

MANUFACTURING CHEMISTS ASSOCIATION

1825 CONNECTICUT AVENUE, N.W. WASHINGTON, D.C. 20009 (202) 483-8126

GEORGE E. BEST
VICE PRESIDENT
TECHNICAL DIRECTOR

RECEIVED
RESEARCH & DEVELOPMENT, May 25, 1973

MAY 29 1973

CERTIFIED MAIL

W. M. SMITH CONFIDENTIAL

To the Management Contacts of Companies Sponsoring
the Vinyl Chloride Research Program
and the
Technical Task Group on Vinyl Chloride Research

Dear Sirs:

As you are already aware, for some time there has been a growing consensus among the companies participating in our program that there should be scientific consultation with appropriate U. S. Government officials concerning the toxicology of vinyl chloride monomer. This attitude stems from the desirability of inviting their advice concerning the conduct of our animal inhalation and epidemiological research so that the findings will have maximum weight and significance, and also a feeling of moral obligation, heightened since the National Institute of Occupational Safety and Health's published invitation in which vinyl chloride was listed as one of the substances of interest.

Accordingly, your technical representatives have drafted a presentation of all the known pertinent information presently available for this purpose. A copy is attached, planned for use by a small delegation in conferring with the Director of NIOSH at a date late in June yet to be determined.

Not included in the draft is information disclosed in confidence from the European project. European company executives have kindly agreed to discuss releasing their information for this purpose at a meeting with U. S. company executives, tentatively scheduled for June 15. It is very much hoped that such permission will be granted. If consent is given, then this additional scientific information will be incorporated. If not, this information will be retained in privileged status.

AP00015607

Management Contacts and
Technical Task Group --
Vinyl Chloride Research Program
May 25, 1973
Page Two

CONFIDENTIAL

If you have constructive suggestions to improve the presentation, may I please have them promptly, since it is in mind to make the initial contact with NIOSH on June 4.

Sincerely,

George E. Burt

GEB:tp

Enclosure

cc: Dr. D. P. Duffield)
Mr. D. M. Elliott)
Dr. Tiziano Garlanda) w/enclosure
Dr. K. D. Johnson)

AP00015608

May 21, 1973

INFORMATION ON VINYL CHLORIDE TO BE PRESENTED ORALLY TO NIOSH

I. Description of the Vinyl Chloride and Polyvinyl Chloride Industry

Vinyl chloride is a commodity used in very large quantities to make polyvinyl chloride resins (polymer and copolymer). The following estimated figures for 1972 illustrate the tremendous importance of this industry which has operated for 35 years in the United States and 40 years in Europe.

	<u>Vinyl Chloride</u>	<u>Polyvinyl Chloride</u>
U.S. Production	5.2 billion lbs (0.65 exported)	4.4 billion lbs
U.S. Capacity	5.9 billion lbs	
World Production	22 billion lbs	
Annual growth last 5 years	14%	
Projected annual growth, U.S.	11%	
U.S. Plants	14	37 (23 companies)
U.S. Workers	1500	5000
World Workers	10,000	

Virtually all (>99%) vinyl chloride is used in production of polyvinyl chloride and other polymers. It is difficult to estimate the number of workers employed in converting, molding, and fabricating these polymers but it is of the order of 10's of thousands, working in thousands of plants. It has been estimated that the wholesale value of fabricated products is over \$3 billion.

Almost all of the vinyl chloride and polyvinyl chloride producers in the U.S. are participants in the MCA Technical Task Group on Vinyl Chloride Research. Over 95% of the

AP00015609

U.S. vinyl chloride capacity and 85% of the PVC capacity are represented by the following 20 companies today.

Air Products	Goodrich
Allied Chemical	Goodyear
Borden Chemical	Monsanto
BP Industries (British)	Olin
Conoco	PPG
Diamond Shamrock	Shell
Dow	Stauffer
Ethyl	Tenneco
Exxon	Union Carbide
Firestone	Uniroyal

II. Summary of Toxicity Studies on Vinyl Chloride Monomer

A. Published Information on VCM

Vinyl chloride monomer was for many years considered to be very low in toxicity, both from single and repeated exposure. Because of its volatility, inhalation was considered the only important route of exposure for workmen. The high flammability of VCM has been considered to be a more serious hazard than inhalation.

