



DOW CHEMICAL U.S.A.

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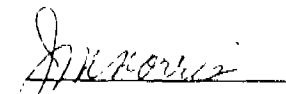
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Technical Proposal
to

INVESTIGATE THE EFFECTS ON LABORATORY ANIMALS OF
INHALED AND INGESTED VINYLIDENE CHLORIDE IN
NINETY DAY AND TWO YEAR TOXICOLOGICAL STUDIES,
TERATOLOGY STUDIES, STUDIES ON ABSORPTION,
DISTRIBUTION, METABOLISM AND EXCRETION, AND
OF INGESTED VINYLIDENE CHLORIDE IN A
REPRODUCTION STUDY

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I. INTRODUCTION

Scientific personnel of The Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan have prepared the protocols, included herein, for a best effort program to evaluate the fate and toxic effects, including the effect on reproductivity, of vinylidene chloride (1,1-dichloroethylene) on laboratory animals.

The program includes (a) toxicological studies on vinylidene chloride incorporated in the drinking water of rats for 90 days and for 2 years in conjunction with a 3 generation reproduction study; (b) a toxicological study on vinylidene chloride in peanut oil administered to dogs for 90 days; (c) a vapor inhalation toxicological study in rats for 90 days and for 2 years; (d) effects of maternally inhaled or ingested vinylidene chloride in rat and rabbit embryonal and fetal development; (e) absorption, distribution, metabolism and excretion of ingested and inhaled vinylidene chloride in rats.

The studies on absorption, distribution, metabolism and excretion of vinylidene chloride are intended as best effort studies to be conducted at a cost of \$50,000, exclusive of the ^{14}C labeled materials cost. Based on the results of these initial studies and the toxicity of vinylidene

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chloride as determined in the other studies included in the program, additional work including studies with a second species may be proposed.

The 2 year vapor inhalation study in rats was started March 20, 1974. No adverse effects were seen after 30 days exposure to 10 ppm or 40 ppm. The vapor concentrations were, therefore, increased to 25 ppm and 75 ppm, respectively. A 90 day vapor inhalation study in rats was started August 8, 1974 at these concentrations. The 2 year toxicological study on vinylidene chloride incorporated in the drinking water of rats was started July 23, 1974 and the 90 day study on July 31, 1974.

II. LABORATORY QUALIFICATIONS

Toxicology Research Laboratory of Health and Environmental Research (formerly known as Chemical Biology Research and the Biochemical Research Laboratory, Dow Chemical U.S.A.), where the studies will be carried out was formally organized in 1933. This laboratory conducts basic and applied research in numerous areas.

Applied experience has been obtained in many aspects of toxicology, environmental research, biochemistry, pharmacology, medicinal chemistry, pathology, and microbiology. In Toxicology, there is the capability for acute and chronic studies of toxicity by all routes of administration - topical, parenteral, oral and inhalation. In Industrial Hygiene, studies of exposure of men to chemicals, radiation, noise and heat are made both in production and use of chemicals. In Pharmacology, the research in the past has been concerned mainly with neuromuscular and cardiovascular processes and anesthetics. Increasing emphasis is now being placed upon the central nervous system and upon fundamental physiological mechanisms.

In Biochemistry, experience relates to enzyme chemistry, carbohydrate chemistry, and the biological mechanisms involving the activity of chemicals, both in animals and in micro-

organisms. In Microbiology, applied work emphasizes biochemical processes and organisms, including degradation and synthesis of substances with some attention to the development of disinfectant and preservative chemicals. The Medicinal Chemistry group, working in cooperation with the Pharmacology group specializes in the synthesis of special organic compounds with various biological activities, with emphasis on therapeutic usefulness.

In Environmental Research, there are facilities for, and experience in determining the fate of chemicals when exposed to natural environmental conditions, studying metabolism, biomagnification, and chemical breakdown. Here there is emphasis on fresh water plant and animal systems, plant metabolism, or microbiological degradation in soils and in soil-water systems, and on air decomposition. The Environmental Health Services section offers to government and to industry outside The Dow Chemical Company means by which adverse environmental factors can be identified and controlled.

Toxicology Research Laboratory of Health and Environmental Research at Midland, Michigan, presently occupies 42,400 square feet of floor space in three buildings. One building, with 6,000 square feet of space is used to house and maintain experimental dogs. The central building has 32,000

square feet of space and contains over 10,000 square feet of floor space for housing smaller laboratory animals, such as rats and mice. Another 5,400 square feet are occupied by laboratory facilities, such as necropsy, pathology, hematology and clinical chemistry sections. A diet preparation room is specifically equipped to handle toxic materials.

Approximately 10,000 square feet is used to accommodate the mechanical service units. These include two main air intake fans, complete with double intake filters, along with two separate high capacity air conditioning units, each of which can adequately serve the entire building. A large diesel power plant is tied into the electrical system, and can assure constant uninterrupted operation of all air intake fans and air conditioning equipment. This duplication of mechanical service units has been provided to insure that constant conditions can be maintained in the animal test areas should equipment or electricity fail.

These mechanical facilities are used to furnish 12 changes of air per hour in each room. Each animal room has an individual air exhaust system which can be filtered, if so desired. No air is recirculated. Each room has its individual environment and humidity control system, which can be adjusted to suit the specific needs. Typically, for rats on chronic tests, a temperature of 72°F and humidity of 45% are routinely maintained.

The animal facilities are inspected every three months by a Federal Veterinary Inspector.

The laboratory is accredited by the American Association for Accreditation of Laboratory Animal Care and also the American Association for Laboratory Animal Science.

Dow has the capability to conduct pathological examinations within Toxicology Research Laboratory of Health and Environmental Research in Midland. This laboratory has three veterinary pathologists on staff. Four veterinary pathologists are also employed in the Dow Human Health Research and Development Laboratory at Zionsville, Indiana. At both locations, Dow has the expertise and equipment to conduct all routine types of gross pathological and histopathological evaluations. In addition, most special pathological evaluations including electron-microscopy are carried out.

The toxicology research facilities in Midland, Michigan are being expanded. A \$3.2 million laboratory to be completed in early 1975 will occupy 47,000 square feet of floor space. The new laboratory will include 25 inhalation rooms, special environmentally-controlled for housing laboratory animals, a surgery facility and an eye examination room, and facilities for metabolism and pharmacokinetic studies. Another major area in the new laboratory will be devoted to industrial hygiene.

