0.23	0.23

Clinical Observations*
8-CB-2
monochlorobenzene (5850 ppm)

Rats: Initial increased activity was accompanied by nose rubbing. There was then a decrease in activity at 10 minutes and ataxia was present at 20 minutes. At 25 minutes, most rats were narcotic, but they recovered rapidly after being removed from the chamber.

Guinea Pigs: The guinea pigs showed initial increased activity with nose rubbing, as did the rats. However, lacrymation and salivation was evident at 10 minutes. All guinea pigs showed narcosis at 30 minutes. Most guinea pigs remained incapacitated until at least 10 minutes after the exposure.

General Comments:

The guinea pigs showed a greater narcotic effect than the rats, but no deaths occurred in either group.

Scores:**

	Increased Activity	Slowed Activity	Nose Rubbing	Lacry-mation	Saliva-tion	Ataxia	Narcosis
Rats	+1 (initially)	+1	+1	+2	+2	+2	+3
Guinea Pigs	+1 (initially)	+2	+1	+2	+2	0	+3

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0			X	X
1-2				
3-9	X			
10		X		

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels
J. A. Burkart, R. J. Voss

Statistical Evaluation of IDLH Experiment 8-CB-2:Monochlorobenzene (5850 ppm)

17 March 1977

77M128

Methods:

Post exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-esposure weight; these changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

No significant differences were observed.

Weight Changes

All groups showed significant weight increases over the period of exposure.

/gj

XC: N. Price
J. A. Nelson
A. F. Toronto

Experiment No. 8-CB-2 : Monochlorobenzene (5850 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	294	6.01	47.08	8.26	3.33	5.14	.20	14.11
		Test	5	282	5.85	50.05	8.28	3.37	4.90	.21	15.05
		t-value		-1.43	-.54	1.50	.09	.22	-.67	1.13	1.15
		significance		NS	NS	NS	NS	NS	NS	NS	NS
	Female	Control	5	199	6.65	40.91	8.08	3.48	6.67	.31	4.57
		Test	5	213	6.66	43.33	7.82	3.73	6.37	.29	4.64
		t-value		1.65	.01	1.14	-.99	1.09	-.71	-.84	.12
		significance		NS	NS	NS	NS	NS	NS	NS	NS
Guinea Pig	Male	Control	5	331	8.28	58.62	10.05	4.20	8.82	.40	4.24
		Test	5	326	8.45	53.45	9.42	4.01	8.63	.40	4.55
		t-value		-.35	.17	-1.03	-1.07	-.67	-.40	-.22	.99
		significance		NS	NS	NS	NS	NS	NS	NS	NS
	Female	Control	5	294	7.78	52.95	9.05	4.45	9.72	.46	4.85
		Test	5	295	7.95	48.77	9.33	3.98	9.84	.49	5.08
		t-value		.26	.27	-1.63	.86	-.72	.36	1.55	.26
		significance		NS	NS	NS	NS	NS	NS	NS	NS

N.S.- Not statistically significant, $\alpha = .05$.

EXP. NO. 8-CB-2: MONOCHLOROBENZENE (5850 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICAN
GROUPS	1	343.22	343.22	.75624	NS
SUBJECTS IN GROUPS	8	3630.8	453.85		
TIMES	4	43386.	10846.	144.79	p<.001
TIMES X GROUPS	4	558.28	139.57	1.8631	NS
TIMES X SUBJECTS IN GROUPS	32	2397.2	74.912		
TOTAL	49	50315.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY10	DAY15	TOTAL
		1	2	3	4	5	
CONTROL	1	-24.400	1.600	23.400	49.200	66.600	23.280
TEST	2	-20.200	3.000	13.400	39.600	54.400	18.040
TOTAL		-22.300	2.300	18.400	44.400	60.500	20.660

77M128 Table 2b.

EXP. NO. 8-CB-2: MONOCHLOROBENZENE (5850 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	212.18	212.18	3.0777	NS
SUBJECTS IN GROUPS	8	551.52	68.940		
TIMES	4	7547.6	1886.9	50.977	p<.001
TIMES X GROUPS	4	176.72	44.180	1.1936	NS
TIMES X SUBJECTS IN GROUPS	32	1184.5	37.015		
TOTAL	49	9672.5			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-16.600	-8.000	1.600	7.000	15.200	-.160
TEST	2	-18.200	-.800	2.800	15.400	20.600	3.960
TOTAL		-17.400	-4.400	2.200	11.200	17.900	1.900

77M128 Table 2c.

EXP. NO. 8-CB-2: MONOCHLOROBENZENE (5850 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	33.625	33.625	.13119	NS
SUBJECTS IN GROUPS	8	2050.4	256.30		
TIMES	4	90848.	22712.	231.70	p<.001
TIMES X GROUPS	4	395.28	98.820	1.0081	NS
TIMES X SUBJECTS IN GROUPS	32	3136.8	98.025		
TOTAL	49	96464.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY10 (4)	DAY15 (5)	TOTAL
CONTROL	1	1.400	17.400	40.200	71.000	113.600	48.720
TEST	2	-2.600	12.000	42.200	78.200	122.000	50.360
TOTAL		-.600	14.700	41.200	74.600	117.800	49.540

EXP. NO. B-CB-2: MONOCHLOROBENZENE (5850 PPM) ON FEMALE GUINEA PIGS
ANALYSIS FO WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1545.7	1545.7	3.9486	NS
SUBJECTS IN GROUPS	8	3131.6	391.45		
TIMES	4	45357.	11339.	159.15	p<.001
TIMES X GROUPS	4	73.516	18.379	.25795	NS
TIMES X SUBJECTS IN GROUPS	32	2280.0	71.250		
TOTAL	49	52388.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY10	DAY15	TOTAL
		1	2	3	4	5	
CONTROL	1	2.800	22.600	42.200	66.600	89.000	44.640
TEST	2	-5.600	13.200	28.800	52.000	79.200	33.520
TOTAL		-1.400	17.900	35.500	59.300	84.100	39.080

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 8-CB-2

Chemical: monochlorobenzene (5850 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
8[illegible]C1M	-	Irreversible: Purulent Meningitis
Test		
-	-	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: monochlorobenzene Exp.#: 16-CB-3 Date: 4/20/77

Chamber Facility Parameters

Room Temperature: 25.8 °C Chamber Temperature: 27.0 °C Barom. Pressure: 643 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 26 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 200 °C Setting: 800 Sensor: 2 Heater Power: 600 watts

Pump Setting: 10.00 Liquid Pressure: 16 psig Liquid Flow: 21.0 ml/min 23.8 g/min

Nitrogen Flow: 0.8 l/min Comp. Air Flow: 65.9 l/min Total Gas Flow: 66.7 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: Adsorption Tubes Collection Medium: charcoal

Sampling Flow: 0.170 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.510 l Rotameter Setting: 30 mm Pres. Drop: < 4 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: 1,1,2,2 - tetrachloroethane Concentration: 12.000 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 215 °C

Analytical Column: 1/8" x 6 ft S.S. 5% Carbowax 20M-TPA 80/100 Mesh Chromosorb W-AW @ 160 °C

Attenuation: 32 Range: 10^3 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 68 ml/min Detector Gasses: H_2 25 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 2.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 0.8 min Internal Standard: 1.2 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: monochlorobenzene Exp.#: 16-CB-3 Date: 4/20/77

Ambient Temperature: 27.0 °C Barometric Pressure: 643 Torr

Analytical Method: Activated charcoal adsorption tube - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	32.2	8290
2	-5.5	31.2	8010

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	+0.5	28.2	7260
4	4.5	30.7	7900
5	8.5	31.5	8090
6	13.0	32.5	8350
7	18.0	31.4	8060
8	21.5	31.1	7990
9	24.0	30.3	7790
10	27.0	32.5	8340
	MEAN:	31.0	7970
	S.D.:	1.38	355
	C.V.:	0.0445	0.0445

Clinical Observations*
16-CB-3
monochlorobenzene (7970 ppm)

Rats: The rats were hyperactive when first exposed and showed some ataxia by five minutes. There was increased ataxia at 10 minutes and most animals were in the lateral recumbent position. By twenty-five minutes, all animals were narcotic and several had twitching movements. This narcosis lasted several minutes after the animals were removed from their exposure cages.

Guinea Pigs: The same signs appeared as those in the rats, but the guinea pigs suffered from severe salivation by five minutes, and narcosis took effect in the guinea pigs at a much earlier time (15 minutes).

General Comments:

Evident narcotic effect was noted in all animals within 15-25 minutes of exposure and guinea pigs appeared to be affected earlier than rats.

Scores:

	Hyperactivity	Ataxia	Narcosis	Tremors	Salivation	Reduced Activity
Rats	+1 (initially)	+3	+4	+2	0	+3
Guinea Pigs	+1 (initially)	+3	+4	+2	+3	+3

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0			X	X
1-2				
3-9				
10	X	X		

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

K. U. Daniels
J. A. Burkart and R. J. Voss RJV

Statistical Evaluation of IDLH Experiment 16-CB-3; Monochlorobenzene (7970 ppm)

3 June 1977

77M253

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

Kidney-to-body weight and brain-to-body weight ratios were significantly higher for the female rat test group than for the control group. The female guinea pig test group had significantly lower heart-to-body weight ratios than the control group.

Weight Changes

All groups showed significant increases in weight over the course of the experiment. Male rats in the control group had significantly larger weight increases than in the test group. The female guinea pig control group showed larger weight increases on day 3, but the test group showed larger increases on days 6, 10, and 15.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

Attachments

77M253 Table 1

Experiment No. 16-CB-3 : Monochlorobenzene (7970 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	300	6.53	50.49	8.45	3.98	4.45	.20	14.70
		Test	5	276	6.55	48.49	7.60	3.58	5.01	.22	14.86
		t-value		-1.59	.06	-.85	-1.72	-1.10	1.25	1.33	.21
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	191	7.39	43.53	6.95	3.75	5.60	.33	5.78
		Test	5	189	7.52	46.51	7.62	4.01	7.08	.33	5.06
		t-value		-.57	.31	1.98	3.11	.98	3.11	0.0	-1.49
		significance		N.S.	N.S.	N.S.	p<.05	N.S.	p<.05	N.S.	N.S.
Guinea Pig	Male	Control	5	281	8.13	49.57	9.56	4.99	9.47	.48	4.38
		Test	5	292	8.44	51.22	9.52	4.90	9.88	.47	4.77
		t-value		.65	.76	.85	-.16	-.24	.52	-.34	.61
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	252	8.38	47.48	10.48	5.29	9.71	.54	4.77
		Test	5	279	8.06	42.83	8.80	4.39	9.66	.48	4.96
		t-value		1.27	-1.07	-1.07	-1.82	-2.41	-.06	-1.25	.20
		significance		N.S.	N.S.	N.S.	N.S.	p<.05	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α=.05.

77M253 Table 2a.

EXP. NO. 16-CB-3: MONOCHLOROBENZENE (7970 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	2112.5	2112.5	18.363	p<.01
SUBJECTS IN GROUPS	8	920.31	115.04		
TIMES	4	39733.	9933.3	216.34	p<.001
TIMES X GROUPS	4	377.60	94.400	2.0560	N.S.
TIMES X SUBJECTS IN GROUPS	32	1469.3	45.915		
TOTAL	49	44613.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	TOTAL
		1	2	3	4	5	
CONTROL	1	-4.600	14.600	31.200	60.600	80.400	36.440
TEST	2	-9.000	1.200	20.400	45.400	59.200	23.440
TOTAL		-6.800	7.900	25.800	53.000	69.800	29.940

77M253 Table 2b.

EXP. NO. 16-CB-3: MONOCHLOROBENZENE (7970 PPM) ON FEMALE RATS

ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	36.980	36.980	.25199	N.S.
SUBJECTS IN GROUPS	8	1174.0	146.75		
TIMES	4	4495.5	1123.9	106.78	p<.001
TIMES X GROUPS	4	90.121	22.530	2.1406	N.S.
TIMES X SUBJECTS IN GROUPS	32	336.00	10.525		
TOTAL	49	6133.4			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-0.000	-1.600	1.600	14.000	14.200	4.040
TEST	2	-10.200	-8.000	2.200	12.200	15.400	2.320
TOTAL		-9.100	-4.000	1.900	13.100	14.800	3.180

77M253 Table 2c.

EXP. NO. 16-CB-3: MONOCHLOROBENZENE (7970 PPM) ON MALE GUINEA PIGS

ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	100.83	100.83	.28370	N.S.
SUBJECTS IN GROUPS	8	2843.2	355.40		
TIMES	4	51362.	12840.	113.54	p<.001
TIMES X GROUPS	4	535.86	133.96	1.1846	N.S.
TIMES X SUBJECTS IN GROUPS	32	3618.8	113.09		
TOTAL	49	59460.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	TOTAL
		1	2	3	4	5	
CONTROL	1	7.200	25.200	50.600	80.800	86.000	49.960
TEST	2	5.400	16.600	45.000	73.200	95.400	47.120
TOTAL		6.300	20.900	47.800	77.000	90.700	48.540

77M253 Table 2d.