From the earliest lists until 1962, the American Conference of Governmental Industrial Hygienists (ACGIH) recommended that the time-weighted average long-term 7-8 hour daily exposure of workmen not exceed 500 ppm v/v ($\sim 1300 \text{ mg/m}^3$). This recommendation was based on single exposure studies on animals and on human experience. Recommended time-weighted average concentrations are published by the ACGIH in an annual list of "Threshold Limit Values" (TLV). A TLV was considered by this group to "represent conditions under which it is believed that nearly all workers may be

repeatedly exposed day after day, without adverse effect." In 1963 the ACGIH modified their recommendation so that 500 ppm was to be a maximum concentration (or ceiling) rather than a time-weighted average. This change was in part due to the publication by Torkelson, Oyen, and Rowe (American Industrial Hygiene Association Journal Vol. 22, p 354, 1961) of the results of six-months repeated inhalation studies on laboratory animals. These authors reported slight reversible liver injury in rats exposed 7-hours per day to 100 ppm and recommended that the time-weighted average exposure of workers not exceed 50 ppm. However, this recommendation was not considered appropriate by Lester, Greenberg, and Adams (ibid. Vol. 24, p 265, 1963) who did not consider significant injury to have resulted in rats which they had repeatedly exposed to 500 ppm v/v.

About 1963-1964 reports appeared in the European literature describing pain and tenderness in the fingers of polyvinyl chloride workers. X-ray examination showed that the bones of the hand were dissolving. This condition known as acroosteolysis was also investigated by American vinyl and polyvinyl chloride companies and similar disease was found in workers who cleaned polymerization vessels. The epidemiological data from this investigation was published in January 1971. (Archives of Environmental Health, Vol. 22, pp 61-91, 1971). This epidemiological study was directed at workers currently employed by the companies. In May 1970, Dr. P. Luigi Viola, an Italian physician, reported in Houston that he had attempted to create a bone condition in rats similar to human acroosteolysis. (Tenth Intl. Cancer Congress, Houston, Texas, May 22-29, 1970). Dr. Viola observed tumors in the ear canals of rats exposed to 30,000 ppm (3%) vinyl chloride vapor 4 hours per day for 12 months. Viola also reported tumors in the lungs and bones of these rats but these later tumors are of questionable significance.

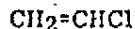
These data were subsequently published (Viola, P. L., Bigotti, A., and Caputo, A., Cancer Research 31, 516-522, May 1971).

In 1970 the ACGIH proposed reduction of their recommended TLV to a time-weighted average of 200 ppm primarily on the basis of work reported by Kramer and Mutchler at a 1968 Gordon Research Conference on Toxicology and subsequently published in January 1972 (American Industrial Hygiene Association Journal, Vol. 33, p 20, 1972). The authors reported evidence of clinical findings in workers exposed to a time-weighted average concentration of 300 ppm VCM for many years.

In 1972 the ACGIH took vinyl chloride from the list of Intended Changes and changed the value in their regular list to 200 ppm. This value remains the recommended value in the 1973 list. A copy of the Documentation for the ACGIH TLV for vinyl chloride as published in 1971 is attached.

In order to get more details of Dr. Viola's experiments, including additional unpublished studies and observations that he might have compiled, he was invited by the Manufacturing Chemists Association to visit Washington and discuss his findings with MCA's Occupational Health Committee. On May 5 and 6, 1971, he discussed data published in 1971 and referred to other studies completed and underway. Dr. Viola was extremely vague and evasive in giving details. He did report that his vinyl chloride was quite impure and claimed to have seen tumors in rats exposed to 5000 ppm. He thought 2500 ppm would be without such an effect. He produced no data and refused to give details. He claimed he would not be concerned about workers exposed to less than 500 ppm. We are not aware of any further publications by Dr. Viola on this subject.