Dow has the capability to conduct cytogenetic studies within the Toxicology Research Laboratory in cooperation with the Biomedical Research Laboratory, located in the Freeport, Texas complex. Cytogenetic testing programs are initiated, performed, and developed in the Biomedical Research Laboratory. This laboratory has the expertise to do animal and human cytogenetic studies.

The Company maintains analytical service and development facilities suited to its operations at each of its major locations. Due to the extensive work carried out at the Midland location with agricultural and organic chemicals, the Midland analytical facilities have developed exceptional competence in handling problems associated with the identity, purity, and assay of all types of organic materials as well as the separation of impurities.

The data obtained in the studies being proposed in this document will be compiled and analyzed by appropriate statistical tests. This phase of the work may involve the Dow Computation Research Laboratory which has been in operation since 1952. Presently, the main computer equipment is an IBM 370-155 for batch processing and time sharing.

As described previously, the Toxicology Research Laboratory of Health and Environmental Research will spearhead the Dow effort in the testing of vinylidene chloride with cooperation from other Dow research groups.

III. TECHNICAL PROGRAM

A. Introduction

Vinylidene chloride is used as a chemical intermediate, particularly as a monomer in the production of plastics. Extensive use is made of polymers, homopolymers and copolymers of vinylidene chloride in commercial resins and films for food packing applications.

There is a need to generate toxicological data since vinylidene chloride is used in food packaging applications. Furthermore, toxicological data on vapor exposure to vinylidene chloride is needed to assess the potential hazard to workers in production or processing plants and to substantiate the proposed standard of 10 ppm (4 mg/M^3)¹ for the permissible exposure to vinylidene chloride in the work atmosphere.

Currently available pertinent toxicological data on vinylidene chloride are limited. Irish (1963) reported liver and kidney injury to animals exposed 8 hours per day, 5 days per week for several months at concentrations of 100 and 50 ppm. Minimal but significant injury was seen to these organs at 25 ppm. Prendergast et al (1967)

¹Threshold limit values for chemical substances in the workroom air adopted by ACGIH (American Conference of Governmental Industrial Hygienists) for 1973.

reported no mortality of animals exposed for 30 days, 8 hours per day, 5 days per week to 100 ppm and significant mortality in monkeys and guinea pigs exposed 24 hours per day for 90 days to concentrations of 47 and 25 ppm and 47, 25 and 15 ppm, respectively. Liver injury was reported in dogs, monkeys and rats and kidney changes in rats continuously exposed for 90 days to 45 ppm. Gage (1970) reported that 20 six-hour daily exposures to 500 ppm vinylidene chloride produced nasal irritation, decreased body weight gain and liver cell degeneration. Siegel et al (1971) reported that the single exposure LC50 for rats was 6350 ppm. Unpublished Dow Chemical Company data concerning acute vinylidene chloride vapor toxicity in rats and guinea pigs are summarized as follows:

Concentration of Vinylidene Chloride Vapor (ppm)	100% Lethal Exposure Time (hours)	
	<u>Rats</u>	<u>Guinea Pigs</u>
500	12	-
1250	11	11
2100	10	9
4500	6	4
24000	3	0.4
55000	2	0.25

B. Test Material

The vinylidene chloride used in the inhalation and ingestion studies has been obtained from The Dow Chemical Company, Freeport, Texas. The material is 99.5% minimum purity and specifications on the material to be used in the toxicological studies is as follows:

Acetylenes (c≡c)	ppm	25 maximum
Acidity as HCl	ppm	15 maximum
H ₂ O	ppm	75 maximum
Peroxides	ppm	25 maximum
Color APHA	ppm	30 maximum
Iron	ppm	0.5 maximum
Specific gravity at 25/25°C		1.202-1.212
Polymer Content		Free; clear
Inhibitors		
MEHQ ¹	ppm	180-220
Di-N-Propylamine	ppm	0.1 maximum

Each sample obtained from production facilities for use in the various toxicological studies will be analyzed for purity and for the level of MEHQ and impurities, including the following:

cis-Dichloroethylene	0.25% maximum
trans-Dichloroethylene	0.25% maximum
1,1-dichloroethane	0.25% maximum
Trichloroethylene	0.25% maximum
Ethylene dichloride	0.25% maximum

The inhibited material will be used for the inhalation studies. Since the vinylidene chloride used to prepare the various polymers and copolymers for food packaging

¹Monomethyl Ether of Hydroquinone

applications is distilled to remove the inhibitor MEHQ, the studies on ingested vinylidene chloride will be conducted on distilled material having residual MEHQ in the 1-5 ppm range. Each sample of distilled vinylidene chloride obtained for use in the ingestion studies will be analyzed for purity, residual MEHQ and the various impurities.

C. Protocol for a Toxicological Study on Vinylidene Chloride Incorporated in the Drinking Water of Rats for 90 Days and for 2 years in Conjunction with a 3 Generation Reproduction Study

1. Introduction

Dow Research personnel have chosen to incorporate vinylidene chloride in the drinking water of animals on ingestion studies since the compound is stable in water for 24 hours but is rapidly lost when incorporated in feed. The standard parameters evaluated in dietary feeding studies have been included in the proposed studies. The 90 day study and interim evaluation of various parameters during the 2 year study are proposed for early indication of possible toxic effects. In addition, the non-protein bound sulfhydryl content of the liver and kidneys will be determined in the animals on the 90 day study to help elucidate the biochemical effect and possible mechanism of toxic action of vinylidene chloride. The major concern of the 2 year study is the incidence of tumor formation in treated rats compared with untreated controls.

A three generation reproduction study using rats on the 2 year study as the F_0 generation parental animals will be conducted to evaluate possible effects of vinylidene chloride on reproductivity.

2. Analysis of Drinking Water

Samples of drinking water taken at designated periods during the course of these studies will be analyzed for vinylidene chloride content to assure that proper dosing is maintained. Aliquots of the aqueous samples will be analyzed using a gas chromatograph under the following conditions:

Column:	1/4" Porapak Q, 80/100, 6 feet.
Oven Temperature:	180°C
Detector:	Dual flame ionization
Carrier gas:	Helium
Flow Rate:	20 ml/minute
Attenuation:	80
Sample Volume:	4.2-4.5 μ l (all normalized to 1 μ l).

The chromatograms obtained will be compared with those from standard solutions of vinylidene chloride in carbon tetrachloride to determine the concentrations of the test solutions.