EXP. NO. 16-CB-3: MONOCHLOROBENZENE (7970 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	890.42	890.42	.47370	N.S.
SUBJECTS IN GROUPS	8	15038.	1879.7		
TIMES	4	32671.	8167.8	61.897	p<.001
TIMES X GROUPS	4	2018.5	504.62	3.8241	p<.05
TIMES X SUBJECTS IN GROUPS	32	4222.7	131.96		
TOTAL	49	54841.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-.600	11.600	31.000	52.600	50.400	29.000
TEST	2	3.200	6.800	33.400	61.000	82.800	37.440
TOTAL		1.300	9.200	32.200	56.800	66.600	33.220

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 16-CB-3

Chemical: monochlorobenzene (7970 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
16RC3M	-	Irreversible:	Spongiosis of the white matter
16GC1M	-	"	in the brain. This may be an
16GC3M	-	"	artifact of processing or inherent in
16GC5M	-	"	animal strain, but apparently is not
16GC2F	-	"	related to exposure.
16GC4F	-	"	
16RC6F	-	Irreversible:	Emphysema
Test			
16RT1M	-	Irreversible:	Spongiosis of the white matter
16RT2F	-	"	in the brain. This may be an
16RT4F	-	"	artifact of processing or inherent in
16RT6F	-	"	animal strain, but apparently is not
16GT1M	-	"	related to exposure.
16GT3M	-	"	
16GT5M	-	"	
16GT2F	-	"	

SPECIFIC METHODS AND RESULTS

1,1,2,2-tetrachloroethane

Experiment #3-TCE-1 (576 ppm)

Experiment #9-TCE-2 (5050 ppm)

Experiment #15-TCE-3 (6310 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,1,2,2-tetrachloroethane Exp.#: 3-TCE-1 Date: 1/12/77

Chamber Facility Parameters

Room Temperature: 24.2 °C Chamber Temperature: 25.6 °C Barom. Pressure: 641 Torr

Chamber Pressure: -10 mm H_2O Chamber Intake Air Supply Relative Humidity 20 %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: 600 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 165 °C Setting: 900 Sensor: 1 Heater Power: 600 watts

Pump Setting: 1.80 Liquid Pressure: 24 psig Liquid Flow: ~4 ml/min ~6 g/min

Nitrogen Flow: 3.1 l/min Comp. Air Flow: 68.6 l/min Total Gas Flow: 71.7 l/min

Comments:

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.805 l/min Sampling Interval: 4.00 min

Total Sample Volume: 3.22 l Rotameter Setting: 100 mm Pres. Drop: <20 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: None Concentration: - ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6' S.S., 5% carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 150 °C

Attenuation: 16 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 63 ml/min Detector Gasses: H_2 29 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 2.4 min Internal Standard: - min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,1,2,2-tetrachloroethane Exp.#: 3-TCE-1 Date: 1/12/77

Ambient Temperature: 25.6 °C Barometric Pressure: 641 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-20.8	3.35	579
2	-16.0	3.60	621

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	+1.6	3.16	545
4	6.4	3.23	557
5	15.2	3.03	523
6	19.6	3.85	664
7	24.2	3.36	580
	MEAN:	3.33	576
	S.D.:	0.32	52
	C.V.:	0.096	0.096

Clinical Observations*
3-TCE-1
1,1,2,2 - tetrachloroethane (576 ppm)

Rats: Rats showed a decrease in activity and alertness as the exposure continued. Upon removal from the chamber, animals returned to normal as evidenced by normal righting reflexes and responses to auditory stimulus.

Guinea Pigs: At 5 minutes, guinea pigs demonstrated eye closure and squinting. By 15 minutes, lacrymation was common. Also, animals were less active.

General Comments:

Slight eye irritation and reduced activity were observed.

Scores:**

	Squinting	Lacrymation	Reduced Activity
Rats	0	0	+1
Guinea Pigs	+2	+1	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES AND ORGAN-TO-BODY WEIGHT RATIOS

Dan Daniels

John A. Burkart / Richard J. Voss

Statistical Evaluation of IDLH Experiment 3-TCE-1: 1,1,2,2 Tetrachloroethane (576 PPM)

16 February 1977

77M085

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

Kidney- to-body weight ratios were lower in the test group than in the control for all four animal groups. The difference was statistically significant only for male guinea pigs.

Weight Changes

All groups showed significant increases in weight over the course of exposure.

xc: N. Price
A. F. Toronto
J. H. Nelson

77M085 Table 1

Experiment No. 3-TCE-1: 1,1,2,2-Tetrachloroethane (576 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	302	5.70	52.80	8.61	3.37	5.44	.21	14.33
		Test	5	289	5.76	50.55	8.32	3.33	5.35	.19	14.51
		t-value		-1.25	0.15	-1.02	-0.93	-0.48	-0.23	-1.01	0.20
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	202	6.72	44.35	7.97	3.69	7.36	.28	4.43
		Test	5	204	6.75	47.24	7.63	3.62	7.00	.29	3.80
		t-value		0.28	0.13	1.43	-1.02	-0.79	-0.67	0.47	-1.41
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	325	8.46	55.73	9.83	3.80	9.30	.43	4.21
		Test	4[a]	319	8.28	61.31[b]	8.92	3.47	9.24	.40	3.59
		t-value		-0.49	-0.31	1.57	-2.24	-1.00	-0.08	-1.07	-1.32
		significance		N.S.	N.S.	N.S.	$p<.05$	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	315	8.27	58.47	9.61	3.55	9.29	.43	5.49
		Test	5	300	8.49	54.26[b]	9.14	3.89	10.08	.46	5.74
		t-value		-1.06	0.49	-1.45	-1.20	1.37	1.51	0.79	0.45
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, $\alpha=.05$.

[a] - Data for one animal omitted due to unreliable body weight estimate

[b] - Unreliable organ weight for one animal omitted.

77M085 TABLE 2a

EXP. NO. 3-TCE-1: 1,1,2,2-TETRACHLOROETHANE (576 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	165.62	165.62	.45474	N.S.
SUBJECTS IN GROUPS	8	2913.8	364.22		
TIMES	4	26633.	6658.4	44.195	p< .001
TIMES X GROUPS	4	89.484	22.371	.14849	N.S.
TIMES X SUBJECTS IN GROUPS	32	4021.0	150.66		
TOTAL	49	34623.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-5.000	17.000	27.800	48.200	62.000	30.000
TEST	2	-6.400	9.800	28.000	43.000	57.400	26.360
TOTAL		-5.700	13.400	27.900	45.600	59.700	28.180

77M085 TABLE 2b

EXP. NO. 3-TCE-1: 1,1,2,2-TETRACHLOROETHANE (576 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	16.820	16.820	.10335	N.S.
SUBJECTS IN GROUPS	8	1302.0	162.75		
TIMES	4	3041.1	760.28	19.981	p< .001
TIMES X GROUPS	4	38.880	9.7199	.25545	N.S.
TIMES X SUBJECTS IN GROUPS	32	1217.6	38.050		
TOTAL	49	5616.4			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-7.000	2.400	5.600	8.400	15.800	5.040
TEST	2	-8.800	-.600	3.800	10.600	14.400	3.880
TOTAL		-7.900	.900	4.700	9.500	15.100	4.460

77M085 TABLE 2c

EXP. NO. 3-TCE-1: 1,1,2,2-TETRACHLOROETHANE (576 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1860.5	1860.5	3.3463	N.S.
SUBJECTS IN GROUPS	8	4447.9	555.99		
TIMES	4	62707.	15677.	49.470	p< .001
TIMES X GROUPS	4	772.17	193.04	.60910	N.S.
TIMES X SUBJECTS IN GROUPS	32	10140.	316.89		
TOTAL	49	79928.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	.800	19.200	47.400	73.200	111.200	50.360
TEST	2	-4.000	13.800	35.800	60.600	84.600	38.160
TOTAL		-1.600	16.500	41.600	66.900	97.900	44.260

77M085 TABLE 2d

EXP. NO. 3-TCE-1: 1,1,2,2-TETRACHLOROETHANE (576 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	3200.0	3200.0	3.3067	N.S.
SUBJECTS IN GROUPS	8	7741.9	967.74		
TIMES	4	51909.	12977.	342.61	p< .001
TIMES X GROUPS	4	37.797	9.4492	.24947	N.S.
TIMES X SUBJECTS IN GROUPS	32	1212.1	37.877		
TOTAL	49	64101.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	4.600	22.800	46.600	62.000	99.800	47.160
TEST	2	-8.200	7.000	28.800	45.600	82.600	31.160
TOTAL		-1.800	14.900	37.700	53.800	91.200	39.160

HISTOPATHOLOGICAL EVALUATION OF ORGAN SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 3-TCE-1

Chemical: 1,1,2,2-tetrachloroethane (576 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
-	-	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,1,2,2-tetrachloroethane Exp.#: 9-TCE-2 Date: 3/2/77

Chamber Facility Parameters

Room Temperature: 23.4 °C Chamber Temperature: 28.6 °C Barom. Pressure: 634 Torr

Chamber Pressure: -8 mm H_2O Chamber Intake Air Supply Relative Humidity 33 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 176 °C Setting: 400 Sensor: 2 Heater Power: 600 watts

Pump Setting: 5.50 Liquid Pressure: 20 psig Liquid Flow: 11.6 ml/min 18.4 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.169 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.507 l Rotameter Setting: 30 mm Pres. Drop: - Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: mono-chlorobenzene Concentration: 250 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6 ' S.S. 5% carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 140 °C

Attenuation: 8 Range: 10^3 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 75 ml/min Detector Gasses: H_2 35 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 2.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.8 min Internal Standard: 1.0 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,1,2,2-tetrachloroethane Exp.#: 9-TCE-2 Date: 3/2/77

Ambient Temperature: 28.6 °C Barometric Pressure: 634 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	30.7	5320
2	-5.0	31.6	5480

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	+1.0	27.6	4790
4	4.5	29.6	5130
5	9.0	29.3	5080
6	13.0	30.0	5200
7	17.0	29.7	5140
8	21.0	29.1	5050
9	24.5	28.4	4920
10	27.0	29.5	5110
	MEAN:	29.2	5050
	S.D.:	0.787	134
	C.V.:	0.027	0.027

Clinical Observations*
9-TCE-2.
1,1,2,2 - tetrachloroethane (5050 ppm)

Rats: When initially exposed, all rats showed increased activity and lacrymation. At five minutes, all animals were narcotic and remained immobile throughout the experiment with the exception of some spontaneous jerking. Respiration became labored as the exposure progressed. After the rats were removed, animals 2 and 7 were found to be dead and animal 9 died a few minutes afterward.

Guinea Pigs: Guinea pigs reacted essentially the same as the rats as the exposure progressed, with the added observation that most of them had tremors along with narcosis. No animals died during the experiment. One guinea pig was noted to have no observable breathing during the last minute of the experiment, but on removal from the chamber it recovered.

General Comments:

This compound produced definite narcotic effects on all animals at this concentration, with a small percentage of immediate fatalities.

Scores:**

	Increased Activity	Narcosis	Lacrymation	Reduced Activity
Rats	+1 (initially)	+4	+1	+4
Guinea Pigs	+1 (initially)	+4	+1	+4

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0			X	X
1-2				
3-9				
10	X	X		

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss

Statistical Evaluation of IDLH Experiment 9-TCE-2: 1,1,2,2-Tetrachloroethane (5050 ppm)

5/3/77

77M206

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes

Three rats (2 male and 1 female) in the test group died at exposure and were not included in these analyses.

Organ-to-Body Weight Ratios

No significant differences were found.

Weight Changes

All groups showed significant increases in weight over the course of exposure. Male rats of the test group had smaller weight increases than the control group. For the female rats, the control group showed larger weight increases than the test group on the 1st and 3rd days after exposure, but for days 6, 10, and 15, the test group showed larger weight increases.

Mortality

In the rat test group (males and females combined), three out of ten animals died on the day of exposure. This is not statistically significant, however, at the .05 level, comparing test with control groups.

XC: N. Price
A. F. Toronto
J. H. Nelson

gj

77M206 Table 1

Experiment No. 9-TCE-2: 1,1,2,2-Tetrachloroethane (5050 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	299	5.15	52.59	7.66	3.27	4.54	.21	13.24
		Test	3[a]	281	5.59	53.33	8.48	3.22	5.11	.22	14.70
		t-value		-1.07	.98	.22	1.81	-.34	1.65	.54	1.03
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	208	6.57	46.37	7.62	3.71	6.37	.31	5.24
		Test	4[b]	214	6.82	48.58	7.53	3.64	6.09	.28	3.58
		t-value		.54	.47	1.19	-.16	-.26	-.59	-1.99	-1.77
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	280	8.18	54.63	9.84	4.56	9.99	.51	4.85
		Test	5	294	8.44	53.91	10.49	4.87	9.29	.47	4.66
		t-value		.79	.50	-.25	.67	.52	-1.65	-1.54	-.41
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	280	7.69	49.75	9.64	4.13	10.24	.49	4.77
		Test	5	267	8.53	47.86	10.14	4.58	10.72	.52	3.99
		t-value		-.49	1.89	-.41	1.00	1.20	.46	.74	-.72
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α=.05.

a - Two animals died at exposure and were excluded from this analysis

b - One animal died at exposure and was excluded from this analysis

EXP. NO. 9-TCE-2: 1,1,2,2-TETRACHLOROETHANE (5050 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1401.5	1401.5	6.2571	p<.05
SUBJECTS IN GROUPS	6	1343.9	223.98		
TIMES	4	33693.	8423.2	196.73	p<.001
TIMES X GROUPS	4	152.17	38.043	.88853	N.S.
TIMES X SUBJECTS IN GROUPS	24	1027.6	42.816		
TOTAL	39	37618.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	3.000	16.400	36.200	57.200	88.000	40.160
TEST	2	-2.667	2.333	26.667	41.333	72.000	27.933
TOTAL		.875	11.125	32.625	51.250	82.000	35.575

EXP. NO. 9-TCE-2: 1,1,2,2-TETRACHLOROETHANE (5050 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICAI
GROUPS	1	14.695	14.695	.63900E-01	
SUBJECTS IN GROUPS	7	1609.7	229.96		N.S.
TIMES	4	4957.0	1239.2	83.131	
TIMES X GROUPS	4	176.82	44.205	2.9654	p<.0(
TIMES X SUBJECTS IN GROUPS	28	417.40	14.907		p<.0!
TOTAL	44	7175.6			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-3.200	4.200	8.600	12.200	24.200	9.200
TEST	2	-6.750	-3.500	9.000	14.000	27.500	8.050
TOTAL		-4.778	.778	8.778	13.000	25.667	8.689

EXP. NO. 9-TCE-2: 1,1,2,2-TETRACHLOROETHANE (5050 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	115.52	115.52	.12278	N.S.
SUBJECTS IN GROUPS	8	7526.6	940.82		
TIMES	4	47922.	11981.	79.807	p<.001
TIMES X GROUPS	4	1426.7	356.67	2.3759	N.S.
TIMES X SUBJECTS IN GROUPS	32	4803.8	150.12		
TOTAL	49	61795.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-4.600	23.800	42.800	66.600	77.400	41.200
TEST	2	-.800	14.200	26.600	59.400	91.400	38.160
TOTAL		-2.700	19.000	34.700	63.000	84.400	39.680

EXP. NO. 9-TCE-2: 1,1,2,2-TETRACHLOROETHANE (5050 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1872.7	1872.7	.30182	N.S.
SUBJECTS IN GROUPS	8	49638.	6204.7		
TIMES	4	42033.	10508.	29.082	p<.001
TIMES X GROUPS	4	885.87	221.47	.61293	N.S.
TIMES X SUBJECTS IN GROUPS	32	11562.	361.33		
TOTAL	49	.10599E+06			

MEANS

TIMES:

		DAY 1	DAY 3	DAY 6	DAY10	DAY15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	-4.400	10.400	21.400	57.000	86.000	34.080
TEST	2	-5.600	2.200	13.800	34.600	64.200	21.840
TOTAL		-5.000	6.300	17.600	45.800	75.100	27.960

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 9-TCE-2

Chemical: 1,1,2,2-tetrachloroethane (5050 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
9GC1M	-	Irreversible:	Generalized neuronal necrosis and spongiosis of white matter.
Test			
9RT7M	Exposure day	Irreversible:	none
9RT9M	Exposure day	Irreversible:	none
9RT2F	Exposure day	Reversible:	Renal degeneration which may have been due to early autolysis.