VINYL CHLORIDE



200 ppm (Approximately 770 mg/m³)

Vinyl chloride is a flammable gas with anesthetic properties at high concentrations. At anesthetic concentrations (8 to 12%) it has serious effects on cardiac muscle resulting in arrhythmias and sensitization in dogs(1,2). There is a wide margin between anesthetic and lethal concentrations (30-40%)(3). Torkelson, Oyen and Rowe(4) reported that repeated seven-hour exposures for six months at 200 ppm resulted in histologic changes in the central lobular region of the livers of rabbits, but not in rats, guinea pigs and dogs. At 100 ppm there was only slight liver enlargement. A subsequent report by Lester, Greenberg and Adams(5) on the effects of repeated exposure of rats to vinyl chloride assaying 99 + percent purity at 2 and 5% concentration stated that, although liver changes were noted, they were not considered to be of pathological significance. In their opinion, a recommended TLV of 500 ppm was satisfactory for worker exposure.

An interim report was made by Mutchler and Kramer(6) of long industrial health experience with vinyl chloride workers. Beginning in 1950, continuous monitoring was begun at five or six stations and the results compared with breath analyses(7) for vinyl chloride for 50 workers. Between 1950 and 1967 the mean air concentration was 160 ppm, with one maximal peak at 600 ppm of vinyl chloride and with great daily variation from 30 to 170 ppm, on the average. During part of this time vinylidene chloride* was an associated air contaminant estimated at around 5 ppm. Twenty-one clinical parameters were measured for comparison with the environmental data, and relationships were obtained by stepwise multiple linear regression analysis. Of medical examinations numbering 404 on 97 workers, 66 were used for comparison with 650 controls. These examinations with final diagnoses made according to the international classification of diseases, failed to reveal any overt condition attributable to vinyl chloride (or vinylidene chloride) exposure. No differences of physiologic significance were found in blood pressure, hemoglobin, electrocardiograms, and no body abnormalities (acro-osteolysis) appeared. No one was clinically ill. Six clinical parameters appeared to be altered, however. Among these, beta lipoprotein, icterus index, and bromosulfalein retention time may have some physiologic significance. The investigators felt, from a review of the interim findings, that a time-weighted average exposure concentration at 300 ppm (combined vinyl chloride and 5 ppm vinylidene chloride) could result in some degree of liver dysfunction. The multiphasic screening tests are continuing on all exposed workers, and analysis of the findings is being made on an individual worker basis. In the meantime, a time-weighted average threshold limit value of 200 ppm vinyl chloride (with a few ppm vinylidene chloride) seems appropriate to prevent adverse systemic effects from long-continued daily exposure.

(* A 90-day animal inhalation exposure to vinylidene chloride at 5 ppm was without observable effect.)

References:

1. Oster, R.H., Carr, C.J., Krantz, J.C., Sauerwald, M.J.: *Anesthesiol.* **3**, 359 (1947).
2. Carr, J., Burgison, R.M., Vitche, I.F., Krantz, J.C.: *J. Pharm. Exptl. Ther.* **97**, 1 (1949).
3. Mastromatteo, E., Fisher, A.M., Christie, H., Danziger, H.: *Am. Ind. Hyg. Assn. J.* **21**, 394 (1960).
4. Torkelson, T.R., Oyen, F., Rowe, V.K.: *ibid.* **22**, 354 (1961).
5. Lester, D., Greenberg, L.A., Adams, W.R.: *ibid.*, **24**, 265 (1963).
6. Mutchler, J.E., Kramer, C.G.: Report on Relation of Exposure to State of Health of Dow Chemical Workers, Gordon Conf., Tilton, N.H. (August, 1968).
7. Barotta, E.D., Stewart, R.D., Mutchler, J.E.: *Am. Ind. Hyg. Assn. J.* **30**, 537 (1969).

(Reprinted from American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio)

III. Toxicity Studies in Progress

1. MCA-Sponsored Inhalation Studies.

As announced in March of 1973, the Manufacturing Chemists Association has contracted with Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois, to conduct two-year studies on rats, mice and hamsters. Groups of 100 male and 100 female of each species (600 total) will be exposed to either 0 (control), 5000 ppm, 500 ppm, or 50 ppm of vinyl chloride monomer, 7 hours per day, 5 days per week for 12 months. They will then be observed for an additional 12 months (6 months for mice). These animals will not have food and water during exposure.