3. 90 Day and 2 Year Studies on Ingested Vinylidene Chloride

a. Design

The design of the 90 day and 2 year feeding studies in rats is indicated in Table 1.

TABLE 1

Design of the 90 Day and 2 Year Studies Incorporating Vinylidene Chloride in the Drinking Water of Rats

Number of Rats: 80/sex for controls (2 years)

48/sex/dose level (2 years)

10/sex/dose level and controls (90 days)

Dose Levels: 0, 200-230, 100-120, 60-70 ppm

Equivalent Range: 0, 16-40, 8-20, 5-12 mg/kg/day¹

<u>Parameter</u>	<u>Days on Test</u>					
	<u>30</u>	<u>90</u>	<u>180</u>	<u>365</u>	<u>540</u>	<u>730</u>
Hematology*	XXX	XXX	XXX	XXX	XXX	XXX
Urinalysis*	XXX	XXX	XXX	XXX	XXX	XXX
Clinical Chemistry**	XXX	XXX	XXX	XXX	XXX	XXX
Necropsy		XXX				XXX
Ophthalmologic Exam		XXX				XXX
Organ Weights		XXX				XXX
Histopathology		XXX				XXX
Non-protein -SH Determinations***	XXX	XXX				XXX

*5/sex/dose level

**5/sex/dose at 30, 180, 365 and 540 days (orbital bleeding)

10/sex/dose at 90 and 730 days.

***2/sex/dose level and also on days 1, 3 and 10.

¹Dosages selected approximate 16,000-40,000, 8,000-20,000 and 5,000-12,000 times the amount of vinylidene chloride that would be ingested by a 75 kg person on a daily diet of 1,500 grams, assuming all the food was contained in food packages containing the compound and migration was 50 ppb into the food.

Male and female Sprague-Dawley, Spartan substrain, SPF (Specific Pathogen Free)-derived rats, 6-7 weeks old, will be used. The rats will be housed in suspended wire-bottomed cages, with 2 rats per cage, having food and water available ad libitum. The food will be untreated ground Laboratory Chow (Purina - Ralston Company). The water will be supplied from 16 ounce wide-mouth glass bottles with plastic screw caps fitted with stainless steel sipper tubes containing stainless steel balls. The bottles will be emptied and refilled daily from freshly prepared stock solutions made up daily. Stock solutions and solutions from water bottles will be analyzed for vinylidene chloride content twice weekly for the first 13 weeks and at least one every month thereafter during the 2 year study to insure proper dosing. This schedule may be modified if more or less frequent analyses are indicated.

b. Observations and Records

Food consumption during the first 90 day period will be determined twice weekly on the 10 rats/sex/dose level on the 90 day study and on 5 designated rats/sex/dose level on the 2 year study. Subsequent to the termination of the 90 day study, 10 replacement rats/sex/dose level will be designated for the continued monitoring of food consumption one week of each month for the remainder of the 2 year study.

Body weights of the 10 rats/sex/dose level on the 90 day study and 5 designated rats/sex/dose level on the 2 year study will be determined each week for the first 90 day period. Subsequent to the termination of the 90 day study, 10 replacement rats/sex/dose level will be designated for the continued monitoring of body weights once each month for the remainder of the 2 year study.

Water consumption will be determined on the same animals used for monitoring food consumption. The water bottles will be weighed when they are filled (full weight) and before being emptied (empty weight) and refilled to determine the water consumption. This procedure will be carried out daily for the first four weeks and twice during one week of each month thereafter. The schedule may be modified if results indicate more or less frequent intervals would be desirable.

The rats will be observed at least twice weekly for signs of toxicity and for any indications of tumor formation during the respective test periods. Gross pathological examination will be conducted on all rats which appear moribound or die spontaneously. Histological examination will be conducted on all tissues needed to determine the cause of death and presence or absence of tumor formation. The first appearance of any suspected tumor will be recorded and subsequently confirmed by histopathological examination.

c. Clinical Observations

Hematological studies involving total erythrocyte count, total and differential leucocyte counts, packed cell volume and hemoglobin determinations will be performed on 5 rats/sex of the control and highest dose groups at days 30, 180, 365, 540 and one week prior to termination of the 90 day and 2 year studies.⁴ Urinalyses involving pH, glucose, ketones, bilirubin, occult blood, albumin and total solids will be performed on 5 rats/sex of the control and highest dose groups at the same times. If changes are noted, animals on the lower doses will be checked.

Clinical chemistry determinations of serum levels of urea nitrogen, alkaline phosphatase activity, and glutamic pyruvic transaminase activity will be made on serum samples from 5 rats/sex/dose level after days 30, 180, 365, and 540 (collected by orbital bleeding) and 10 rats/sex/dose level at the termination of the 90 day and 2 year studies (collected from cervical vessels after decapitation). The non-protein free sulfhydryl content of the livers and kidneys of 2 rats/sex/dose level and controls will be determined on days 1, 3, 10, 30 and at the termination of the 90 day study.

d. Necropsy and Pathology

The 10 rats/sex/dose level on the 90 day study and all surviving rats in the 2 year study will be necropsied at the respective termination times. Organ weights of brain, heart, liver, kidneys and testes (males) of all

sacrificed rats will be recorded. The following organs and tissues will be collected and preserved in buffered 10% formalin: accessory male sex glands, adipose tissue, adrenal glands, aorta, brain (cerebellum, cerebrum, brain stem), colon, cecum, duodenum, esophagus, gonads, heart, ileum, integument, jejunum, kidneys, liver, lungs, mesenteric lymph nodes, pancreas, parathyroid gland, peripheral nerve, pituitary gland, salivary glands, skeletal muscle, spinal cord, spleen, sternbrae, stomach, thymus, thyroid gland, trachea, urinary bladder and uterus. Microscopic examination of all of the above tissues from 10 rats/sex of the control and highest dose on the 90 day and 2 year studies will be made by a veterinary pathologist. Examination of any affected tissues will be extended to lower dose levels where indicated. Additional histopathological evaluations will be conducted on all lesions suggestive of a tumor or a tumor-like process observed in all rats at terminal necropsy. Data on tumor incidence over the two-year period will be compiled.

e. Ophthalmologic Examination

Both eyes from rats necropsied at the termination of the 90 day and 2 year studies will be examined grossly. The eyes from 5 rats/sex/dose level will be fixed in Zenker's solution. Eyes from the remaining rats will be preserved in buffered 10% formalin. The eyes from the 5 rats/sex of the control and top dose levels will be processed

and examined histologically. The same procedure will be carried out on eyes from the rats on the lower dose, if indicated. Eyes of all rats dying during the course of the study will also be examined grossly and be preserved in formalin fixative.

f. Statistical Evaluations

Hematology, clinical chemistry, food consumption, water consumption, organ weight and body weight data will be evaluated using an analysis of variance and Dunnett's test (Steel and Torrie, 1960). The incidence of tumors will be evaluated by the Fisher exact probability test (Siegel, 1956). The level of significance chosen for all cases is $P < 0.05$.