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,1,2,2-tetrachloroethane Exp.#: 15-TCE-3 Date: 4/13/77

Chamber Facility Parameters

Room Temperature: 27.0 °C Chamber Temperature: 29.0 °C Barom. Pressure: 639 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 28 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 210 °C Setting: 800 Sensor: 2 Heater Power: 600 watts

Pump Setting: 9.00 Liquid Pressure: 45-55 psig Liquid Flow: 18.9 ml/min 30.2 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments:

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.153 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.459 l Rotameter Setting: 30 mm. Pres. Drop: <4 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: mono-chlorobenzene Concentration: 220 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 215 °C

Analytical Column: 1/8" x 6' S.S. 5% carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 140 °C

Attenuation: 32 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 53 ml/min Detector Gasses: H_2 30 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 2.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.6 min Internal Standard: 0.8 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,1,2,2-tetrachloroethane Exp.#: 1S-TCE-3 Date: 4/13/77

Ambient Temperature: 29.0 °C Barometric Pressure: 639 Torr

Analytical Method: Activ[illegible]d charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	39.1	6790
2	-5.0	36.5	6330

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	36.1	6260
4	5.0	36.9	6400
5	9.0	36.6	6350
6	13.0	38.0	6590
7	17.0	37.3	6460
8	20.5	35.4	6140
9	25.0	34.6	6010
10	27.5	36.3	6290
	MEAN:	36.4	6310
	S.D.:	1.07	186
	C.V.:	0.0294	0.0294

Clinical Observations*
15-TCE-3
1,1,2,2 - tetrachloroethane (6310 ppm)

Rats: Ataxia was noted after initial exposure and the animals were in the lateral recumbent position by two minutes. All animals were narcotic by five minutes, and respiration was very shallow and labored throughout the rest of the exposure. Animals 2, 5, 7, and 9 were noted to be dead before the experiment ended, with the deaths beginning after 15 minutes of exposure. Animal 1 died soon after he was removed from the exposure chamber.

Guinea Pigs: Same as the rats but lacrymation was noted to be present. No animals died during the exposure, but three died soon afterward (numbers 2, 6, 9).

General Comments:

Definite narcosis was observed in all animals after 5 minutes of exposure with some deaths during and after exposure.

Scores:**

	Ataxia	Narcosis	Lacrymation	Reduced Activity
Rats	+4	+4	0	+4
Guinea Pigs	+4	+4	+3	+4

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0			X	X
1-2				
3-9				
10	X	X		

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss RJV

Statistical Evaluation of IDLH Experiment 15-TCE-3: 1,1,2,2-Tetrachloroethane (6310 ppm)

3 June 1977

77M254

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

The female guinea pig test group had significantly lower mean body weight at autopsy and significantly higher brain-to-body weight ratios than the control group.

Weight Changes

All groups showed significant weight increases over the course of the experiment. The female guinea pig test group showed significantly smaller weight increases than the control group.

Mortality

Combining male and female rats, 5 out of 10 in the test group died at exposure, as opposed to 0 out of 10 in the control group. This is significant at the $\alpha = .05$ level using Fisher's exact test. For guinea pigs, 3 out of 10 in the test group died, 0 out of 10 in the control group (not statistically significant).

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

Attachments

77M254 Table 1

Experiment No. 15-TCE-3 : 1,1,2,2-Tetrachloroethane (6310 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	313	6.25	50.41	8.28	3.27	4.27	.20	13.84
		Test	1	302	5.99	49.97	7.58	3.31	4.64	.20	13.64
		t-value		*	*	*	*	*	*	*	*
		significance									
	Female	Control	5	205	7.70	50.02	8.39	3.75	6.70	.29	4.96
		Test	4	203	7.11	47.53	7.72	3.44	6.32	.30	5.03
		t-value		-.25	-.90	-.72	-1.45	-1.28	-.51	.26	.08
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	295	8.86	56.14	9.77	4.89	9.74	.47	4.60
		Test	3	282	9.31	53.30	9.91	4.67	10.05	.48	3.83
		t-value		-.65	.56	-.57	.23	-.41	.47	.36	-2.07
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	290	8.17	45.43	8.93	5.26	9.26	.46	5.43
		Test	4	268	8.60	50.90	9.86	5.24	11.00	.49	5.38
		t-value		-2.52	1.18	1.82	2.17	-.16	2.59	1.86	-.04
		significance		$p<.05$	N.S.	N.S.	N.S.	N.S.	$p<.05$	N.S.	N.S.

N.S.- Not statistically significant, $\alpha=.05$.

*t-test not applicable.

77M254 Table 2a.

EXP. NO. 15-TCE-3: 1,1,2,2-TETRACHLOROETHANE (6310 PPM) ON MALE RATS

ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	156.06	156.06	.24633	N.S.
SUBJECTS IN GROUPS	4	2534.2	633.56		
TIMES	4	27361.	6840.3	206.43	p<.001
TIMES X GROUPS	4	124.70	31.176	.94005	N.S.
TIMES X SUBJECTS IN GROUPS	16	530.17	33.136		
TOTAL	29	30706.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
		1	2	3	4	5	TOTAL
CONTROL	1	-4.200	25.600	38.200	61.200	65.800	41.320
TEST	2	-1.000	13.000	34.000	51.000	79.000	35.200
TOTAL		-3.667	23.500	37.500	59.500	84.667	40.300

77M254 Table 2b.

EXP. NO. 15-TCE-3: 1,1,2,2-TETRACHLOROETHANE (6310 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	358.47	358.47	1.7123	N.S.
SUBJECTS IN GROUPS	7	1465.4	209.35		
TIMES	4	5380.4	1345.1	29.237	p<.001
TIMES X GROUPS	4	379.49	94.871	2.0622	N.S.
TIMES X SUBJECTS IN GROUPS	28	1288.2	46.006		
TOTAL	44	8871.9			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-9.200	4.200	9.800	17.000	21.600	8.680
TEST	2	-8.250	-7.750	-3.250	12.750	21.500	3.000
TOTAL		-8.778	-1.111	4.000	15.111	21.556	6.156

77M254 Table 2c.

EXP. NO. 15-TCE-3: 1,1,2,2-TETRACHLOROETHANE (6310 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	2517.4	2517.4	2.7553	N.S.
SUBJECTS IN GROUPS	6	5482.0	913.66		
TIMES	4	46061.	11515.	50.795	p<.001
TIMES X GROUPS	4	322.42	80.605	.35556	N.S.
TIMES X SUBJECTS IN GROUPS	24	5440.8	226.70		
TOTAL	39	59824.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	4.400	24.200	39.400	72.800	102.800	48.720
TEST	2	-1.667	2.667	25.000	50.667	85.000	32.333
TOTAL		2.125	16.125	34.000	64.500	96.125	42.575

77M254 Table 2d.

EXP. NO. 15-TCE-3: 1,1,2,2-TETRACHLOROETHANE (6310 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	3914.6	3914.6	15.545	P<.01
SUBJECTS IN GROUPS	7	1762.8	251.83		
TIMES	4	44304.	11076.	188.78	p<.001
TIMES X GROUPS	4	571.19	142.80	2.4339	N.S.
TIMES X SUBJECTS IN GROUPS	28	1642.7	58.670		
TOTAL	44	52195.			

MEANS

TIMES:

		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	9.600	23.000	46.000	71.800	101.200	50.320
TEST	2	5.000	-.250	25.000	40.000	80.000	31.550
TOTAL		7.556	12.667	36.667	61.222	91.778	41.978

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 15-TCE-3

Chemical: 1,1,2,2-tetrachloroethane (6310 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
-	-	Irreversible:	none
Test			
15RT1M	Exposure day	Irreversible:	Myocardial damage
15RT4F	-	"	Deposits of unknown material in brain
15RT6F	-	"	Deposits of unknown material in brain
15GT4F	-	"	Pulmonary granulomata
15RT5M	Exposure day	Irreversible:	none
15RT7M	"	"	"
15RT9M	"	"	"
15RT2F	"	"	"
15GT9M	"	"	"
15GT2F	"	"	"
15GT6F	"	"	"

SPECIFIC METHODS AND RESULTS

3-chloropropene

Experiment #4-3CP-1 (1300 ppm)

Experiment #13-3CP-2 (11800 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 3-chloropropene Exp.#: 4-3CP-1 Date: 1/26/77

Chamber Facility Parameters

Room Temperature: 25.2 °C Chamber Temperature: 24.3 °C Barom. Pressure: 645 Torr

Chamber Pressure: -13 mm H_2O Chamber Intake Air Supply Relative Humidity - %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: 960 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 82 °C Setting: 0000 Sensor: 1 Heater Power: 600 watts

Pump Setting: 3.50 Liquid Pressure: 12-14 psig Liquid Flow: ~7.4ml/min 6.9 g/min

Nitrogen Flow: 1.5 l/min Comp. Air Flow: 0.0 l/min Total Gas Flow: 1.5 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: Adsorption Tubes Collection Medium: Charcoal

Sampling Flow: 0.839 l/min Sampling Interval: 4.00 min

Total Sample Volume: 3.36 l Rotameter Setting: 100 mm Pres. Drop: 49 Torr

Desorption Solvent: Carbon Disulfide Desorption Volume: 1.00 ml

Internal Standard Used: n-Pentane Concentration: 100 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 12 ft S.S. 5% Carbowax 20 M - TPA 80/100 Mesh Chromosorb W-AW @ 60 °C

Attenuation: 8 Range: 10^3 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 30 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 2.2 min Internal Standard: 0.8 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 3-chloropropene Exp.#: 4-3CP-1 Date: 1/26/77

Ambient Temperature: 24.3 °C Barometric Pressure: 645 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments: This exposure lasted only 25 minutes due to timing errors.

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-	-	-
2	-11.4	3.08	1160
3	-4.4	2.92	1100

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
4	2.0	3.43	1300
5	7.8	3.25	1230
6	12.7	3.46	1310
7	17.8	3.28	1240
8	22.7	3.71	1400
	MEAN:	3.43	1300
	S.D.:	0.18	68.0
	C.V.:	0.053	0.053

Clinical Observations *
4-3CP-1
3-chloropropene (1300 ppm)

Rats: No abnormalities were noted in comparing clinical behavior of control and test animals during first 20 minutes of experiment. Mild squinting was noted at 25 minutes.

Guinea Pigs: No differences were noted in the test and control guinea pigs except for some slight squinting during the last 10 minutes of exposure.

General Comments:

Slight degree of eye irritation was observed near the end of exposure in all animals.

Scores:**

	Squinting
Rats	+1/2 (after 20 minutes)
Guinea Pigs	+1/2 (after 20 minutes)

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

to A. U. Daniels
from John A. Burkart/Richard J. Voss
subject Statistical Evalution of IDLH Experiment 4-3CP-1: 3-Chloropropene (1300 ppm)
date 11 March 1977
refer to 77M116

Methods

Post exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight; these changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

The female rat test group had significantly lower kidney weight ratios than did the control group.

Weight Changes

All groups showed significant increases in weight over the period of exposure. The female guinea pig test group showed significantly greater weight increases than the control group.

RJV:lg

xc: N. Price
J. Nelson
A. F. Toronto

77M116 Table 1.

Experiment No. 4-3CP-1 : 3-Chloropropene (1300 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	310	5.36	49.62	8.54	3.41	5.46	.19	13.97
		Test	5	310	5.98	51.74	8.72	3.53	5.49	.19	14.48
		t-value		.20	1.52	1.09	.36	.52	.07	.00	.76
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	216	6.60	45.86	8.12	3.78	6.95	.24	4.38
		Test	5	215	6.64	46.22	7.43	3.70	6.92	.25	3.84
		t-value		-.32	.09	.14	-3.19	- .36	- .05	.64	-1.58
		significance		N.S.	N.S.	N.S.	$p<.05$	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	339	7.45	59.83	9.95	4.08	9.84	.42	4.19
		Test	5	301	8.41	53.14	10.12	3.77	10.79	.45	3.78
		t-value		-1.65	-1.53	-2.27	.37	-1.20	1.11	1.23	-1.27
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	307	7.98	54.21	9.50	3.94	9.29	.43	4.03
		Test	5	315	8.09	46.86	9.77	3.77	9.73	.44	5.18
		t-value		.35	.16	-1.88	.39	-.48	.55	.33	1.55
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, $\alpha = .05$.

77M116 Table 2a. Male Rats

EXP. NO. 4-3CP-1: 3-CHLOROPROPENE (1300 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	36.984	36.984	.18318	N.S.
SUBJECTS IN GROUPS	8	1615.2	201.90		
TIMES	4	57688.	14422.	286.51	p <.001
TIMES X GROUPS	4	3.5156	.87891	.17460E-01	N.S.
TIMES X SUBJECTS IN GROUPS	32	1610.8	50.338		
TOTAL	49	60955.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	5.400	27.000	52.600	71.800	102.600	51.880
TEST	2	3.400	25.400	51.400	69.200	101.400	50.160
TOTAL		4.400	26.200	52.000	70.500	102.000	51.020

77M116 Table 2b. Female Rats

EXP. NO. 4-3CP-1: 3-CHLOROPROPENE (1300 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	21.781	21.781	.47714	N.S.
SUBJECTS IN GROUPS	8	365.20	45.650		
TIMES	4	5416.5	1354.1	82.568	p <.001
TIMES X GROUPS	4	19.918	4.9795	.30363	N.S.
TIMES X SUBJECTS IN GROUPS	32	524.80	16.400		
TOTAL	49	6348.2			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-.600	10.800	19.800	24.200	29.600	16.760
TEST	2	2.600	10.200	21.800	25.400	30.400	18.080
TOTAL		1.000	10.500	20.800	24.800	30.000	17.420

77M116 Table 2c. Male Guinea Pig

EXP. NO. 4-3CP-1: 3-CHLOROPROPENE (1300 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	5020.0	5020.0	2.7022	N.S.
SUBJECTS IN GROUPS	8	14862.	1857.7		
TIMES	4	57477.	14369.	30.061	p <.001
TIMES X GROUPS	4	1560.7	390.17	.81625	N.S.
TIMES X SUBJECTS IN GROUPS	32	15296.	478.00		
TOTAL	49	94215.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	3.400	26.400	55.800	77.000	110.800	54.680
TEST	2	-1.000	15.000	28.800	41.000	89.400	34.640
TOTAL		1.200	20.700	42.300	59.000	100.100	44.660