This "lifetime" study is designed primarily as a cancer study and will include only a limited amount of other observations. Because Viola's studies involved simultaneous exposure of food, water, and bedding, a separate group of 100 rats of one sex will receive exposures to 5000 ppm along with their food, water, and bedding. This group is included to determine the possibility that a reaction of the vinyl chloride monomer with either the food, water, or bedding results in a toxic reaction product.

Exposure of these animals started in May 1973. A complete copy of the protocol is attached.

2. MCA-Sponsored Epidemiological Studies

The Manufacturing Chemists Association has contracted with Tabershaw-Cooper Associates, Berkeley, Calif., to conduct a study of the cause of death in vinyl chloride and polyvinyl chloride workers. The objectives are:

- (a) to compare the mortality of individuals who have worked in vinyl chloride plants with that of the general population, corrected for area, age, and sex;
- (b) to compare mortality patterns within the population of vinyl chloride workers, based upon estimated occupational exposure; and
- (c) to compare mortality among vinyl chloride workers with the mortality of other occupational groups which TCA, Inc., has studied.

This study is expected to require at least 8 months for completion and reporting. A copy of the protocol is attached.

IV. Recommendations for Action

1. VCM producers and consumers will undertake plant atmospheric monitoring programs.
2. The industry members have agreed to exchange analytical test methods to expedite the above program.
3. The industry members of the MCA group have agreed to implement the 1972 ACGIH adopted TLV value of 200 ppm for VCM in producing and consuming plants and to work towards lower levels such as 50 ppm.
4. The results of the animal and epidemiological test programs now in progress will be followed closely by the MCA Technical Task Group on Vinyl Chloride Research and the results referred to a qualified group such as the National Academy of Sciences for review.
5. The MCA group welcomes the participation of NIOSH in the evaluation of the test data and the formulation of additional programs which may be indicated.

PROTOCOL

MANUFACTURING CHEMISTS' ASSOCIATION, INC. Y

CHRONIC VAPOR INHALATION TOXICITY STUDY WITH
VINYL CHLORIDE

I. Outline of Investigation

- A. Type and Length*: 12-month vapor inhalation in
White Mice
12-month vapor inhalation in
Albino Rats
12-month vapor inhalation in
Hamsters
- B. Number of Animals: 800 Mice
800 Rats
800 Hamsters
- C. Exposure Schedule: Seven Hours per Day
Five Days per Week
- D. Test Materials: Vinyl Chloride (Ethylene derived).
- E. Organization: See Table I
- F. Dose Levels: See Table I

*After exposure, animals will be maintained
for observation and sacrifice at end of
two-year period (18 months for mice).

TABLE I

Chronic Vapor Inhalation Toxicity Study

Organization of Groups

<u>Test Material</u>	<u>Group</u>	<u>Number of Animals</u>					
		<u>Mice</u>		<u>Rats</u>		<u>Hamsters</u>	
		<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>	<u>Males</u>	<u>Females</u>
None	Control	100	100	100	100	100	100
Vinyl Chloride (Ethylene derived)	TE-I Low level - 50 ppm	100	100	100	100	100	100
	TE-II Intermediate level - 500 ppm	100	100	100	100	100	100
	TE-III High level - 5000 ppm	100	100	100	100	100	100

II. Chamber Parameters

Each group of animals will be exposed in a specially constructed plexiglas inhalation chamber having a capacity of approximately 6.0 M³, allowing animal loading of less than 2% when the animals reach maturity. Flow rate through the chamber will be at least 0.60 M³/min. providing a theoretical air change every ten minutes. The chamber supply air will be filtered and maintained at 40% to 60% relative humidity and 70° to 75°F.