4. Reproduction Study

a. Design

The design for the 3 generation reproduction study is outlined in Table 2. F₀ generation male and female rats for each dose level of vinylidene chloride and controls will be randomly selected from the corresponding treatment groups in the 2 year study, excluding those animals used in monitoring food or water consumption or body weight changes. The selected animals will be mated after 90 days on the various treatment doses.

TABLE 2
3 Generation Reproduction Study on
Vinylidene Chloride Incorporated
in the Drinking Water of Rats

Number of Rats: 10 males/dose levels plus 15 control males
20 females/dose level plus 30 control females

Dose Levels: 0, 200-230, 100-120, 60-70 ppm

Parameter	Days on Test		
	Generation		
	<u>F₀</u>	<u>F₁</u>	<u>F₂</u>
Mating Period (3 estrus cycles)	90-105	110-125	110-125
Delivery Days	111-126	131-146	131-146
Weanling Days	132-147	152-167	152-167
Necropsy (approximate)	147 ¹	167 ²	167 ³
Ophthalmologic Examination		167	167
Organ Weights	147	167	167
Gross and Histopathology	147	167	167

¹Weanlings not designated for use as F₁ parental animals.

²F₁ parental animals and weanlings not designated for use as F₂ parental animals

³F₂ parental animals and all weanlings.

Test water solutions of vinylidene chloride will be prepared daily for the animals on the 3 generation reproduction study as described in the section on the 2 year study. During the 15 day mating period (3 estrus cycles), the rats will be grouped 2 females/male/cage. Water consumption will be determined daily and food consumption and body weights determined twice each week during this period. After the mating period, the males will return to the regular schedule on the 2 year study. The females will be placed singly in delivery cages containing ground corn cob litter for nesting. At the end of the first week of lactation, the corn cob litter will be removed and a screened bottom will be placed in each cage. The dams and pups will remain in these cages until the pups are weaned (21 days old). At that time the dams of the F_0 generation will be returned to their original cages. The parental animals for the F_1 generation will be selected at random from the weanlings. At 110 days of age, the males and females of the F_1 generation will be mated as described above to produce the F_2 generation. Males and females of the F_2 generation will be randomly selected and mated at 110 days of age to produce the F_3 generation.

b. Observations and Records

The animals will be observed daily for changes in appearance and demeanor. After mating, the females will be observed daily for signs of normal or abnormal parturition. The

following records will be maintained:

- 1) Weekly body weights and food consumption of rats throughout the study.
- 2) Date of parturition.
- 3) Number of live and dead newborn.
- 4) Number of live pups at days 1, 7, 14 and 21.
- 5) Litter weights at days 1, 7 and 14.
- 6) Individual weanling weights at day 21.
- 7) Sex of each weanling at day 21.
- 8) Any abnormalities noted on day 21.

The following indices will be calculated for each group of rats:

- | | |
|--------------------------|---|
| Fertility Index | - The proportion of females that were pregnant. |
| Gestation Survival Index | - The proportion of newborn pups that were alive. |
| 24-Hour Survival Index | - The proportion of liveborn pups that survived 24 hours. |
| 7-Day Survival Index | - The proportion of liveborn pups that survived 7 days. |
| 14-Day Survival Index | - The proportion of liveborn pups that survived 14 days. |
| 21-Day Survival Index | - The proportion of liveborn pups that survived 21 days. |

If a significant percent of pups begin to die among litters of vinylidene chloride treated dams, 5 typical litters from treated dams will be exchanged with litters of 5 control dams. The observations will continue as described above.

c. Necropsy and Pathology

Parental animals of the F_1 and F_2 generations will be necropsied after the pups have been weaned and gross pathological examinations will be made. Organ weights

of brain, heart, liver and kidneys will be recorded. The same organs and tissues as in the 90 day and 2 year studies will be collected and preserved in buffered 10% formalin. Microscopic examination of those tissues from 10 rats/sex on the control and top dose level will be made by a veterinary pathologist. The tissues from rats on the lower dose levels will be examined only if histological changes are observed at the high dose level.

The pups of the F_0 and F_1 generations that are not selected for use as parental animals in the subsequent generation and all the pups of the F_2 generation will be sacrificed at weaning. Gross pathological examination will be made on all sacrificed pups. Bone marrow preparations will be made on 4 pups/sex/dose level for cytogenetic evaluation. Organ weights of kidney and liver from 5 pups of different litters/sex/dose level will be recorded. Sections of these organs will be preserved for microscopic examination. The tissues from rats on the lower dose levels will be examined only if histopathological changes are observed at the high dose level.

d. Statistical Analysis

Statistical evaluation of the indices of the reproduction study will be made by the Fisher exact probability test

(Siegel, 1956). Analysis of maternal food consumption and maternal and neonatal body and organ weights will be made by an analysis of variance and Dunnett's test (Steel and Torrie, 1960). The level of significance shown for all cases is $P < 0.05$.

REFERENCES

Steel, R.G.D. and H. H. Torrie Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York pp 101-105 and 111-112 (1960).

Siegel, S. Non-Parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, Inc., New York (1956).

D. Protocol for a Toxicological Study on Vinylidene Chloride
in Peanut Oil Administered to Dogs for 90 Days

1. Introduction

Dow research personnel have chosen to incorporate the vinylidene chloride in peanut oil for administration in gelatin capsules to the dogs since supplying vinylidene treated drinking water bottles with sipper tubes to the dogs has drawbacks and incorporation into feed is not feasible due to the volatility of the compound. The standard parameters evaluated in dietary feeding studies have been included in this protocol. In addition, the nonprotein bound sulfhydryl content of the liver and kidneys will be determined to elucidate the biochemical effect and possible mechanism of toxic action of vinylidene chloride.