77M116 Table 2d. Female Guinea Pig

EXP. NO. 4-3CP-1: 3-CHLOROPROPENE (1300 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	3595.5	3595.5	7.5932	p <.05
SUBJECTS IN GROUPS	8	3788.2	473.52		
TIMES	4	53671.	13418.	207.76	p <.001
TIMES X GROUPS	4	561.86	140.46	2.1750	N.S.
TIMES X SUBJECTS IN GROUPS	32	2066.7	64.583		
TOTAL	49	63683.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
		1	2	3	4	5	TOTAL
GROUPS:							
CONTROL	1	-8.600	12.800	28.800	52.000	79.200	32.840
TEST	2	6.600	17.400	49.200	74.200	101.600	49.800
TOTAL		-1.000	15.100	39.000	63.100	90.400	41.320

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 4-3CP-1

Chemical: 3-chloropropene (1300 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
4GC2F	-	Reversible: myocardial damage to myofibrils in sarcolemma
Test		
-	-	Irreversible: none
4RT2F	-	Reversible: renal tubular calcification

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 3-chloropropene Exp.#: 13-3CP-2 Date: 3/30/77

Chamber Facility Parameters

Room Temperature: 23.8 °C Chamber Temperature: 26.0 °C Barom. Pressure: 644 Torr

Chamber Pressure: -13 mm H_2O Chamber Intake Air Supply Relative Humidity 26 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 82 °C Setting: 0000 Sensor: 1 Heater Power: 600 watts

Pump Setting: 10.00 Liquid Pressure: 25 psig Liquid Flow: 20.7 ml/min 19.4 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments:

Test Atmosphere Sampling Parameters

Collection Method: Adsorption Tubes Collection Medium: Charcoal

Sampling Flow: 0.0907 l/min Sampling Interval: 2.67 min

Total Sample Volume: 0.242 l Rotameter Setting: 19 mm Pres. Drop: < 3 Torr

Desorption Solvent: Carbon Disulfide Desorption Volume: 1.00 ml

Internal Standard Used: n-Pentane Concentration: 100 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 12 ft S.S. 5% Carbowax 20M - TPA 80/100 Mesh Chromosorb W-AW @ 60 °C

Attenuation: 8 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 70 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.4 min Internal Standard: 0.4 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 3-chloropropene Exp.#: 13-3CP-2 Date: 3/30/77

Ambient Temperature: 26.0 °C Barometric Pressure: 644 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-8.0	30.8	11,700
2	-4.1	31.4	11,900

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	30.1	11,400
4	4.5	31.6	12,000
5	7.5	30.9	11,700
6	11.0	31.3	11,900
7	16.8	30.6	11,600
8	20.0	31.7	12,000
9	23.5	31.5	11,900
10	27.0	31.1	11,800
	MEAN:	31.1	11,800
	S.D.:	0.548	207
	C.V.:	0.0176	0.0176

Clinical Observations*
13-3CP-2
3 - chloropropene (11,800 ppm)

Rats: Increased initial activity was apparent. All animals were squinting and activity was slowed at 5 minutes. At 10 minutes, there was slight lacrymation in some of the animals, but no other effects were observed during the exposure.

Guinea Pigs: All animals showed initial increased activity and nose rubbing. There was squinting in all animals at 5 minutes and slight lacrymation at 10 minutes. Slight ataxia and escape behavior was noted at 20 minutes.

General Comments:

Some nasal and eye irritation was observed in most animals, and guinea pigs appeared to have slight ataxia near the end of exposure. One guinea pig died postexposure, and one had to be sacrificed due to a broken leg received while in the exposure cage.

Scores:**

	Increased Activity	Nose Rubbing	Lacrymation	Ataxia	Escape Behavior
Rats	+1	+1	+1		0
Guinea Pigs	+1	+1	+1	+1	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss

Statistical Evaluation of IDLH Experiment 13-3CP-2: 3-Chloropropane (11,800 ppm)

6 May 1977

77M212

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

One female guinea pig died of causes unrelated to this study and was not included in these analyses.

Organ-to-Body Weight Ratios

Female rats of the test group were significantly lighter than the control group in body weight at autopsy. Liver-to-body weight ratios were significantly lower for the test female guinea pig group than for the control group. For female rats, the test group had significantly larger adrenal-to-body weight ratios than the control group.

Weight Changes

All groups showed significant increases in weight over the course of exposure. The male guinea pig test group showed significantly smaller weight increases than the control. The male rat test group increased weight slower than the control up to the third day after exposure, but increased weight faster than the control from the third day to the end of the experiment.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

77M212 Table 1

Experiment No. 13-3CP-2 : 3-Chloropropene (11,800 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	298	6.41	47.02	7.94	3.56	4.81	.19	14.73
		Test	5	305	6.40	50.65	8.67	3.75	4.52	.19	14.30
		t-value		.78	-.02	1.28	2.02	.62	-.43	-.41	-.68
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	207	7.38	44.50	7.01	3.93	6.97	.29	4.59
		Test	5	190	7.74	44.19	8.08	3.96	6.96	.32	5.00
		t-value		-3.73	.68	-.13	2.06	.14	-.04	4.33	.84
		significance		p<.01	N.S.	N.S.	N.S.	N.S.	N.S.	p<.01	N.S.
Guinea Pig	Male	Control	4	316	8.01	51.78	9.30	5.66	8.59	.43	5.05
		Test	5	271	7.78	56.19	10.25	5.56	9.14[a]	.48	4.14
		t-value		-1.72	-.45	2.00	1.71	-.11	1.82	2.14	-1.49
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	6	275	10.02	46.98	10.46	5.83	10.14	.52	5.59
		Test	4[b]	279	9.01	39.96	9.44	4.77	9.29	.46	4.78
		t-value		.18	-.84	-2.32	-1.59	-.99	-1.02	-.70	-.73
		significance		N.S.	N.S.	p<.05	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. - Not statistically significant. $\alpha = .05$.

a - Based on 4 animals, as one value was lost.

b - One animal died due to non-study-related causes.

77M212 Table 2a

EXP. NO. 13-3CP-2: 3-CHLOROPROPENE (11,000 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	259.92	259.92	.93259	N.S.
SUBJECTS IN GROUPS	8	2229.7	270.71		
TIMES	4	44943.	11211.	327.34	p<.001
TIMES X GROUPS	4	749.89	107.47	5.4741	p<.01
TIMES X SUBJECTS IN GROUPS	32	1095.9	34.247		
TOTAL	49	49178.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-4.800	15.400	29.200	53.000	74.600	33.460
TEST	2	-4.800	-.200	19.000	49.400	81.200	28.920
TOTAL		-4.800	7.600	24.100	51.200	77.900	31.200

77M212 Table 2b

EXP. NO. 13-3CP-2: 3-CHLOROPROPENE (11,800 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANC
GROUPS	1	865.28	865.28	2.5231	N.S.
SUBJECTS IN GROUPS	8	2743.5	342.94		
TIMES	4	3914.2	978.55	29.230	$p<.001$
TIMES X GROUPS	4	151.72	37.930	1.1330	N.S.
TIMES X SUBJECTS IN GROUPS	32	1071.3	33.478		
TOTAL	49	8746.0			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-1.800	6.200	4.600	16.800	26.000	10.360
TEST	2	-6.600	-5.400	-1.200	10.000	12.600	2.040
TOTAL		-4.200	.400	1.700	13.800	19.300	6.200

EXP. NO. 13-3CP-2: 3-CHLOROPROPENE (11,800 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	7784.1	7784.1	11.033	p<.05
SUBJECTS IN GROUPS	6	4233.0	705.50		
TIMES	4	58359.	14590.	88.804	p<.001
TIMES X GROUPS	4	1371.6	342.91	2.0872	N.S.
TIMES X SUBJECTS IN GROUPS	24	3943.0	164.29		
TOTAL	39	75691.			

MEANS

TIMES:

		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	5.500	15.250	47.500	84.250	110.500	52.600
TEST	2	-.250	-23.250	16.000	48.000	83.000	24.700
TOTAL		2.625	-4.000	31.750	66.125	96.750	38.650

EXP. NO. 13-3CP-2: 3-CHLOROPROPENE (11,000 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	14.961	14.961	.30028E-02	N.S.
SUBJECTS IN GROUPS	8	39858.	4982.3		
TIMES	4	38909.	9747.4	33.130	p<.001
TIMES X GROUPS	4	1167.2	291.81	.99101	N.S.
TIMES X SUBJECTS IN GROUPS	32	9414.9	294.22		
TOTAL	49	89445.			

MEANS

TIMES:

		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	-3.667	6.833	21.167	42.167	70.167	27.333
TEST	2	1.750	-11.500	30.250	46.500	75.250	20.450
TOTAL		-1.500	-.500	24.800	43.900	72.200	27.780

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 13-3CP-2

Chemical: 3-chloropropene (11,800 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
13GT9M	2 days	Irreversible: autolysis prevented conclusive findings.
13GT2F	day of exposure	This animal broke its leg while in the exposure cage and was therefore sacrificed. No organs were submitted for histopathology.

SPECIFIC METHODS AND RESULTS

N,N-dimethylaniline

Experiment #5-NNDMA-1 (194 ppm)

Experiment 12-NNDMA-2 (953 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: N,N-dimethylaniline Exp.#: 5-NNDMA-1 Date: 2/2/77

Chamber Facility Parameters

Room Temperature: 25.3 °C Chamber Temperature: 26.2 °C Barom. Pressure: 646 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 24 %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: ~600 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 199 °C Setting: 620 Sensor: 2 Heater Power: 600 watts

Pump Setting: 0.50 Liquid Pressure: 10 psig Liquid Flow: ~1 ml/min ~1 g/min

Nitrogen Flow: 3.1 l/min Comp. Air Flow: 71.3 l/min Total Gas Flow: 74.4 l/min

Comments:

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.792 l/min Sampling Interval: 4.00 min

Total Sample Volume: 3.17 l Rotameter Setting: 100 mm Pres. Drop: <22 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: 1,1,2,2-tetra-chloroethane Concentration: 200 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6' S.S. 5% Carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 160 °C

Attenuation: 32 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 60 ml/min Detector Gasses: H_2 55 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 2.2 min Internal Standard: 1.6 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: N,N-dimethylaniline Exp.#: 5-NNDMA-1 Date: 2/2/77

Ambient Temperature: 26.2 °C Barometric Pressure: 646 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-24.0	0.691	164
2	-17.3	0.729	173
3	-9.1	0.836	198

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
4	2.2	0.740	176
5	9.3	1.08	256
6	14.7	0.780	185
7	19.3	0.809	192
8	23.9	0.655	156
9	26.9	0.836	199
	MEAN:	0.817	194
	S.D.:	0.144	34
	C.V.:	0.176	0.176

Clinical Observations*
5-NNDMA-1
N,N - dimethylaniline (194 ppm)

Rats: Few clinical signs were noted in this group. Slight squinting without lacrymation was noted and some decrease in general activity. Upon removal from the test chamber, squinting disappeared immediately without apparent after effects.

Guinea Pigs: Guinea Pigs appeared to show a decrease in activity. An occasional animal was noted wiping or rubbing its nose and all animals demonstrated marked squinting, again without lacrymation. Upon removal of animals from the chambers, squinting immediately disappeared and eyes appeared normal.

General Comment

Slight nasal and eye irritation along with some slowed activity was noted.

Scores:**

	Squinting	Eye Closure	Reduced Activity	Nose Rubbing	Increased Activity
Rats	+1	0	+1 (late)	0	+1 (only initially)
Guinea Pigs	+1	+1	+1 (late)	+1	+1 (only initially)

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

to A. U. Daniels

from J. A. Burkart/R. J. Voss

subject Statistical Evaluation of IDLH Experiment 5-NNDMA-1: N,N-Dimethylaniline (194 ppm)

date 11 March 1977

refer to 77M118

Methods

Post exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight; these changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

The final body weight of the test female rat group was significantly higher than the control group. The female rat test group also had significantly lower brain weight ratios than the control group. The female guinea pig test group had significantly lower gonad weight ratios than the control.

Weight Changes

All groups showed significant increases in weight over the period of exposure. Both male and female rats showed significant differences between test and control groups considering weight changes over time; for males the control group had greater increases, for females the test group had greater increases.

RJV:lg

xc: N. Price
J. Nelson
A. F. Toronto

77M118 Table 1.

Experiment No. 5-NNDMA-1 : N,N-Dimethylaniline (194 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	292	6.01	50.14	7.42	4.04	4.93	.22	14.25
		Test	5	278	5.94	48.14	8.03	3.47	5.30	.23	14.70
		t-value		-2.19	-.19	-1.24	.72	-.99	1.30	.60	.59
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	196	7.09	41.55	7.64	3.92	7.32	.31	4.91
		Test	5	208	6.33	45.13	7.49	3.60	6.55	.29	4.05
		t-value		3.38	-1.53	1.67	-.36	-.88	-2.68	-1.06	-1.62
		significance		$p<.01$	N.S.	N.S.	N.S.	N.S.	$p<.05$	N.S.	N.S.
Guinea Pig	Male	Control	5	339	8.33	52.15	8.96	4.00	9.47	.40	4.39
		Test	6	318	8.28	50.90	9.42	3.78	9.59	.42	3.90
		t-value		-1.61	-.12	- .44	1.33	-.79	.27	.80	-2.10
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	329	8.22	48.60	8.86	4.15	8.82	.41	5.96
		Test	4	312	8.05	48.42	8.61	3.70	9.17	.43	4.21
		t-value		-.97	-.15	- .08	-.59	-.78	.45	.61	-2.92
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	$p<.05$

N.S.- Not statistically significant, $\alpha=.05$.