III. Animal Parameters

A. Clinical Observations

All animals will be observed daily for lesions and behavioral changes attributable to the test material. The time of appearance and location of all tumors that occur among both control and test animals will be recorded. Mortality records will be kept on all groups of animals. All animals which die during the study will be necropsied and their tissues processed in accordance with the methods given in the Anatomic Pathology Section. Care will be exercised to minimize loss of tissues through cannibalism or autolysis. Animals in a moribund state will be sacrificed in extremis when death is imminent.

B. Body Weights

Individual body weights will be recorded once before exposure and after 1, 2, 3, and 4 weeks of testing. Thereafter, mean group body weights will be determined monthly up to the 12-month point of the study.

C. Clinical Pathology

Hemoglobin, hematocrit, total erythrocyte and total leukocyte counts will be performed at 18 and 24 months on 30 (15 male and 15 female) rats of the control and each test group. Differential leukocyte counts will be performed on all animals having high total leukocyte counts.

D. Anatomic Pathology

1. Methods of Sacrifice

Upon completion of the study, all survivors will be sacrificed by exsanguination following carbon dioxide anesthesia.

2. Gross Pathology

Complete necropsies will be performed on all animals which die or are sacrificed and all macroscopic lesions will be recorded. The lungs will be inflated with formalin fixative.

3. Histopathology

Representative specimens of the following organs

and tissues will be taken from all animals at time of sacrifice
and fixed in 10.0% neutral buffered formalin:

Adrenal Glands
All Gross Lesions
Bone (femur, tarsal and metatarsal, including long bones of
all four limbs)
Bone Marrow (sternal)
Brain
Both Ears (external auditory canal with ceruminal (Zymbal's)
glands)
Esophagus
Eye
Gonads (testes and ovaries)
Kidneys
Large Intestine (caecum and colon)
Liver
Lungs
Lymph Nodes (tracheobronchial, cervical and mesenteric)
Optic Nerve
Pancreas
Parathyroid Gland
Pituitary Gland
Prostate
Salivary Gland
Seminal Vesicles
Skin
Small Intestine (duodenum, jejunum and ileum)
Spleen
Stomach
Thymus
Thyroid Gland
Tracheo
Urinary Bladder
Uterus.

The above tissues, from animals of the control,
TE-II, and TE-III will be processed by conventional methods,
embedded in paraplast, sectioned (4-6 μ), stained with hema-
toxylin and eosin, and evaluated by light microscopy. If

drug-related lesions are detected in tissues from the high or intermediate dose (TE-II or TE-III) animals, affected tissues from the TE-I group animals will be processed and examined in the same manner as the above.

Only major organs (liver, kidney, spleen, heart, lungs) and neoplasms from animals which die and are found in an advanced state of autolysis will be processed for histopathologic evaluation.

IV. Reports

Quarterly summaries of mortalities, clinical observations, clinicopathologic and anatomopathologic findings will be prepared. Upon completion of the study, a complete report will be prepared and issued.

February 1, 1973

APPENDIX

TEST PROTOCOLS
extracted from
the
Industrial BIO-TEST Contract Extension

"This experimental group is to consist of 100 rats of the same sex, to be exposed to vinyl chloride vapors at a concentration of 5,000 ppm v/v. Food, water, and bedding are to be present in the cages in which the animals are exposed during the entire exposure period. Otherwise, experimental procedures, including exposure conditions, their documentation and control, and the handling, observation and necropsies of animals shall conform to those described in the Protocol incorporated by reference in the subject agreement, and hereby so incorporated in this agreement extension."

KDJ:mb

THE EFFECTS OF EXPOSURE OF RATS TO 50, 500 AND 5,000 PPM VINYL
CHLORIDE VAPOR ON THE SULFHYDRYL LEVELS OF LIVER

R. E. Hefner, Jr. and P. J. Gehring
Toxicology Department
The Dow Chemical Company
Midland, Michigan 48640

ABSTRACT

Exposure of rats to 50, 500 or 5,000 ppm vinyl chloride vapors for 7 hr/day for 5 consecutive days causes a decrease in the non-protein sulfhydryl content of the liver. The degree of this response was essentially equivalent at these exposure concentrations suggesting that this response is mediated via a "zero" order mechanism.