2. Analysis of Vinylidene Chloride in Peanut Oil

The stability of the vinylidene chloride-peanut oil solutions in the gelatin capsule will be determined prior to the starting of the study. Subsequent analyses of the vinylidene chloride-peanut oil stock solutions will be made once weekly for the first 4 weeks and at least once each month thereafter to assure that proper dosing is maintained. Aliquots of the peanut oil solutions will be analyzed using a gas chromatograph under the following conditions:

Column: 1/4" Porapak Q, 80/100, 6 feet.
Oven temp.: 180°C
Detector: Dual flame ionization
Carrier gas: Helium
Flow Rate: 20 ml/min
Attenuation: 10
Sample volume: 1-1.5 µl (normalized to 1.0 µl)

3. Experimental Procedure

a. Design

Sixteen male and sixteen female purebred beagle dogs, 8 months of age, obtained from a commercial breeder and acclimated to the kennels of the Toxicology Research Laboratory will be randomly selected for one of the following treatment groups:

<u>Dose¹</u> <u>mg/kg/day</u>	<u>Number of Dogs</u>	
	<u>Male</u>	<u>Female</u>
25	4	4
12.5	4	4
6.25	4	4
0	4	4

¹Dosages selected approximate 25,000, 12,500 and 6,250 times the amount of vinylidene chloride that would be ingested by a 75 kg person on a daily diet of 1,500 grams, assuming all the food was contained in food packages containing the compound and migration was 50 ppb into the food.

The dogs will be housed 4 dogs per pen according to the treatment groups, and supplied commercial laboratory feed (Purina-Ralston Company) fortified with 1% peanut oil, and water ad libitum.

b. Observations and Records

Body weights will be recorded weekly during the first month of the study and every two weeks thereafter. Food consumption per pen will be recorded twice each week throughout the study. The average amount consumed/dog/day will be calculated. Clinical observations will be made daily to discern any changes in general appearance and demeanor, and for signs of toxicity.

c. Clinical Studies

Hematological studies involving total erythrocyte count, total and differential leukocyte counts, packed cell volume and hemoglobin determinations will be conducted on all dogs prior to starting on the test, after 30 days on test, and one week prior to the termination of the study.

Urinalysis involving pH, glucose, ketones, bilirubin, occult blood, albumin, total solids and microscopic examination of sediment will be conducted on all dogs prior to starting on test and one week prior to termination of the study.

Clinical chemistry determinations of urea nitrogen, alkaline phosphatase activity, glutamic pyruvic transaminase activity, and glutamic oxalacetic transaminase activity will be made on serum samples from all dogs prior to and at the termination of the study. The dogs will be fasted overnight before

collecting the blood samples for these determinations. The nonprotein free sulfhydryl content of the livers and kidneys will be determined on all dogs at the termination of the study.

d. Pathology

At the termination of the study, the dogs will be fasted overnight, weighed, anesthetized with pentobarbital sodium and killed by exsanguination. A complete gross pathological examination will be made by a veterinary pathologist at necropsy. The heart, brain, liver, kidneys and testes (males) will be removed and weighed. Representative portions of the following tissues will be excised and preserved in buffered 10% formalin: adrenal gland, aorta (thoracic), bone, bone marrow (sternum), brain (cerebrum, cerebellum, brain stem), cecum, colon, epididymis (males), esophagus, gall bladder, heart, kidneys, larynx, liver, lungs, lymph nodes (cervical, mediastinal, mesenteric), mesenteric adipose tissue, muscle (skeletal), parathyroid gland, pancreas, peripheral nerve (sciatic and brachial plexus), pituitary gland, prostate gland (males), salivary gland (submaxillary), small intestine (duodenum, jejunum, ileum), spinal cord, spleen, skin, mammary tissue (females), stomach (cardia, fundus, pylorus), testes or ovaries, thymus, thyroid gland, tonsil, trachea, uterus (female) and urinary bladder, as

well as any tissue which appears abnormal. Microscopic examination will be conducted on all tissues from dogs on the control and top dose levels by a veterinary pathologist. The right sciatic nerve and the left brachial plexus will be removed and pinned to a nonflexible surface before fixation in buffered formalin. Special stains will be utilized to evaluate possible alterations in the peripheral nerves. If histopathological changes are observed, selected tissues from the dogs on the lower dose levels will also be examined.

e. Ophthalmologic Examination

Ophthalmological examinations (slit lamp and ophthalmoscope) will be conducted on all animals prior to starting and at the termination of the study. At necropsy, both eyes will be examined grossly and fixed in Zenker's solution. The eyes from the dogs on the control and top dose will be cut into calottes and examined histologically. Eyes from dogs on the lower doses will also be examined in this manner, if indicated.

f. Statistical Analysis

Statistical evaluation of body weights, organ weights, organ and body weight ratios, hematology and clinical chemistry data will be made using an analysis of variance and Dunnett's test (Steel and Torrie, 1960). Food consumption will not be tested by an analysis of variance since the 4 dogs/group

will be housed together ($n = 1$) but trends will be tested for by regression analysis. The level of significance in all cases will be $p < 0.05$.

REFERENCE

Steel, R.G.D. and H. H. Torrie Principles and Procedures of Statistics. McGraw-Hell Book Company, Inc., New York pp 101-105 and 111-112 (1960).

E. Protocol for a Vapor Inhalation Study of Vinylidene Chloride in Rats

1. Introduction

These studies involve the exposure of rats to the vapors of vinylidene chloride for 64 exposures in 90 days and for 261 exposures in 365 days in a 2 year study and observation of the animals in the latter study for an additional 12 months. The 2 year study will be started first with an interim kill after 30 days to determine the dose levels for the remainder of the exposure period in the 2 year study and for the 90 day study.

2. Vapor Generation and Analysis

The vinylidene chloride vapor will be generated by vaporizing the the liquid compound in a temperature regulated vaporization flask at a controlled rate. This vapor will be swept by air at a calculated rate into the air inlet of the exposure chamber where the vapor will be further diluted with room air to the desired concentration.

The nominal concentration of vinylidene chloride will be determined from the ratio of the rate of compound vaporization to the rate of total chamber airflow (volume of air ejected from the generator plus the volume of make-up air). The analytical concentration will be determined by infrared spectrometry (Beckman IR 10) analysis. The wave length for analysis will be 12.6 μ . The chamber concentrations

will be analyzed continuously during the exposures.

3. Experimental Procedures

a. Design

The design for the 90 day and 2 year inhalation studies in rats is outlined in Table 1.