77M118 Table 2a. Male Rats

EXP. NO. 5-NNDMA-1: N,N-DIMETHYLANILINE (194 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1/	1458.0	1458.0	10.470	p<.05
SUBJECTS IN GROUPS	8	1114.1	139.26		
TIMES	4	40946.	10237.	753.53	p<.001
TIMES X GROUPS	4	196.80	49.199	3.621G	p<.05
TIMES X SUBJECTS IN GROUPS	32	434.72	13.585		
TOTAL	49	44150.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	.600	18.800	38.600	65.400	85.000	41.680
TEST	2	-6.400	11.800	29.000	52.400	67.600	30.880
TOTAL		-2.900	15.300	33.800	58.900	76.300	36.280

77M118 Table 2b. Female Rats

EXP. NO. 5-NNDMA-1: N,N-DIMETHYLANILINE (194 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	985.68	985.68	4.2865	N.S.
SUBJECTS IN GROUPS	8	1839.6	229.95		
TIMES	4	4340.5	1085.1	73.692	p<.001
TIMES X GROUPS	4	295.52	73.880	5.0173	p<.01
TIMES X SUBJECTS IN GROUPS	32	471.20	14.725		
TOTAL	49	7932.5			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-10.000	.200	6.800	8.200	10.200	3.080
TEST	2	-5.800	5.000	12.600	22.800	25.200	11.960
TOTAL		-7.900	2.600	9.700	15.500	17.700	7.520

77M118 Table 2c. Male Guinea Pig

EXP. NO. 5-NNDMA-1: N,N-DIMETHYLANILINE (194 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	408.52	408.52	.71160	N.S.
SUBJECTS IN GROUPS	9	5166.7	574.08		
TIMES	4	86291.	21573.	248.01	p<.001
TIMES X GROUPS	4	416.11	104.03	1.1960	N.S.
TIMES X SUBJECTS IN GROUPS	36	3131.4	86.983		
TOTAL	54	95414.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DA 10	DAY15	TOTAL
		1	2	3	4	5	
CONTROL	1	-1.800	13.200	44.600	75.000	116.200	49.440
TEST	2	-.833	12.000	39.833	67.500	101.333	43.967
TOTAL		-1.273	12.545	42.000	70.909	108.091	46.455

77M118 Table 2d. Female Guinea Pig

EXP. NO. 5-NNDMA-1: N,N-DIMETHYLANILINE (194 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	1515.8	1515.8	3.0615	N.S.
SUBJECTS IN GROUPS	7	3465.8	495.12		
TIMES	4	66843.	16711.	135.70	p<.001
TIMES X GROUPS	4	223.55	55.887	.45384	N.S.
TIMES X SUBJECTS IN GROUPS	28	3447.9	123.14		
TOTAL	44	75496.			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY10	DAY15	TOTAL
		1	2	3	4	5	TOTAL
CONTROL	1	.200	24.000	46.000	75.800	113.400	51.880
TEST	2	-5.000	7.750	35.250	66.750	96.250	40.200
TOTAL		-2.111	16.778	41.222	71.778	105.778	46.689

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 5-NNDMA-1

Chemical: N,N-dimethylaniline (194 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
-	-	Irreversible:	none
Test			
5GT6F	-	Irreversible:	mild focal non-suppurative meningitis

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: N,N-dimethylaniline Exp.#: 12-NNDMA-2 Date: 3/23/77

Chamber Facility Parameters

Room Temperature: 25.0 °C Chamber Temperature: 28.0 °C Barom. Pressure: 634 Torr

Chamber Pressure: -18 mm H_2O Chamber Intake Air Supply Relative Humidity 25 %

Air Velocity Meter Reading 300 ft/min Intake Duct Air Flow: 821 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 204 °C Setting: 700 Sensor: 2 Heater Power: 600 watts

Pump Setting: 3.00 Liquid Pressure: 5 psig Liquid Flow: 6.07 ml/min 5.80 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.170 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.510 l Rotameter Setting: 30 mm. Pres. Drop: .4 Torr

Desorption Solvent: carbon disulfide Desorption Volume: 1.00 ml

Internal Standard Used: 1,1,2,2-tetra-chloroethane Concentration: 500 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6' S.S. 5% Carbowax 20M-TPA

80/100 mesh Chromosorb W-AW @ 160 °C

Attenuation: 16 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 70 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 1.0 ul Solvent Flush Volume: 1.0 ul

Elution times: Test Compound: 1.8 min Internal Standard: 1.2 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: N,N-dimethylaniline Exp.#: 12-NNDMA-2 Date: 3/23/77

Ambient Temperature: 28.0 °C Barometric Pressure: 634 Torr

Analytical Method: Activated charcoal adsorption tubes - carbon disulfide desorption - FID/GC analysis

Comments: Test compound condensed on the wall of inhalation chambers during exposure.

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	3.89	940
2	-5.0	4.09	989

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	3.86	933
4	5.5	4.26	1030
5	9.0	2.98	722
6	14.0	3.92	949
7	17.3	3.99	966
8	20.8	4.29	1040
9	23.1	4.01	970
10	26.0	4.17	1010
	MEAN:	3.94	953
	S.D.:	0.416	101
	C.V.:	0.106	0.106

Clinical Observations*
12-NNDMA-2
N,N - dimethylaniline (953 ppm)

Rats: Initial hyperactivity was noted. At five minutes, some animals were squinting and lacrymating. No other changes were observed.

Guinea Pigs: There was also some initial hyperactivity. All animals were squinting and lacrymating at 5 minutes. At 15 minutes, all animals had their eyes closed and were relatively inactive.

General Comments:

The rats seemed less affected than the guinea pigs and the main effect observed was eye irritation.

Scores:**

	Increased Activity	Lacrymation	Squinting	Eye Closure
Rats	+2 (initially)	+2	+2	0
Guinea Pigs	+2 (initially)	+2	+2	+2

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss

Statistical Evaluation of IDLH Experiment 12-NNDMA-2: N,N-Dimethylaniline (953 ppm)

6 May 1977

77M211

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

Lung-to-body weight ratios were significantly lower in the test group than in the control group for female guinea pigs.

Weight Changes

All groups showed significant increases in weight over the course of exposure. For rats, both males and females, the test groups showed significantly smaller weight increases than the control groups.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

77M211 Table 1

Experiment No. 12-NNDMA-2 : N,N-Dimethylaniline (953 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	305	6.43	46.45	7.97	3.33	4.74	.20	14.82
		Test	5	292	5.58	50.66	*	3.40	4.75	.21	14.73
		t-value		-.98	-2.12	1.35		.52	.02	1.60	-.19
		significance		N.S.	N.S.	N.S.		N.S.	N.S.	N.S.	N.S.
	Female	Control	5	202	7.72	42.96	7.66	3.67	6.28	.30	4.56
		Test	5	201	6.72	45.10	*	3.54	6.74	.30	4.04
		t-value		-.08	-1.76	.87		-.45	1.42	0.0	-1.17
		significance		N.S.	N.S.	N.S.		N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	345	8.06	50.39	8.79	4.65	8.28	.39	4.69
		Test	5	315	8.49	50.33	9.03	4.24	8.9	.43	4.95
		t-value		-1.55	.57	-.01	.63	-.85	1.11	1.41	.33
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	316	8.19	48.61	8.85	4.75	8.53	.44	5.04
		Test	5	311	7.56	46.35	8.94	4.52	8.99	.43	6.09
		t-value		-.60	-3.0	-.46	.23	-.46	.60	-.51	1.65
		significance		N.S.	$p<.05$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, $\alpha=.05$.

* - Missing data

EXP. NO. 12-NNDMA-2: N,N - DIMETHYLANILINE (953 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	669.78	669.78	19.397	p<.01
SUBJECTS IN GROUPS	8	276.24	34.530		
TIMES	4	38260.	9565.1	390.14	p<.001
TIMES X GROUPS	4	229.92	57.480	2.3445	N.S.
TIMES X SUBJECTS IN GROUPS	32	784.55	24.517		
TOTAL	49	40221.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-5.200	20.200	29.600	54.200	79.800	35.720
TEST	2	-8.200	17.200	22.200	39.600	71.200	28.400
TOTAL		-6.700	18.700	25.900	46.900	75.500	32.060

77M211 Table 2b

EXP. NO. 12-NNDMA-2: N,N-DIMETHYLANILINE (953 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	390.80	380.88	9.7938	p<.05
SUBJECTS IN GROUPS	8	311.12	38.890		
TIMES	4	5781.6	1445.4	47.270	p<.001
TIMES X GROUPS	4	95.921	23.980	.78424	N.S.
TIMES X SUBJECTS IN GROUPS	32	978.40	30.577		
TOTAL	49	7540.0			

MEANS

TIMES:

GROUPS:		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	TOTAL
		1	2	3	4	5	
CONTROL	1	-7.800	6.400	5.000	15.400	20.800	7.960
TEST	2	-14.000	-.600	-3.600	10.000	20.400	2.440
TOTAL		-10.900	2.900	.700	12.700	20.600	5.200

77M211 Table 2c

EXP. NO. 12-NNDMA-2: N,N-DIMETHYLANILINE (953 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	409.00	409.00	.81727	N.S.
SUBJECTS IN GROUPS	8	4003.6	500.45		
TIMES	4	78563.	19641.	103.18	p<.001
TIMES X GROUPS	4	1332.9	333.23	1.7505	N.S.
TIMES X SUBJECTS IN GROUPS	32	6091.6	190.36		
TOTAL	49	90401.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	4.400	20.400	45.200	78.600	129.600	55.640
TEST	2	9.000	24.000	39.000	70.800	106.000	49.920
TOTAL		6.700	22.600	42.100	74.700	117.800	52.780

77M211 Table 2d

EXP. NO. 12-NNDMA-2: N,N-DIMETHYLANILINE (953 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	180.50	180.50	.61093	N.S.
SUBJECTS IN GROUPS	8	2363.6	295.45		
TIMES	4	58030.	14507.	299.51	p<.001
TIMES X GROUPS	4	172.59	43.148	.89081	N.S.
TIMES X SUBJECTS IN GROUPS	32	1550.0	48.437		
TOTAL	49	62296.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	7.200	24.400	40.800	68.200	103.400	48.800
TEST	2	5.000	18.000	39.800	58.000	103.400	45.000
TOTAL		6.100	21.600	40.300	63.100	103.400	46.900

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 12-NNDMA-2

Chemical: N,N-dimethylaniline (953 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
-	-	Irreversible: none

SPECIFIC METHODS AND RESULTS

1,1,2,2-tetrabromoethane

Experiment #6-TBE-1 (19.9 ppm)

Experiment #11-TBE-2 (91.1 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,1,2,2-tetrabromoethane Exp.#: 6-TBE-1 Date: 2/9/77

Chamber Facility Parameters

Room Temperature: 23.2 °C Chamber Temperature: 25.6 °C Barom. Pressure: 645 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity ____ %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: ~950 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 199 °C Setting: 630 Sensor: 2 Heater Power: 600 watts

Pump Setting: 0.70 Liquid Pressure: 16 psig Liquid Flow: <1 ml/min <3 g/min

Nitrogen Flow: 2.8 l/min Comp. Air Flow: 47.0 l/min Total Gas Flow: 49.8 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: silica gel

Sampling Flow: 0.830 l/min Sampling Interval: 4.00 min

Total Sample Volume: 3.32 l Rotameter Setting: 100 mm Pres. Drop: <32 Torr

Desorption Solvent: acetone Desorption Volume: 1.00 ml

Internal Standard Used: 1-bromo-napthalene Concentration: 30 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 12' S.S. 10% UC-W98

80/100 mesh Chromosorb W-HP @ 170 °C

Attenuation: 4 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 63 ml/min Detector Gasses: H_2 30 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 3.0 ul Solvent Flush Volume: 2.0 ul

Elution times: Test Compound: 2.4 min Internal Standard: 4.4 min

*Dodecane was substituted for 1-bromonapthalene in subsequent experiments to reduce analysis time and to obtain a sharper peak for the internal standard.

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,1,2,2-tetrabromoethane Exp.#: 6-TBE-1 Date: 2/9/77

Ambient Temperature: 25.6 °C Barometric Pressure: 645 Torr

Analytical Method: Silica gel adsorption tubes - acetone desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-16	0.235	18.2
2	-8	0.237	18.4

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	0.251	19.4
4	5.2	0.211	16.3
5	10.0	0.261	20.2
6	15.0	0.267	20.7
7	20.0	0.257	19.9
8	25.0	0.296	22.9
	MEAN:	0.257	19.9
	S.D.:	0.028	2.14
	C.V.:	0.109	0.109

Clinical Observations*
6-TBE-1
1,1,2,2 - tetrabromoethane (19.9 ppm)

Rats: After a very brief period of increased activity, all the rats appeared to be abnormally quiet. Near total inactivity was noted during the entire exposure. Animals squinted only slightly during the test.

Guinea Pigs: As the guinea pigs were exposed to the test atmosphere, most of the animals appeared nervous, excited, and several began wiping their noses with their forearms. After 5 minutes of exposure to the test atmosphere, the nose, chin, and chest appeared to be wet from nasal and oral secretions. By the end of the experiment, seven of ten animals showed marked salivating. At the 10-minute mark in exposure, two animals had a momentary period of excitement. This excitement could be characterized as a jerking, jumping escape behavior. Mild isolated muscle group jerkings were noted in some of the guinea pigs. A progression of abnormal behavioral signs did not appear to extend beyond the 10-minute mark in the 30-minute exposure. Eye squinting was mild.

General Comments:

The guinea pigs appeared to have more nasal/oral irritation than the rats.

Scores:**

	Squinting	Slowed Activity	Nasal and/or Oral Secretion	Hyperactivity	Escape Behavior	Jerking
Rats	+1	+2	0	+1 (initially)	0	0
Guinea Pigs	+1	0	+3	+2 (initially)	+1	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.
**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

to A. U. Daniels
from J. A. Burkart/R. J. Voss
subject Statistical Evaluation of IDLH Experiment 6-TBE-1: 1,1,2,2 Tetrabromoethane (19.9 ppm)
date 11 March 1977
refer to 77M117

Methods

Post exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight; these changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

Among the female guinea pigs the test group had significantly lower lung weight ratios. Among the female rats, the test group had significantly lower weight ratios for both brain and adrenals.

Weight Changes

All groups showed significant weight increases over the period of exposure.

RJV:lg

xc: N. Price
J. Nelson
A. F. Toronto

77M117 Table 1.

Experiment No. 6-TBE-1 : 1,1,2,2 - Tetrabromoethane (19.9 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	292	5.71	47.25	8.09	3.59	5.31	.21	14.10
		Test	5	288	5.86	48.22	8.34	3.59	5.26	.21	14.67
		t-value		-.50	.29	.72	.36	.01	-.14	-.43	.85
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	196	7.57	41.21	8.29	4.26	8.01	.31	4.60
		Test	5	204	6.73	44.98	7.45	3.83	6.79	.29	3.75
		t-value		1.61	-1.37	2.23	-1.17	-1.01	-2.40	-4.00	-1.81
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	p<.05	p<.01	N.S.
Guinea Pig	Male	Control	5	313	7.50	46.78	9.16	3.96	9.43	.43	4.26
		Test	4[a]	305	7.93	46.17	9.30	4.09	9.89	.45	4.90
		t-value		-.41	1.30	-.25	.22	.54	.76	1.04	1.88
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	295	9.76	46.77	8.94	4.41	9.32	.46	5.06
		Test	5	292	7.46	47.67	8.39	4.18	9.05	.45	4.66
		t-value		-.18	-2.63	.25	-.82	-.57	-.47	-.89	-.43
		significance		N.S.	p<.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, $\alpha = .05$.

a - One animal found to have sex organs of both sexes, hence omitted.