2-hydroxyethyl-L-cysteine and monochloroacetic acid were found in the urine of rats exposed to 5,000 ppm vinyl chloride. It is proposed that in vivo vinyl chloride is sequentially oxidized to 2-chloroethanol, chloroacetaldehyde and chloroacetic acid. The lack of dependency of the depression of the non-protein sulfhydryl content of the liver on exposure concentration of vinyl chloride is attributed to the "zero" order oxidation of 2-chloroethanol to chloroacetaldehyde via alcohol dehydrogenase. Chloroacetaldehyde spontaneously alkylates cysteine while other oxidation products of vinyl chloride do not.

CONFIDENTIAL

C O N F I D E N T I A L

THE EFFECTS OF EXPOSURE OF RATS TO 50, 500 AND 5,000 PPM
VINYL CHLORIDE ON THE SULFHYDRYL LEVELS OF LIVER

R. E. HEFNER, JR. AND P. J. GEHRING
TOXICOLOGY DEPARTMENT
THE DOW CHEMICAL COMPANY
MIDLAND, MICHIGAN 48640

CONFIDENTIAL

(THIS REPORT PREPARED FOR THE MANUFACTURING CHEMISTS ASSOCIATION
TECHNICAL TASK GROUP ON VINYL CHLORIDE RESEARCH SUMMARIZES
PRELIMINARY STUDIES AND IS TO BE CONSIDERED HIGHLY CONFIDENTIAL.
RELEASE OF THIS INFORMATION IS TO OCCUR ONLY WITH THE CONSENT
OF THE AUTHORS AND THE DOW CHEMICAL COMPANY).

AP00015625

INTRODUCTION

In 1971, Viola et al. reported that tumors of the skin, lungs, and bones developed in rats exposed to an atmosphere containing 30 000 ppm vinyl chloride for 4 hours per day, 5 days per week for 12 months. In the study described herein the zero order kinetics of vinyl chloride metabolism would indicate that a dose response curve for tumor induction by various concentrations of vinyl chloride might be expected to have a very shallow slope. Thus, if this hypothesis is valid, tumor induction may be mediated via the metabolism of vinyl chloride.

Consideration of the possible fate of vinyl chloride in the body suggests that it may be oxidized sequentially to 2-chloroethanol, chloroacetaldehyde, and chloroacetic acid. In vivo oxidation of 2-chloroethanol to chloroaldehyde occurs via the same pathway as the oxidation of ethanol (Johnson, 1967). Both oxidations are mediated by alcohol dehydrogenase. Associated with the injection of 2-chloroethanol in rats is a reduction in the glutathione content of the liver, which is mediated via an alkylation of the free sulfhydryl group of the cysteine portion of this tripeptide (Johnson, 1967). Cysteine, per se, may also be alkylated. Neither 2-chloroethanol (Johnson, 1967) or chloroacetic acid (Gibson, 1973) alkylate glutathione in vitro, however, chloroacetaldehyde does, suggesting that the zero order oxidation of 2-chloroethanol to chloroacetaldehyde via alcohol dehydrogenase precedes the alkylation of glutathione and/or cysteine.

CONFIDENTIAL

AP00015626

Using the aforementioned rationale, we hypothesized that vinyl chloride will be oxidized to chloroacetaldehyde in vivo and cause a reduction in the non-protein sulfhydryl content, glutathione and cysteine, of various tissues. In a previous probe study, the non-protein sulfhydryl content of the liver of rats exposed to 15,000 ppm vinyl chloride was reduced (Hefner, and Gehring, NB T13.4-16-8).

How may such reactions be associated with tumor induction? It has been reported that the administration of cysteine or glutathione provides protection against the untoward effects of various aliphatic and aromatic mustards, triethylenemelamine, x-rays, and ionizing radiation (Calcutt, et al., 1963; Patt, 1964; Goldenthal, et al., 1959; Stacey, et al., 1958; Ball, 1966). Conceivably, cysteine and glutathione may provide a natural defense against tumor producing free radicals generated within the body, as well as synthetic or naturally occurring alkylating agents which are absorbed into the body. Thus, it is our hypothesis that the induction of tumors in rats exposed to vinyl chloride may be secondary to an induced reduction in the non-protein sulfhydryl content of various tissues.