TABLE 1

90 Day and 2 Year Vapor Inhalation Studies Vinylidene Chloride in Rats

Number of Rats: 20/sex/exposure level (90 day study).

100/sex/exposure level (2 year study).

Dose Levels: 0, 10 and 40 ppm for 30 days.

0, 25 and 75 ppm if increased concentrations are indicated.

Parameter	Number of Rats/Sex							
	Days on Study							
	90 Day Study		2 Year Study					
	30 ¹	90	15	30 ¹	44	180 ¹	365 ¹	730
Hematology		5				5	5	10
Urinalysis		5				5	5	10
Clinical Chemistry	8	12		4		5	5	10
SGPT (only) ²			5		4			
Necropsy	8	12		4		5	5	all
Ophthalmologic Examination	8	12		4		5	5	all
Organ Weights	8	12		4		5	5	all
Histopathology	as indicated			4		5	5	all
Electron Microscopy								as indicated
Cytogenetics						4		
Non-Protein SH Determinations		4						

¹Interim kills

²Glutamic Pyruvic Transaminase

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Male and female Sprague-Dawley, Spartan substrain, SPF-derived weanling rats on the 2 year study will initially be exposed to vapor atmospheres of 10 ppm and 40 ppm 6 hours per day, 5 days per week, for a total of 21 exposures in 30 days. Control rats will be maintained at ambient conditions throughout both studies. Commercial feed (Purina - Ralston Company) and water will be supplied to the animals only during the time when they are not being exposed to the vinylidene chloride vapors. All exposures will be carried out in 3.7 cubic meter stainless steel chamber in two work shifts. The decision to increase the exposure concentrations will be made on the basis of the SGPT determinations taken on days 15 and 30 and pathological examination of 4 rats/sex/exposure level killed after 30 days on test. The subsequent exposures will be 6 hours per day, 5 days per week for the designated periods.

b. Observations and Records

The rats will be observed at least twice weekly for signs of toxicity and changes in appearance and demeanor during the respective periods of exposure. Body weights will be recorded weekly for the first month of the studies and bimonthly thereafter. This schedule will be altered if more or less frequent intervals are indicated. Gross

pathological examination will be conducted on all rats which appear moribund or die spontaneously. Histological examination will be conducted on all tissues needed to determine the probable cause of death and presence or absence of tumor formation. The first appearance of any suspected tumor will be recorded and subsequently confirmed by histological examination.

c. Clinical Studies

Hematological studies involving total erythrocyte count total and differential leucocyte counts, packed cell volume and hemoglobin determinations will be performed on 5 rats/sex of the control and top exposure level at 6 months, one year and one week prior to termination of the 90 day studies and on 10 rats/sex one week prior to the termination of the 2 year study. Urinalyses involving pH, glucose, ketones, bilirubin, occult blood, albumin and total solids will be performed on the same number of rats from the control and top exposure level at the same times. If changes are noted, animals on the lower level will be checked. Non-protein bound sulfhydryl determinations will be made at the termination of the 90 day study on 4 rats/sex of the control and top exposure level. If changes are noted, the animals on the lower level will be checked.

Clinical chemistry determinations of serum levels of urea nitrogen, alkaline phosphatase activity, and glutamic pyruvic transaminase activity (SGPT) will be made on

serum samples from 5 rats/sex/exposure level after 6 months and one year (collected by orbital bleeding) and at termination of the 90 day studies and on 10 rats/sex at the termination of the 2 year study (collected from cervical vessels after decapitation). In the event the exposure concentrations are increased, a 30 day interim kill of animals in the 90 day study will be scheduled and clinical chemistry determination will be made.

d. Cytogenetic Studies

Bone marrow preparations will be made on 4 rats/sex/exposure concentration at the 6 month kill for cytogenetic evaluation

e. Necropsy and Pathology

At the termination of the 90 day and 2 year studies and at interim kills on days 30, 180 and 365 rats will be deprived of food overnight, weighed and anesthetized with methoxyflurane prior to clamping of the trachea and decapitation. The lungs and trachea will be removed as a unit and expanded with 10% buffered formalin. Organ weights of brain, heart, liver, kidney and testes (males) will be recorded. The following organs and tissues will be collected from all animals and preserved in buffered 10% formalin: accessory male sex glands, adipose tissue, adrenal glands, aorta, brain (cerebellum, cerebrum, brain stem), colon, cecum, duodenum, esophagus, gonads, heart, ileum, integument, jejunum, kidneys, liver, lungs, mesenteric lymph nodes, bone marrow, ear canal, nasal turbinate, pancreas, parathyroid

gland, peripheral nerve, pituitary gland, salivary glands, skeletal muscle, spinal cord, spleen, sternbrae, stomach, thymus, thyroid gland, trachea, urinary bladder, and uterus. The peripheral (sciatic) nerve will be removed and pinned to a non-flexible surface before fixation in buffered formalin and processing for histological examination. Microscopic examination of all of the above tissues from rats on the control and top exposure level will be made by a veterinary pathologist. Examination of any affected organ will be extended to the lower exposure level, where indicated. Additional histopathological evaluations will be conducted on all lesions suggestive of a tumor or a tumor-like process observed in all rats at terminal necropsy. Data on tumor incidence over the two-year period will be compiled. Representative samples of liver, kidney and other tissues from selected animals as indicated by the results of the biological studies will be preserved with appropriate methods for possible electron microscopic examination in the future.

f. Ophthalmologic Examination

Both eyes of all rats necropsied at the interim kills and at the termination of the 90 day and 2 year studies will be examined using a glass microscope slide technique. The eyes from the rats at the interim kills and a

representative number from the rats at the terminal necropsies will be preserved in Zenker's fixative. Eyes from the remaining rats at the terminal necropsies will be preserved in buffered 10% formalin. The eyes from rats on the control and top exposure level will be processed and examined histologically. Eyes of rats on the lower exposure level will be examined, if indicated.

g. Statistical Evaluation

Hematology, clinical chemistry, organ weight and body weight data will be evaluated using an analysis of variance and Dunnett's test (Steel and Torrie, 1960). The incidence of tumors will be evaluated by the Fisher exact probability test (Siegel, 1965). The level of significance chosen for all cases is $P < 0.05$.

REFERENCES

Steel, R.G.D. and H. H. Torrie Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York pp 101-105 and 111-112 (1960).

Siegel, S. Non-Parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, Inc., New York (1956).