77M117 Table 2a. Male Rats

EXP. NO. 6-TBE-1: 1,1,2,2-TETRABROMOETHANE (19.9 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significanc
GROUPS	1	48.023	48.023	.85893E-01	N.S.
SUBJECTS IN GROUPS	8	4472.9	559.11		
TIMES	4	33309.	8327.1	103.43	p <.001
TIMES X GROUPS	4	118.68	29.670	.36852	N.S.
TIMES X SUBJECTS IN GROUPS	32	2576.3	80.510		
TOTAL	49	40524.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-1.400	15.800	32.000	52.000	67.000	33.080
TEST	2	-5.800	11.400	27.400	54.200	68.400	31.120
TOTAL		-3.600	13.600	29.700	53.100	67.700	32.100

EXP. NO. 6-TBE-1: 1,1,2,2-TETRABROMOETHANE (19.9 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	158.42	158.42	1.2760	N.S.
SUBJECTS IN GROUPS	8	993.20	124.15		
TIMES	4	2793.3	698.33	8.1248	p <.001
TIMES X GROUPS	4	331.48	82.870	.96416	N.S.
TIMES X SUBJECTS IN GROUPS	32	2750.4	85.950		
TOTAL	49	7026.8			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	-4.800	3.800	7.600	9.600	14.600	6.160
TEST	2	-7.800	11.400	18.600	12.400	14.000	9.720
TOTAL		-6.300	7.600	13.100	11.000	14.300	7.940

77M117 Table 2c. Male Guinea Pig

EXP. NO. 6-TBE-1: 1,1,2,2-TETRABROMOETHANE (19.9 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	51.359	51.359	.75743E-01	N.S.
SUBJECTS IN GROUPS	7	4746.5	678.08		
TIMES	4	59772.	14943.	127.14	p <.001
TIMES X GROUPS	4	255.20	63.801	.54284	N.S.
TIMES X SUBJECTS IN GROUPS	28	3290.9	117.53		
TOTAL	44	68116.			

MEANS

TIMES:

		DAY 1	DAY 3	DAY D	AY10 D	AY15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	2.400	9.000	41.200	78.400	100.000	46.200
TEST	2	6.750	14.500	46.500	81.250	92.750	48.350
TOTAL		4.333	11.444	43.556	79.667	96.778	47.156

77M117 Table 2d. Female Guinea Pig

EXP. NO. 6-TBE-1: 1,1,2,2-TETRABROMOETHANE (19.9 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	Significance
GROUPS	1	959.22	959.22	2.2784	N.S.
SUBJECTS IN GROUPS	8	3368.0	421.00		
TIMES	4	50059.	12515.	138.44	p <.001
TIMES X GROUPS	4	810.69	202.67	2.2419	N.S.
TIMES X SUBJECTS IN GROUPS	32	2892.8	90.400		
TOTAL	49	58090.			

MEANS

TIMES:

	DAY 1	DAY 3	DAY 6	DAY10	DAY15	
GROUPS:	1	2	3	4	5	TOTAL
1	-.400	12.800	34.000	77.000	84.200	41.520
2	-7.800	11.200	30.800	52.800	76.800	32.760
TOTAL	-4.100	12.000	32.400	64.900	80.500	37.140

HISTOPATHOLOGICAL EVALUATION OF ORGAN SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 6-TBE-1

Chemical: 1,1,2,2-tetrabromoethane (19.9 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
-	-	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: 1,1,2,2-tetrabromoethane Exp.#: 11-TBE-2 Date: 3/16/77

Chamber Facility Parameters

Room Temperature: 24.5 °C Chamber Temperature: 27.7 °C Barom. Pressure: 638 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 30 %

Air Velocity Meter Reading 300 ft/min Intake Duct Air Flow: 821 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 202 °C Setting: 650 Sensor: 2 Heater Power: 600 watts

Pump Setting: 0.85 Liquid Pressure: 5 psig Liquid Flow ~1.46 ml/min ~4.33 g/min

Nitrogen Flow: 1.6 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 50.2 l/min

Comments: _____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: silica gel

Sampling Flow: 0.179 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.537 l Rotameter Setting: 30 mm Pres. Drop: <6 Torr

Desorption Solvent: acetone Desorption Volume: 1.00 ml

Internal Standard Used: dodecane Concentration: 40 ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 12' S.S. 10% UC-W98

80/100 mesh Chromosorb W-HP @ 170 °C

Attenuation: 64 Range: 10 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 68 ml/min Detector Gasses: H_2 60 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 3.0 ul Solvent Flush Volume: 2.0 ul

Elution times: Test Compound: 2.2 min Internal Standard: 3.0 min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: 1,1,2,2-tetrabromoethane Exp.#: 11-TBE-2 Date: 3/16/77

Ambient Temperature: 27.7 °C Barometric Pressure: 638 Torr

Analytical Method: Silica gel adsorption tubes - acetone desorption - FID/GC analysis

Comments: Test compound condensed on the walls of the inhalation chamber during exposure.

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-8.0	1.02	85.9
2	-4.0	1.14	95.7

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.5	1.01	84.9
4	5.5	1.01	84.9
5	9.5	1.20	101
6	13.0	1.00	84.5
7	17.0	0.942	79.3
8	20.5	1.16	97.6
9	24.0	1.17	98.3
10	27.0	1.17	98.1
	MEAN:	1.08	91.1
	S.D.:	0.10	8.5
	C.V.:	0.093	0.093

Clinical Observations*
11-TBE-2
1,1,2,2 - tetrabromoethane (91.1 ppm)

Rats: Increased activity was noted initially with some squinting and nose rubbing. At five minutes, there was a slowed degree of activity and lacrymation in some. There was very little or no change noted after five minutes.

Guinea Pigs: The guinea pigs also showed initial increased activity. Activity slowed after five minutes and profuse lacrymation was noted. Some shaking was apparent in a few animals at 10 minutes, but no other changes were noted during the remainder of the experiment.

General Comments:

Guinea pigs were affected differently than rats and some tremors were observed in guinea pigs.

Scores:**

	Increased Activity	Lacrymation	Nose Rubbing	Tremors	Eye Closure
Rats	+2 (initially)	+2	+2	0	0
Guinea Pigs	+2 (initially)	+3	+2	+1	+2

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels
J. A. Burkart and R. J. Voss

Statistical Evaluation of IDLH Experiment 11-TBE-2: 1,1,2,2-Tetrabromoethane (91.1 ppm)

6 May 1977

77M210

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight ratios

The female rat test group was found to have significantly larger liver-to-body weight ratios than the control group.

Weight Changes

All groups showed significant increases in weight over the course of exposure.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

77M210 Table 1

Experiment No. 11-TBE-2 : 1,1,2,2-Tetrabromethane (91.1 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	302	6.22	48.32	8.01	3.45	4.74	.20	14.10
		Test	5	287	6.07	48.79	7.77	3.42	4.91	.21	13.85
		t-value		-1.06	-.43	.24	-.53	-.20	.70	1.35	-.26
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	202	7.30	42.69	7.50	3.79	7.24	.31	4.96
		Test	5	204	6.51	48.87	7.76	3.45	6.72	.31	4.48
		t-value		.38	-1.91	3.31	.96	-.98	-1.01	0.0	-.75
		significance		N.S.	N.S.	p<.05	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	323	7.88	55.36	9.59	4.57	8.39	.45	5.26
		Test	5	300	7.70	56.79	9.74	4.49	10.02	.54	4.52
		t-value		-1.32	-.37	.32	.25	-.18	1.97	2.08	-1.96
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	314	8.35	54.65	9.19	5.08	8.76	.46	5.13
		Test	5	296	7.92	47.74	9.65	4.28	9.50	.52	5.08
		t-value		-.99	-.69	-1.33	.91	-1.89	1.23	1.50	-.05
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α=.05.

EXP. NO. 11-TBE-2: 1,1,2,2 - TETRABROMOETHANE (91.1 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	752.72	752.72	2.2516	N.S.
SUBJECTS IN GROUPS	8	2674.5	334.31		
TIMES	4	37562.	9395.5	222.79	p<.001
TIMES X GROUPS	4	91.281	22.820	.54112	N.S.
TIMES X SUBJECTS IN GROUPS	32	1349.5	42.172		
TOTAL	49	42450.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	1.800	23.000	43.600	64.600	82.400	43.080
TEST	2	-2.400	17.600	34.600	56.200	70.600	35.320
TOTAL		-.300	20.300	39.100	60.400	76.500	39.200

77M210 Table 2b

EXP. NO. 11-TBE-2: 1,1,2,2 - TETRABROMOETHANE (91.1 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	14.580	14.500	.33410	N.S.
SUBJECTS IN GROUPS	8	349.12	43.640		
TIMES	4	3791.8	947.95	26.692	p<.001
TIMES X GROUPS	4	88.521	22.130	.62312	N.S.
TIMES X SUBJECTS IN GROUPS	32	1136.5	35.515		
TOTAL	49	5388.5			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-6.000	2.200	7.200	19.200	19.200	8.360
TEST	2	-3.400	7.000	9.000	16.600	18.000	9.440
TOTAL		-4.700	4.600	8.100	17.900	18.600	8.900

77M210 Table 2c

EXP. NO. 11-TBE-2: 1,1,2,2-TETRABROMOETHANE (91.1 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	4122.3	4122.3	2.2470	N.S.
SUBJECTS IN GROUPS	8	14676.	1834.6		
TIMES	4	56061.	14015.	79.211	p<.001
TIMES X GROUPS	4	500.69	125.17	.70744	N.S.
TIMES X SUBJECTS IN GROUPS	32	5662.0	176.94		
TOTAL	49	81023.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	13.400	28.200	53.000	83.400	108.200	57.240
TEST	2	1.800	16.200	27.200	57.000	92.400	39.080
TOTAL		7.600	22.200	40.100	70.600	100.300	48.160

EXP. NO. 11-TDE-2: 1,1,2,2-TETRABROMOETHANE (91.1 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	255.37	255.37	.60533	N.S.
SUBJECTS IN GROUPS	8	3375.0	421.88		
TIMES	4	57153.	14288.	172.47	p<.001
TIMES X GROUPS	4	412.72	103.18	1.2455	N.S.
TIMES X SUBJECTS IN GROUPS	32	2651.0	82.843		
TOTAL	49	63847.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	8.400	23.800	49.200	80.800	107.400	53.920
TEST	2	7.200	25.000	46.400	76.400	92.000	49.400
TOTAL		7.800	24.400	47.800	78.600	99.700	51.660

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 11-TBE-2

Chemical: 1,1,2,2-tetrabromoethane (91.1 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
11GC2F	-	Irreversible:	focal loss of myocardial fibrils
11GC5M	-	"	myocardial degeneration in ventricular walls
Test			
—	—	Irreversible:	none

SPECIFIC METHODS AND RESULTS

dimethoxymethane

Experiment #7-DMM-1 (2810 ppm)

Experiment #14-DMM-2 (8540 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: dimethoxymethane Exp.#: 7-DMM-1 Date: 2/16/77

Chamber Facility Parameters

Room Temperature: 25.2 °C Chamber Temperature: 26.9 °C Barom. Pressure: 650 Torr

Chamber Pressure: <3 mm H_2O Chamber Intake Air Supply Relative Humidity 27 %

Air Velocity Meter Reading - ft/min Intake Duct Air Flow: ~200 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 93 °C Setting: 300 Sensor: 1 Heater Power: 600 watts

Pump Setting: 5.32 Liquid Pressure: 25 psig Liquid Flow: ~7 ml/min 6 g/min

Nitrogen Flow: 0.6 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.2 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.268 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.805 l Rotameter Setting: 40 mm Pres. Drop: - Torr

Desorption Solvent: hexane Desorption Volume: 1.00 ml

Internal Standard Used: none Concentration: - ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6' S.S. Porapak Q 100/120 mesh

_____ @ 200 °C

Attenuation: 16 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 80 ml/min Detector Gasses: H_2 40 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 2.0 ul Solvent Flush Volume: 2.0 ul

Elution times: Test Compound: 1.2 min Internal Standard: - min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: dimethoxymethane Exp.#: 7-DMM-1 Date: 2/16/77

Ambient Temperature: 26.9 °C Barometric Pressure: 650 Torr

Analytical Method: Activated charcoal adsorption tubes - hexane desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-15	7.71	2900
2	-10	6.72	2530

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	7.46	2800
4	6.0	7.14	2680
5	8.5	7.46	2800
6	12.0	7.04	2650
7	15.0	6.94	2610
8	19.0	7.76	2920
9	23.0	7.87	2960
10	26.5	8.11	3050
	MEAN:	7.47	2810
	S.D.:	0.419	157
	C.V.:	0.0561	0.0561

Clinical Observations*
7-DMM-1
dimethoxymethane (2810 ppm)

Rats: Little abnormal behavior was noticed during the experiment. There was slight, initial increased activity at first and a slowing of activity toward the end of the exposure, but all animals returned immediately to normal after they were removed from the chamber. There was also a slightly increased amount of nose rubbing.

Guinea Pigs: Same signs were observed in the guinea pigs as in the rats.

General Comments:

Slight nasal irritation was present along with a slight decrease in activity near the end of exposure.

Scores:**

	Increased Activity	Decreased Activity	Nose Rubbing
Rats	+1 (initially)	+1	+1
Guinea Pigs	+1 (initially)	+1	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

to A. U. Daniels

from J. A. Burkart/R. J. Voss

subject Statistical Evaluation of IDLH Experiment 7-DMM-1: Dimethoxymethane (2810 ppm)

date 15 March 1977

refer to 77M121

Methods

Post exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight; these changes were analyzed using an analysis of variance for repeated measures design. Tables 2a to 2d present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratio

The male rat test group had significantly higher gonad weight ratios than did the control group. Among female rats, the test group had significantly higher lung and brain weight ratios than the control group. The male guinea pig test group was significantly lighter in body weight and heavier in brain weight ratios than the control.

Weight Changes

All groups showed significant weight increases over the period of exposure. The male guinea pig test group was significantly lighter than the control group and showed significantly lower weight increases over the measurement times.

RJV:lg

xc: N. Price
J. H. Nelson
A. F. Toronto

77M121 Table 1

Experiment No. 7-DMM-1 : Dimethoxymethane (2810 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	301	5.57	48.56	7.73	3.75	5.04	.20	13.77
		Test	5	293	5.93	49.31	8.28	3.49	5.13	.21	14.72
		t-value		-.68	.86	.25	1.58	-2.05	.27	1.00	2.36
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	p<.05
	Female	Control	5	215	6.70	44.43	7.79	3.51	5.80	.27	4.16
		Test	5	217	7.35	43.80	7.10	3.80	7.16	.26	3.86
		t-value		.27	2.49	- .45	-.60	1.28	4.07	-.38	- .42
		significance		N.S.	p<.05	N.S.	N.S.	N.S.	p<.01	N.S.	N.S.
Guinea Pig	Male	Control	5	356	8.26	58.45	8.77	4.00	7.83	.40	4.98
		Test	5	322	8.05	56.33	9.56	4.01	9.66	.42	4.27
		t-value		-2.99	-.28	- .46	1.69	.05	4.52	1.15	-1.96
		significance		p<.05	N.S.	N.S.	N.S.	N.S.	p<.01	N.S.	N.S.
	Female	Control	5	306	8.23	48.76	9.28	3.81	9.67	.46	4.14
		Test	5	299	8.14	49.84	9.07	4.27	9.74	.46	4.53
		t-value		-.45	-.14	.21	-.63	1.37	.16	.15	.48
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α = .05.