The primary objective of the study reported herein was to characterize the reduction of the non-protein sulfhydryl content of the liver of rats exposed to various concentrations of vinyl chloride. If our hypothesis is valid, the magnitude

CONFIDENTIAL

AP00015627

of such reductions should not be markedly dependent on the exposure concentration. Secondary objectives were to establish some temporal relationships for the reduction of the non-protein sulfhydryl content of the liver of rats exposed daily and to attempt preliminary identification of reaction products of cysteine and vinyl chloride in the urine of exposed rats.

METHODS

Groups of male Sprague-Dawley albino rats of Spartan strain weighing from 193 to 250 grams at initiation of the experiment were exposed to nominal concentrations of 5,000, 500, or 50 ppm vinyl chloride, for 1, 3, or 7 weeks and to 50 ppm for 1 week. The exposures were carried out in a glass-walled 160L chamber under dynamic conditions with the vinyl chloride being metered into the chamber airstream. Seven hour exposures were conducted on the first four of five consecutive days each week. On the fifth day, the duration of the exposure was reduced to 5 to 6 hours.

Body weights and food consumption were determined before each exposure and the rats were observed periodically for signs of toxicity. Between 1 and 2 PM, immediately following the fifth exposure of the designated week, the rats were killed and the livers removed and prepared for assay of sulfhydryl groups. Gross pathological examinations were conducted.

CONFIDENTIAL

AP00015628

Sulfhydryl Assay. The method used for free sulfhydryl assay was a modification of that described by Sedlak and Lindsay (1968). Exactly 500 mg of liver from each rat was homogenized for 1 minute in a Dounce tissue homogenizer containing 8 ml of 0.02M disodium EDTA. For the total sulfhydryl assay, a 0.5 ml aliquot of each homogenate was mixed with 1.5 ml of 0.2M tris pH 9.2 buffer, 0.1 ml of 0.01M DTNB [5,5'-dithiobis(2-nitrobenzoic acid)], and 7.9 ml of methanol. A reagent blank without liver homogenate, and a sample blank, without DTNB, were also prepared. The color generated via the release of nitro-mercaptobenzoic acid anion was allowed to develop for 15 minutes, and the samples were centrifuged for 15 minutes at 4,000 g. Absorbance of each sample was read against the respective sample blank at 412 nm with a Beckman DB spectrophotometer. Subsequently, the molar concentration of total sulfhydryl in the sample was calculated using an extinction coefficient determined from standards of known concentrations of glutathione or cysteine. A plot of absorbance versus concentration of cysteine or glutathione is shown in Figure 2. This plot coincides with that reported by Sedlak and Lindsay (1968).

The non-protein sulfhydryl content of liver was determined after precipitating out the protein by addition of 1 ml of 50% trichloroacetic acid to a 5 ml sample of liver homogenate. Each sample was diluted with 4 ml of distilled water and after 15 minutes centrifuged at 4,000 g. A 2 ml aliquot of the

CONFIDENTIAL

AP00015629

supernatant was mixed with 4 ml of 0.4M tris pH 8.9 buffer. Immediately before reading the absorbance against a reagent blank, 0.1 ml of 0.01M DTNB was added. Subtraction of the non-protein sulfhydryl content from the total sulfhydryl content yielded a value for protein-bound sulfhydryl.

Metabolite Identification. Urine samples collected from rats exposed to 5,000 ppm vinyl chloride for 4, 5, and 7 weeks were analyzed for the presence of 2-chloroethyl-L-cysteine, 2-hydroxyethyl-L-cysteine, and 2-carboxymethyl-L-cysteine (5 and 7 week samples only). Urine was collected for analysis by applying pressure to the posterior abdomen between the fifth and sixth hour of the fifth daily exposure on the designated week. Urine collected on the same day was pooled. For the three aforementioned compounds, 2 to 15 μ l of urine were spotted directly on a 5 by 20 cm Baker-flex® silica gel plate. Also spotted were samples of urine collected from control rats and standard aqueous solutions as well as control urine to which approximately 1 μ g/ μ l of each of the compounds had been added. The chromatograms were developed for 5 hours in a sealed glass tank containing n-butanol, acetic acid, water (80:10:10). After being air dried, the plates were sprayed with Ninspray® ninhydrin reagent and heated for 2 minutes at 80°C. The color of the spots and their Rf values were used to identify the compounds.