F Protocol for a Study of the Effects of Maternally Inhaled or Ingested Vinylidene Chloride on Rat and Rabbit Embryonal and Fetal Development

1. Experimental Procedures

Since humans are exposed to vinylidene chloride both by inhalation and by ingestion, these teratology studies will be conducted using both routes of exposure. Bred rats and rabbits will be exposed in the inhalation study and rats will be administered vinylidene chloride in the drinking water to simulate exposure by ingestion.

a. Design

Exposure via inhalation. In an initial study, bred rats and rabbits will be exposed to twice the maximum excursion limit of vinylidene chloride (TLV = 10 ppm). Twice the maximum excursion limit of vinylidene chloride is 40 ppm (10 ppm x 2 x 2). Rabbits will be exposed on days 6-18 and rats on days 6-15 of gestation for 7 hours on each day. Groups of 30 rats and 15 to 20 rabbits will be exposed. A group of control rats and rabbits will be exposed in a chamber to filtered room air. If exposure to 40 ppm causes no evidence of maternal toxicity, embryotoxicity or teratogenicity, additional groups of rats and rabbits will be exposed to 80 ppm on the same days of gestation. If maternal toxicity is observed at 40 ppm, additional higher dose levels will not be studied, regardless of the

effect on the embryo and fetus. If embryotoxicity or teratogenicity is observed at 40 ppm, additional lower concentrations will be studied at half-fold decrements until no embryotoxicity or fetaltotoxicity is evident. Thus, concentrations of 20, 10 or 5 ppm, etc., will be studied if a significant embryotoxic effect is observed at 40 ppm in the initial experiment.

Pregnant animals will be exposed in stainless steel chambers of 3.7 cubic meter volume under dynamic airflow conditions. The chamber atmosphere will be generated by metering vinylidene chloride at a calculated rate, with a precision syringe pump, into the air stream being drawn into the exposure chamber. The concentration of vinylidene chloride in the chamber atmosphere will be calculated from the ratio of the material deliver rate and the total chamber airflow rate. The analytical concentration will be determined by infrared spectrometry (Beckman IR 10). The wave length for analysis will be 12.6 μ . The chamber concentration will be analyzed continuously during exposure.

Exposure via drinking water. Groups of 30 rats each will be given vinylidene chloride in the drinking water at concentrations of 60-70, 100-120 or 200-230 ppm on days 6-15 of gestation. A group of 40 control rats will be given drinking water without vinylidene chloride. Stock solutions of vinylidene

chloride in water will be prepared daily and each rat will be given a fresh supply each day.

b. Animals

Adult New Zealand white rabbits and Sprague-Dawley rats will be used. The day of natural mating will be considered day 0 of pregnancy. Animals will be housed individually in wire-bottom cages and maintained on commercially available laboratory animal chow free choice. Animals will not have access to water or food in the inhalation chamber during exposure in the inhalation study. Food consumption and water consumption will be measured at 3 day intervals among the rats during the experimental period.

c. Maternal Observations

Animals will be observed daily throughout the gestation period for indications of toxicity from the test material. The maternal body weight of rabbits will be recorded on days 6, 12 and 19 of gestation. The body weight of rats will be recorded on days 6, 10 and 16 of gestation. In addition, maternal body weights and the weight of the maternal liver will be recorded at the time of cesarean section, day 21 in rats and day 29 in rabbits.

d. Teratological Examination

On gestation days 21 and 29 in rats and rabbits, respectively, the pregnant females will be sacrificed by carbon dioxide

inhalation and the fetuses will be removed by cesarean section. The following data will be recorded: (1) position and number of fetuses in utero, (2) number of live and dead fetuses, (3) number of resorptions, (4) number of corpora lutea, (5) individual pup weight and crown-rump length and (6) gross external abnormalities. The sex of each rat fetus will be determined by external examination while the sex of each rabbit fetus will be determined by examination of the internal genitalia.

One-third of each litter will be examined immediately by dissection under a low power microscope for evidence of soft tissue abnormalities. Each pup in each litter will be eviscerated and placed in 95% ethanol, cleared and stained with alizarin red-S for subsequent examination for skeletal anomalies.

e. Statistical Evaluation

Statistical evaluation of the frequency of anomalies and resorptions among litters and the fetal population will be made by the Fisher exact probability test (Siegel, 1956). Analyses of fetal body weights and body measurements, maternal weight gains and maternal liver weights will be made by an analysis of variance. Group means will be compared to control values using Dunnett's test (Steel and Torrie, 1960). The level of significance chosen for all cases is $p < 0.05$.

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REFERENCES

Steel, R.G.D. and H. H. Torrie Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York pp 101-105 and 111-112 (1960).

Siegel, S. Non-Parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Company, Inc., New York (1956).

G. Protocol for Absorption, Distribution, Metabolism and Excretion of Ingested and Inhaled Vinylidene Chloride in Rats

1. Introduction

The objectives of this study are to determine the rate of absorption, distribution and excretion of vinylidene chloride and the metabolism of vinylidene chloride in rats exposed to various concentrations via inhalation and by oral gavage. Jaeger et al (1974,a,b) have reported that the toxicity of vinylidene chloride in rats is related to hepatic glutathione levels and that phenobarbital pretreated rats may be protected against the acute hepatotoxic effects of vinylidene chloride. Since diurnal fluctuation of hepatic glutathione may affect the metabolism of vinylidene chloride, all experiments will be run at a fixed time of day.

Animals will be exposed in a recirculating inhalation system to initial concentrations of 25, 100 and 300 ppm vinylidene chloride for one hour (Hefner et al, 1974). ^{14}C -labeled vinylidene chloride will be administered orally at dose levels of 0.1 mg/kg and 10 mg/kg. The investigation will be divided into three parts:

- a) Exposure via inhalation to varying levels of vinylidene chloride (non-labeled) and monitoring of the rate of decline in the inhalation chamber concentration.
- b) Exposure to ^{14}C -vinylidene chloride to study the elimination kinetics and distribution of ^{14}C -activity after inhalation exposure.
- c) Oral administration of ^{14}C -vinylidene chloride to study the absorption and elimination kinetics and the distribution of radioactivity after oral exposure.

Analysis of the rate of decline of vinylidene chloride concentration in the closed recirculating inhalation system will give information on the uptake kinetics of labeled vinylidene chloride. The kinetics of elimination of inhaled and ingested vinylidene chloride will be determined by analyzing sequential samples of excreta after administration of ^{14}C -labeled vinylidene chloride. Selected organs and tissues will be analyzed at termination of the experiments to evaluate tissue deposition of vinylidene chloride or its metabolic products.