77M121 Table 2a.

EXP. NO. 7-DMM-1: DIMETHOXYMETHANE (2810 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	432.19	432.19	5.0109	N.S. (p=.06)
SUBJECTS IN GROUPS	8	690.00	86.250		
TIMES	4	46187.	11547.	336.65	p <.001
TIMES X GROUPS	4	63.719	15.930	.46443	N.S.
TIMES X SUBJECTS IN GROUPS	32	1097.6	34.300		
TOTAL	49	48471.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	7.400	19.400	52.000	74.000	87.000	47.960
TEST	2	-.400	17.800	44.400	67.400	81.200	42.080
TOTAL		3.500	18.600	48.200	70.700	84.100	45.020

77M121 Table 2b.

EXP. NO. 7-DMM-1: DIMETHOXYMETHANE (2810 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	.50098	.50098	.41355E-02	N.S.
SUBJECTS IN GROUPS	8	969.12	121.14		
TIMES	4	6272.9	1568.2	68.964	p< .001
TIMES X GROUPS	4	19.399	4.8499	.21327	N.S.
TIMES X SUBJECTS IN GROUPS	32	727.68	22.740		
TOTAL	49	7989.6			

MEANS

TIMES:

		DAY 1	DAY 3	DAY 6	DAY10	DAY15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	-5.800	5.000	10.600	23.200	25.200	11.640
TEST	2	-5.400	5.600	12.800	22.400	23.800	11.840
TOTAL		-5.600	5.300	11.700	22.800	24.500	11.740

EXP. NO. 7-DMM-1: DIMETHOXYMETHANE (2810 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1885.0	1885.0	7.6460	p<.05
SUBJECTS IN GROUPS	8	1972.2	246.53		
TIMES	4	74546.	18636.	381.47	p<.001
TIMES X GROUPS	4	1264.1	316.03	6.4688	p<.001
TIMES X SUBJECTS IN GROUPS	32	1563.3	48.854		
TOTAL	49	81230.			

MEANS

TIMES:

ROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY10 4	DAY15 5	TOTAL
CONTROL	1	1.400	22.200	57.000	84.000	123.400	57.600
TEST	2	.400	16.000	48.600	68.200	93.400	45.320
TOTAL		.900	19.100	52.800	76.100	108.400	51.460

EXP. NO. 7-DMM-1: DIMETHOXYMETHANE (2810 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	269.12	269.12	.57491	N.S.
SUBJECTS IN GROUPS	8	3745.0	468.12		
TIMES	4	45666.	11417.	129.19	p .001
TIMES X GROUPS	4	505.87	126.47	1.4311	N.S.
TIMES X SUBJECTS IN GROUPS	32	2827.8	88.370		
TOTAL	49	53014.			

MEANS

		TIMES: DAY 1	DAY 3	DAY 6	DAY10	DAY15	
OUPS:		1	2	3	4	5	TOTAL
CONTROL	1	.400	15.400	36.400	62.400	90.600	41.040
TEST	2	-2.800	14.200	41.000	52.000	77.600	36.400
TOTAL		-1.200	14.800	38.700	57.200	84.100	38.720

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 7-DMM-1

Chemical: dimethoxymethane (2810 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
-	-	Irreversible: none
Test		
-	-	Irreversible: none

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: dimethoxymethane Exp.#: 14-DMM-2 Date: 4/6/77

Chamber Facility Parameters

Room Temperature: 24.9 °C Chamber Temperature: 26.0 °C Barom. Pressure: 649 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 22 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 93 °C Setting: 300 Sensor: 1 Heater Power: 600 watts

Pump Setting: 10.00 Liquid Pressure: 30 psig Liquid Flow: 20.4 ml/min 17.6 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 48.6 l/min Total Gas Flow: 49.7 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: adsorption tubes Collection Medium: charcoal

Sampling Flow: 0.116 l/min Sampling Interval: 3.00 min

Total Sample Volume: 0.348 l Rotameter Setting: 22 mm Pres. Drop: <3 Torr

Desorption Solvent: hexane Desorption Volume: 2.00 ml

Internal Standard Used: none Concentration: - ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 215 °C

Analytical Column: 1/8" x 6' S.S. Porapak Q 100/120 mesh

_____ @ 200 °C

Attenuation: 32 Range: 10^2 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: + Pulse Interval: - usec

Carrier Gas: He 68 ml/min Detector Gasses: H_2 30 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 3.0 ul Solvent Flush Volume: 2.0 ul

Elution times: Test Compound: 0.8 min Internal Standard: - min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: dimethoxymethane Exp.#: 14-DMM-2 Date: 4/6/77

Ambient Temperature: 26.0 °C Barometric Pressure: 649 Torr

Analytical Method: Activated charcoal adsorption tubes - hexane desorption - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	22.9	8600
2	-5.0	23.5	8850

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	22.8	8580
4	5.0	23.2	8720
5	9.3	22.7	8550
6	13.6	22.9	8620
7	18.5	25.7	9670
8	23.0	22.4	8420
9	25.5	19.5	7330
10	28.0	22.5	8450
	MEAN:	22.7	8540
	S.D.:	1.68	632
	C.V.:	0.074	0.074

Clinical Observations*
14-DMM-2
dimethoxymethane (8540 ppm)

Rats: The rats showed mild initial hyperactivity which subsided after five minutes. There was some slight nose rubbing but no other effects were noted.

Guinea Pigs: The guinea pigs showed the same initial signs as the rats, but some shaking was noticed at 5 minutes. No other effects were noticed.

General Comments:

Little effect was observed in test animals during the exposure.

Scores:**

	Hyperactivity	Nose Rubbing	Shaking
Rats	+1	+1	0
Guinea Pigs	+1	0	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

to A. U. Daniels

from J. A. Burkart, and R. J. Voss

subject Statistical Evaluation of IDLH Experiment 14-DMM-2: Dimethoxymethane (8540 ppm)

date 6 May 1977

refer to 77M213

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

No significant differences were found.

Weight Changes

All groups showed significant increases in weight over the course of exposure.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

77M213 Table 1

Experiment No. 14-DMM-2 : Dimethoxymethane (8540 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	304	6.70	44.82	8.07	3.32	4.34	.20	14.62
		Test	5	290	6.43	47.59	7.80	3.40	4.66	.21	14.34
		t-value		-1.11	-.49	1.25	-.84	.66	.96	.32	-.48
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	210	6.75	43.52	7.16	3.79	6.43	.29	3.95
		Test	5	209	7.02	45.76	7.21	3.53	6.27	.30	3.63
		t-value		-.11	.29	1.83	.13	-.76	-.57	.84	-.65
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	296	8.59	55.08	9.36	4.17	9.28	.45	4.91
		Test	5	268	7.64	55.28	9.88	4.37	10.75	.55	4.48
		t-value		-.75	-1.71	.04	.66	.59	.87	1.21	-.85
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	274	8.65	46.65	9.64	5.00	11.32	.50	5.09
		Test	5	302	8.01	50.68	9.08	4.46	9.88	.45	5.51
		t-value		1.11	-.86	.92	-.66	-1.34	-1.46	-.85	.51
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α=.05.

77M213 Table 2a

EXP. NO. 14-DMM-2: DIMETHOXYMETHANE (8540 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	873.62	873.62	2.2404	N.S.
SUBJECTS IN GROUPS	8	3119.6	389.95		
TIMES	4	33004.	8250.9	250.22	p<.001
TIMES X GROUPS	4	62.281	15.570	.47218	N.S.
TIMES X SUBJECTS IN GROUPS	32	1055.2	32.975		
TOTAL	49	38114.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-.400	20.600	32.000	57.000	74.400	36.720
TEST	2	-6.600	10.800	26.400	48.400	62.800	28.360
TOTAL		-3.500	15.700	29.200	52.700	68.600	32.540

77M213 Table 2b

EXP. NO. 14-DMM-2: DIMETHOXYMETHANE (8540 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	141.12	141.12	2.0839	N.S.
SUBJECTS IN GROUPS	8	541.76	67.720		
TIMES	4	5697.3	1424.3	63.849	p<.001
TIMES X GROUPS	4	124.88	31.220	1.3995	N.S.
TIMES X SUBJECTS IN GROUPS	32	713.84	22.307		
TOTAL	49	7218.9			

MEANS

TIMES:

OUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-11.600	1.000	7.000	19.400	17.400	6.640
TEST	2	-4.600	1.800	11.600	18.000	23.200	10.000
TOTAL		-8.100	1.400	9.300	18.700	20.300	8.320

77M213 Table 2c

EXP. NO. 14-DMM-2: DIMETHOXYMETHANE (8540 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	369.92	369.92	.90532E-01	N.S.
SUBJECTS IN GROUPS	8	32689.	4086.1		
TIMES	4	31743.	7935.8	10.050	p<.001
TIMES X GROUPS	4	2023.9	505.97	.64078	N.S.
TIMES X SUBJECTS IN GROUPS	32	25267.	709.61		
TOTAL	49	92093.			

MEANS

TIMES:

ROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	1.800	13.000	27.800	47.400	90.200	36.040
TEST	2	3.800	16.800	32.800	38.200	61.400	30.600
TOTAL		2.800	14.900	30.300	42.800	75.800	33.320

77M213 Table 2d

EXP. NO. 14-DMM-2: DIMETHOXYMETHANE (8540 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1341.6	1341.6	1.1699	N.S.
SUBJECTS IN GROUPS	8	9174.6	1146.8		
TIMES	4	40823.	10206.	59.321	$p<.001$
TIMES X GROUPS	4	758.28	189.57	1.1019	N.S.
TIMES X SUBJECTS IN GROUPS	32	5505.4	172.05		
TOTAL	49	57603.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-2.400	1.800	30.200	49.400	64.200	28.640
TEST	2	.800	14.000	33.600	58.000	88.600	39.000
TOTAL		-.800	7.900	31.900	53.700	76.400	33.820

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 14-DMM-2

Chemical: dimethoxymethane (8540 ppm)

Animal #	Time of Death Post Exposure	Lesions Found
Control		
14RC3M	-	Irreversible: deposits in cerebral white matter
14RC4F	-	" "
Test		
14RT1M	-	Irreversible: deposits in cerebral white matter
14RT3M	-	" "
14RT5M	-	" "
14RT2F	-	" "

SPECIFIC METHODS AND RESULTS

phenol

Experiment #P-17-1 (187 ppm)

Experiment #P-18-2 (540 ppm)

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: Phenol Exp.#: 17-P-1 Date: 4/27/77

Chamber Facility Parameters

Room Temperature: 25.0 °C Cnamber Temperature: 28.4 °C Barom. Pressure: 640 Torr

Chamber Pressure: -18 mm H_2O Chamber Intake Air Supply Relative Humidity 30 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 199 °C Setting: 700 Sensor: 2 Heater Power: 600 watts

Pump Setting: 1.00 Liquid Pressure: 7 psig Liquid Flow: 1.87 ml/min 1.78 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 65.9 l/min Total Gas Flow: 67.0 l/min

Comments: Apparently test compound intermittantly plugged the vaporizer output dispersion nozzle holes - thus causing large variations in the concentration of test compound in the inhalation chamber.

Test Atmosphere Sampling Parameters

Collection Method: Bubbler Collection Medium: 0.1 N NaOH

Sampling Flow: 1.00 l/min Sampling Interval: 3.00 min

Total Sample Volume: 3.00 l Rotameter Setting: 130 mm Pres. Drop: < 58 Torr

Desorption Solvent: - Collection Volume: 15.0 ml

Internal Standard Used: none Concentration: - ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 215 °C

Analytical Column: 1/8" x 6 ft Tenax G.C. 60/80 Mesh @ 200 °C

Attenuation: 32 Range: 1 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 70 ml/min Detector Gasses: H_2 30 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 5.0 ul Solvent Flush Volume: 2.0 ul

Elution times: Test Compound: 1.6 min Internal Standard: - min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: Phenol Exp.#: 17-P-1 Date: 4/27/77

Ambient Temperature: 28.4 °C Barometric Pressure: 640 Torr

Analytical Method: Bubble sampling with 0.1 N sodium hydroxide - adjust pH and dilute - FID/GC analysis

Comments:

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	0.751	232
2	-5.0	0.681	210

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	0.479	148
4	5.5	0.604	187
5	10.0	0.872	269
6	14.5	0.534	165
7	17.5	0.732	226
8	21.0	0.301	92.8
9	24.0	0.203	62.7
10	27.0	1.13	348
	MEAN:	0.607	187
	S.D.:	0.302	93.0
	C.V.:	0.497	0.497

Clinical Observations*
17-P-1
phenol (187 ppm)

Rats: Initially hyperactivity was noted, but the animals calmed down to a normal activity level after five minutes and no other effects were noted.

Guinea Pigs: Increased initial activity was noted with slight lacrymation and salivation at five minutes. No other effects noted.

General Comments:

Little effect upon test animals was observed.

Scores:**

	Hyperactivity	Salivation	Lacrymation	Eye Closure
Rats	+1 (initially)	0	0	0
Guinea Pigs	+1 (initially)	+1	+1	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0	X	X	X	X
1-2				
3-9				
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.

**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels

J. A. Burkart and R. J. Voss RJV

Statistical Evaluation of IDLH Experiment 17-P-1: Phenol (187 ppm)

7 June 1977

77M261

Methods

Post-exposure organ-to-body weight ratios were compared between test and control groups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

Male rats in the test group had significantly lower heart-to-body weight ratios than in the control group.

Weight Changes

All groups showed significant increases in weight over the course of the experiment.