2. Animals

Male Sprague-Dawley (Spartan Substrain) rats weighing 180-200 g will be used. The rats will be acclimated to the experimental environment for at least three days before initiation of the experiment. Food and water will be provided ad libitum.

3. Test Material

Vinylidene chloride, non-inhibited, will be obtained and checked for chemical purity by gas chromatography. Vinylidene chloride labeled with ^{14}C in the number one carbon with a specific activity of at least 5 mCi/mmmole (51 $\mu\text{Ci/mg}$) will be obtained. ^{14}C -vinylidene chloride will be stored in an ultracold freezer (-80°C). The chemical purity of the tracer will be checked by gas chromatography, and the radiochemical purity will be determined by liquid

scintillation counting of trapped eluant fractions from the gas chromatograph.

Experience derived from handling a pilot preparation of ^{14}C -vinylidene chloride will determine whether the labeled material is stable enough to be used throughout the experiment, or whether the ^{14}C -vinylidene chloride must be synthesized in a series of preparations immediately before each use.

4. Experimental Procedure

a. Design

Inhalation Exposure (non-labeled vinylidene chloride). Rats will be exposed to initial concentrations of vinylidene chloride gas ranging from 25 to 300 ppm in a closed, recirculating 4.7ℓ inhalation apparatus in which a group of four rats can be concurrently maintained. Exposure to larger concentrations (up to 1000 ppm) may be used to test dose dependent effects. To minimize contamination of fur and skin, only the nares of the rats will protrude through a rubber membrane into the chamber. Expired carbon dioxide will be continuously removed from the chamber by absorption on an ASCARITE® column in the recirculating system. Oxygen will be metered into the chamber to replace the expired CO_2 .

The chamber atmosphere will be continuous / analyzed for vinylidene chloride (10.6 μ) using an in-line Miran-1 infrared analyzer (Wilks). Oxygen consumption for each experiment will be determined directly by measuring the oxygen metered into the system using a dry test meter (American Meter Co.).

If the decline in the concentration is in accordance with first order kinetics it will be described by

$$\frac{dC}{dt} = -kC$$

k is the rate constant for the decline in vinylidene chloride concentration from the recirculating system. Corrections will be made for leakage from the chamber. Animals may be pretreated with metabolic inducers or inhibitors (phenobarbital, SKF-525-A) in an attempt to alter the rate of disappearance from the system.

All inhalation chambers will be operated in hoods to minimize personnel exposure. Air samples from the room will be analyzed periodically by the Industrial Hygiene Department of The Dow Chemical Company.

Inhalation Exposure (^{14}C -vinylidene chloride). Rats will be exposed to ^{14}C -vinylidene chloride in the inhalation chamber as described above. Immediately following exposure the animals will be placed in modified Roth-type all glass

metabolism cages. Urine and feces will be collected separately and maintained at dry-ice temperature during each collection interval. The cages will be kept in an air-conditioned room with a controlled light cycle of 12 hours on, 12 hours off. Room air will be pulled through the cages at 500 ml/min, and air exiting the cages will be passed through activated charcoal to trap expired vinylidene chloride. Air leaving this trap will be bubbled through a trap containing 120 ml 5M ethanolamine in 2-methoxyethanol to trap expired CO₂. Glass or polyethylene connections will be utilized throughout the apparatus.

Upon termination of the experiment, the brain, heart, lung, liver, kidney, thymus, spleen, adrenal glands, whole blood and plasma will be sampled. All samples collected during the course of the experiment, as well as the selected tissues and the remaining carcass, will be frozen until analyzed.

Oral Administration (¹⁴C-vinylidene chloride). Dosing solutions will be prepared by transferring the desired quantity of liquid ¹⁴C-vinylidene chloride with a chilled syringe into a measured quantity of U.S.P. corn oil. The concentration of vinylidene chloride in the dosing solution will be adjusted by addition of the appropriate amount of unlabeled vinylidene chloride. The concentrations will be such that rats will receive 2 ml/kg and at least 5 μ Ci/kg

at both the 0.1 mg/kg and 10 mg/kg dose levels. The ^{14}C -vinylidene chloride in corn oil will be administered to the rats by syringe and feeding needle.

Following administration, the rats will be maintained in the Roth-type metabolism cages and urine and feces samples will be collected as described above. Heparinized whole blood samples will be obtained either via indwelling jugular cannulas or by tail vein bleeding.

b. Carbon-14 Assay

The concentration of ^{14}C in blood plasma, urine, and trapped CO_2 will be determined directly by liquid scintillation counting in a Nuclear Chicago Mark II liquid scintillation system equipped with photon monitor and external channels ratio standardization. All calculations will be made on the basis of disintegrations per minute, and the external standard calibration will be spot-checked by the use of ^{14}C -toluene as an internal standard. Radioactivity in the activated charcoal traps will also be determined by liquid scintillation counting. The amount of ^{14}C in feces, tissues, and selected organs will be determined by counting $^{14}\text{CO}_2$ released upon oxidation of appropriate aliquots of aqueous homogenates of these samples (Harvey Biological Material Oxidizer). Overall recovery of administered ^{14}C will be determined.

c. Metabolite Identification

Major quantities of ^{14}C activity in excreta and ^{14}C that may accumulate in tissues or organs to an appreciable extent will be isolated, and an attempt will be made to determine if it is contained in ^{14}C -vinylidene chloride or in a bio-transformation product of the starting material. It is expected that gas chromatography - mass spectrometry (LKB 90005 or Finnigan Quad 3000) will be the major tool utilized in metabolite identification. However, it may occur that ^{14}C activity will be incorporated in nonvolatile constituents; in this case thin-layer chromatography may be the method of choice. Thin-layer chromatograms will be analyzed for ^{14}C with a Panax Nucleonics automated thin-layer plate scanner. If extensive efforts become necessary in order to identify a metabolite, further consultation may be necessary to justify the added cost of such experiments.

d. Data Analysis and Interpretation

Indications on the disposition kinetics of vinylidene chloride in rats will be obtained from analysis of the disappearance rate constant from the inhalation system under varying conditions of exposure level and drug altered metabolism. The kinetics of elimination of vinylidene chloride in rats will be determined by analyzing the time course of concentration of ^{14}C in excreta coupled with

identification and quantitation of any major metabolites that may be formed.

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