XC: N. Price
A. F. Toronto
J. H. Nelson

/gj

Attachments

77M261 Table 1

Experiment No. 17-P-1 : Phenol (187 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	324	6.31	48.24	8.40	3.54	4.27	.20	14.29
		Test	5	322	6.49	53.10	7.95	3.18	4.16	.19	13.52
		t-value		-.15	.57	1.47	-1.21	-2.47	-.27	-1.13	-.98
		significance		N.S.	N.S.	N.S.	N.S.	p<.05	N.S.	N.S.	N.S.
	Female	Control	5	230	6.94	42.56	6.97	3.80	5.65	.27	4.67
		Test	5	211	7.33	45.87	7.03	4.09	5.84	.28	4.61
		t-value		-1.23	.93	1.95	.07	1.11	.39	1.11	-.13
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	311	8.03	64.58	9.35	4.67	9.45	.45	4.41
		Test	5	302	7.75	59.78	9.25	4.46	9.23	.46	4.57
		t-value		-.52	-1.13	-.80	-.23	-.68	-.28	.07	.36
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	288	8.53	62.47	9.83	4.91	9.41	.46	4.99
		Test	5	293	8.28	50.92	9.34	4.16	9.82	.47	4.97
		t-value		.24	-.30	-1.88	-.66	-1.95	.78	.42	-.02
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, α=.05.

EXP. NO. 17-P-1: PHENOL (187 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	292.83	292.83	1.8120	N.S.
SUBJECTS IN GROUPS	8	1292.9	161.61		
TIMES	4	68985.	17246.	350.37	p<.001
TIMES X GROUPS	4	42.266	10.566	.21467	N.S.
TIMES X SUBJECTS IN GROUPS	32	1575.1	49.223		
TOTAL	49	72188.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	-5.200	20.200	41.200	70.400	100.000	45.320
TEST	2	-10.600	17.400	34.000	64.200	97.400	40.480
TOTAL		-7.900	18.800	37.600	67.300	98.700	42.900

EXP. NO. 17-P-1: PHENOL (187 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	456.02	456.02	1.3228	N.S.
SUBJECTS IN GROUPS	8	2758.0	344.75		
TIMES	4	9243.3	2310.8	22.332	p<.001
TIMES X GROUPS	4	279.48	69.870	.67524	N.S.
TIMES X SUBJECTS IN GROUPS	32	3311.2	103.47		
TOTAL	49	16048.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-6.600	4.400	11.400	22.000	38.200	13.880
TEST	2	-11.000	1.800	7.000	18.600	22.800	7.840
TOTAL		-8.800	3.100	9.200	20.300	30.500	10.860

77M261 Table 2c.

EXP. NO. 17-P-1: PHENOL (187 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	348.48	348.48	.35540	N.S.
SUBJECTS IN GROUPS	8	7844.2	980.53		
TIMES	4	43760.	10940.	75.025	p<.001
TIMES X GROUPS	4	147.12	36.781	.25224	N.S.
TIMES X SUBJECTS IN GROUPS	32	4666.2	145.82		
TOTAL	49	56766.			

MEANS

TIMES:

		DAY 1	DAY 3	DAY 6	DAY 10	DAY 15	
GROUPS:		1	2	3	4	5	TOTAL
CONTROL	1	5.200	17.200	30.400	58.600	92.000	42.280
TEST	2	1.000	17.000	31.000	54.400	81.600	37.000
TOTAL		3.100	17.100	34.700	56.500	86.800	39.640

EXP. NO. 17-P-1: PHENOL (187 PPM) ON FEMALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1.6250	1.6250	.27954E-02	N.S.
SUBJECTS IN GROUPS	8	4650.6	581.32		
TIMES	4	27160.	6790.0	25.631	p<.001
TIMES X GROUPS	4	386.69	96.672	.36493	N.S.
TIMES X SUBJECTS IN GROUPS	32	8477.0	264.91		
TOTAL	49	40676.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	.400	15.000	32.400	50.200	63.000	32.200
TEST	2	2.400	16.800	26.200	45.200	72.200	32.560
TOTAL		1.400	15.900	29.300	47.700	67.600	32.380

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 17-P-1

Chemical: phenol (187 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
17RC1M	-	Irreversible:	mild spongiosis of the white
17RC3M	-	"	matter in the brain. This
17RC5M	-	"	may be an artifact of processing
17RC2F	-	"	or inherent in the animal strain,
17GC1M	-	"	but apparently is not related
17GC2F	-	"	to exposure.
Test			
17RT1M	-	Irreversible:	mild spongiosis of the white
17RT3M	-	"	matter in the brain. This
17RT2F	-	"	may be an artifact of processing
17GT2F	-	"	or inherent in the animal strain,
17GT4F	-	"	but apparently is not related
			to exposure.

INHALATION CHAMBER TEST ATMOSPHERE GENERATION AND MONITORING PARAMETERS

Test Compound: Phenol Exp.#: 18-P-2 Date: 4/27/77

Chamber Facility Parameters

Room Temperature: 25.2 °C Chamber Temperature: 28.7 °C Barom. Pressure: 639 Torr

Chamber Pressure: -19 mm H_2O Chamber Intake Air Supply Relative Humidity 20 %

Air Velocity Meter Reading 200 ft/min Intake Duct Air Flow: 552 l/min(calc.)

Vapor Generator Parameters

Oven Temperature: 199 °C Setting: 750 Sensor: 2 Heater Power: 600 watts

Pump Setting: 2.00 Liquid Pressure: 10 psig Liquid Flow: 4.12 ml/min 3.93 g/min

Nitrogen Flow: 1.1 l/min Comp. Air Flow: 65.9 l/min Total Gas Flow: 67.0 l/min

Comments: _____

_____

Test Atmosphere Sampling Parameters

Collection Method: Bubbler Collection Medium: 0.1 N NaOH

Sampling Flow: 1.06 l/min Sampling Interval: 3.00 min

Total Sample Volume: 3.18 l Rotameter Setting: 130 mm Pres. Drop: < 58 Torr

Desorption Solvent: - Collection Volume: 15.0 ml

Internal Standard Used: none Concentration: - ul/100 ml solvent

G. C. Analysis Parameters

Detector: FID @ 265 °C Injection Port Temperature: 210 °C

Analytical Column: 1/8" x 6 ft Tenax G.C. 60/80 Mesh

_____ @ 200 °C

Attenuation: 16 Range: 10 Chartspeed: 6.4 mm/min

Bridge Current: - Polarity: - Pulse Interval: - usec

Carrier Gas: He 75 ml/min Detector Gasses: H_2 25 ml/min Air 380 ml/min

- % CH_4/A - ml/min

Injection Volume: 6.0 ul Solvent Flush Volume: 2.0 ul

Elution times: Test Compound: 1.6 min Internal Standard: - min

SUMMARY OF INHALATION CHAMBER TEST ATMOSPHERE ANALYTICAL RESULTS

Test Compound: Phenol Exp.#: 18-P-2 Date: 5/4/77

Ambient Temperature: 28.7 °C Barometric Pressure: 639 Torr

Analytical Method: Bubbler sampling with 0.1 N NaOH - pH adjustment and dilution - FID/GC analysis

Comments: Test compound condensed and crystallized on the wall of inhalation chamber during exposure

Pre-Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
1	-10.0	1.72	531
2	-5.0	1.74	537

Animal Exposure Inhalation Chamber Atmosphere Analysis

Sample #	Time (min)	Test Compound (mg/l)	Concentration (ppm)
3	1.0	1.75	541
4	5.0	1.85	572
5	9.0	1.76	542
6	13.0	1.72	530
7	17.0	1.70	525
8	22.0	1.84	569
9	26.5	1.63	502
	MEAN:	1.75	540
	S.D.:	0.0775	23.9
	C.V.:	0.0443	0.0443

Clinical Observations*
18-P-2
phenol (540 ppm)

Rats: Initial increased activity was noted in the rats as they were placed in the chamber. At five minutes, some nose rubbing was observed and most of the animals had slight tremors. These tremors became more pronounced throughout the exposure and when the animals were removed from the chamber, the tremors continued for more than an hour. These tremors caused a lack of muscle coordination and the animals seemed quite oblivious to their surroundings when removed from the chamber.

Guinea Pigs: Squinting was noted initially in the guinea pigs with slight lacrymation and salivation at five minutes. Tremors in most guinea pigs started at ten minutes after exposure began and increased with time until the animals were removed from the chamber. This effect of tremors also lasted more than one hour post-exposure.

General Comments:

Tremors observed in most animals which would appear to inhibit their ability to escape from exposure areas.

Scores:**

	Hyperactivity	Nose Rubbing	Tremors	Lacrymation	Salivation	Eye Closure
Rats	+1	+1	+4	0	0	0
Guinea Pigs	+1	0	+4	+1	+1	+1

Animals Judged to Have Impaired Escape Ability:

Number of Animals (10 possible)	Test		Control	
	R	G	R	G
0			X	X
1-2				
3-9	X	X		
10				

* For 10 animals: "few" = 1-2; "some" = 3-5; "most" = 6-9; and "all" = 10.
**Rating Scale: 0 (not present) to +4 (profound).

STATISTICAL ANALYSIS OF BODY WEIGHT CHANGES
AND ORGAN-TO-BODY WEIGHT RATIOS

A. U. Daniels
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Statistical Evaluation of IDLH Experiment 18-P-2: Phenol (540 ppm)

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77M262

Methods

Post-exposure organ-to-body weight ratios were compared between test and control goups using Student's t-test. Mean values are given in Table 1. Weight changes were computed by subtracting pre-exposure weight from each post-exposure weight. These changes were analyzed using an analysis of variance for a repeated measures design. Tables 2a-2b present the results of this analysis plus the mean weight changes.

Organ-to-Body Weight Ratios

No significant differences were observed.

Weight Changes

All groups showed significant increases in weight over the course of the experiment. Female guinea pigs of the test group had significantly lower body weight at autopsy and showed significantly smaller weight increases than those of the control group.

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Attachments

77M262 Table 1

Experiment No. 18-P-2 : Phenol (540 ppm)

Animal	Sex	Group	Number of Animals	Mean Weight (grams)	Mean Organ-to-Body Weight Ratio (x 1000)						
					Lungs	Liver	Kidneys	Heart	Brain	Adrenals	Gonads
Rats	Male	Control	5	302	6.75	50.39	8.10	3.77	5.25	.21	14.88
		Test	5	290	6.81	50.16	7.96	3.56	5.04	.22	15.23
		t-value		-1.23	.13	-.08	-.42	-.73	-.56	.65	.46
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	223	7.44	48.79	7.65	3.93	6.32	.29	5.70
		Test	5	211	7.16	45.58	7.29	3.81	6.33	.29	5.29
		t-value		-.69	-.38	-1.35	-.75	-.42	0.0	-.04	-.83
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Guinea Pig	Male	Control	5	310	8.80	53.58	9.80	4.91	8.96	.43	4.91
		Test	5	316	8.21	52.85	9.44	4.53	8.84	.43	4.97
		t-value		.37	-.75	-.14	-1.25	-.65	-.30	-.10	.17
		significance		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Female	Control	5	317	8.56	48.34	9.32	4.42	8.54	.45	5.67
		Test	5	288	7.60	42.01	9.01	5.23	9.05	.47	5.58
		t-value		-2.92	-1.41	-1.43	-.67	1.73	1.29	1.24	-.09
		significance		$p<.05$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.- Not statistically significant, $\alpha=.05$.

77M262 Table 2a.

EXP. NO. 18-P-2: PHENOL (540 PPM) ON MALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	2693.8	2693.8	1.9174	N.S.
SUBJECTS IN GROUPS	8	11239.	1404.9		
TIMES	4	43770.	10942.	147.09	p<.001
TIMES X GROUPS	4	384.52	96.129	1.2922	N.S.
TIMES X SUBJECTS IN GROUPS	32	2380.6	74.392		
TOTAL	49	60468.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	-1.400	19.200	29.800	61.800	86.600	39.200
TEST	2	-15.200	5.200	22.000	48.800	61.800	24.520
TOTAL		-8.300	12.200	25.900	55.300	74.200	31.860

77M262 Table 2b.

EXP. NO. 18-P-2: PHENOL (540 PPM) ON FEMALE RATS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	1076.5	1076.5	.53393	N.S.
SUBJECTS IN GROUPS	8	16129.	2016.1		
TIMES	4	9516.7	2379.2	23.462	p<.001
TIMES X GROUPS	4	69.520	17.380	.17139	N.S.
TIMES X SUBJECTS IN GROUPS	32	3245.0	101.40		
TOTAL	49	30037.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	-1.200	10.000	20.400	34.000	39.200	20.480
TEST	2	-7.600	3.400	10.200	23.000	27.000	11.200
TOTAL		-4.400	6.700	15.300	28.500	33.100	15.840

EXP. NO. 18-P-2: PHENOL (540 PPM) ON MALE GUINEA PIGS
ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	13.516	13.516	.21645E-01	N.S.
SUBJECTS IN GROUPS	8	4995.4	624.42		
TIMES	4	54437.	13609.	187.11	p<.001
TIMES X GROUPS	4	109.89	27.473	.37772	N.S.
TIMES X SUBJECTS IN GROUPS	32	2327.4	72.732		
TOTAL	49	61883.			

MEANS

TIMES:

GROUPS:		DAY 1 (1)	DAY 3 (2)	DAY 6 (3)	DAY 10 (4)	DAY 15 (5)	TOTAL
CONTROL	1	8.400	23.600	42.200	69.000	97.800	48.200
TEST	2	5.800	22.800	38.600	66.200	102.400	47.160
TOTAL		7.100	23.200	40.400	67.600	100.100	47.680

77M262 Table 2d.

EXP. NO. 18-P-2: PHENOL (540 PPM) ON FEMALE GUINEA PIGS

ANALYSIS OF WEIGHT CHANGE FROM PRE-EXPOSURE

ANALYSIS OF VARIANCE TABLE FOR REPEATED MEASURES DESIGN

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO	SIGNIFICANCE
GROUPS	1	2478.1	2478.1	8.5752	p<.05
SUBJECTS IN GROUPS	8	2311.8	288.98		
TIMES	4	50015.	12504.	261.56	p<.001
TIMES X GROUPS	4	316.52	79.129	1.6553	N.S.
TIMES X SUBJECTS IN GROUPS	32	1529.7	47.805		
TOTAL	49	56652.			

MEANS

TIMES:

GROUPS:		DAY 1 1	DAY 3 2	DAY 6 3	DAY 10 4	DAY 15 5	TOTAL
CONTROL	1	10.200	30.800	49.600	73.400	108.000	54.400
TEST	2	4.400	19.000	33.200	57.800	87.200	40.320
TOTAL		7.300	24.900	41.400	65.600	97.600	47.360

HISTOPATHOLOGICAL EVALUATION OF ORGAN
SPECIMENS FOR IRREVERSIBLE LESIONS

Experiment #: 18-P-2

Chemical: Phenol (540 ppm)

Animal #	Time of Death Post Exposure	Lesions Found	
Control			
18RC2F	-	Irreversible:	possible calcification in one kidney
18RC6F	-	"	inflammation of kidney - scarring would follow
Test			
-	-	Irreversible:	none

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