CONFIDENTIAL

AP00015630

A urine sample collected from rats exposed to 5,000 ppm vinyl chloride for 9 weeks was analyzed for the presence of chloroacetic acid. The 10 ml urine sample was acidified with 0.1 ml of 50% v/v H_2SO_4 . Subsequently, the urine sample was extracted 3 times with 2 ml of diethyl ether. By evaporation, the diethyl ether extract was concentrated to 0.1 ml and 2 to 15 μ l of the concentrated extract and an aqueous standard containing 5% w/v chloroacetic acid were spotted on an Eastman fluorescent silica gel plate. The chromatograms were developed in a sealed glass tank for 5 hours using the aforementioned solvent system. After drying the plates, they were examined under ultraviolet light to determine the location of spots. Subsequently, the plates were treated with ninhydrin as described previously.

RESULTS

No antemortem or postmortem signs of toxicity were noted in rats exposed to any concentration of vinyl chloride used in these experiments. Analysis of food consumption data of rats exposed to nominal concentrations of 5,000 and 500 ppm vinyl chloride, and controls revealed no statistically significant differences. In addition, no significant difference was found in the water consumption of rats exposed to 5,000 ppm and controls. Throughout the durations of observation, the mean body weights of rats exposed to the various concentrations of vinyl chloride were essentially the same as those of the respective controls,

Figure 3. No gross pathological lesions were found in any of the rats exposed to vinyl chloride.

Sulfhydryl Content of Liver. In Tables 2, 3, and 4, respectively, are shown the total, protein, and non-protein sulfhydryl content of the liver of rats exposed to 50, 500, or 5,000 ppm vinyl chloride for the indicated durations. Values for concurrent controls are also given. The results in Table 4 show that exposure to either 500 or 5,000 ppm vinyl chloride was consistently associated with a statistically significant decrease in the non-protein sulfhydryl content of the liver. Even in the group of rats exposed to 50 ppm vinyl chloride for one week, there appears to be some reduction. However, because of a low value for the non-protein sulfhydryl content of one of the controls the reduction was not statistically significant. To resolve this, a second group of rats was exposed to 50 ppm vinyl chloride for one week and a statistically significant decrease in the non-protein sulfhydryl content of the liver was found, Table 5. The relative non-protein sulfhydryl value, for both control and exposed rats are higher than those found in previous assays. The samples of liver were homogenized more thoroughly in this study, 2 minutes instead of 1 minute, which may account for this apparent discrepancy.

Figure 4 illustrates the percent depression of the non-protein sulfhydryl content of the liver as a function of exposure concentration and duration of exposure. The results indicate

that the reduction in the non-protein sulfhydryl content of the liver of rats exposed to 50, 500, 5,000 and 15,000 ppm vinyl chloride is independent of the exposure concentration. The result for rats exposed to 15,000 ppm was taken from a previous report (Hefner, Jr. and Gehring, NB T13.4-16-8).

Although not definitive, the depression of the non-protein sulfhydryl content of the liver of rats exposed to 500 and 5,000 ppm vinyl chloride appears to decrease as the duration of the exposure is lengthened. This suggests that repeated exposure to vinyl chloride may induce a metabolic adaptation which decreases the response.

With regard to the total sulfhydryl and protein sulfhydryl content of the liver, the changes in rats exposed to vinyl chloride were sporadic and inconsistent, thus negating any definitive conclusions. This was not unexpected because the sulfhydryl groups of protein have been shown not to be readily alkylated unless denaturation of the protein renders them available for alkylation (Stacey et al., 1958).

Metabolite Identification. Figure 5 depicts a representative thin-layer chromatogram of urine collected from rats exposed to 5,000 ppm vinyl chloride. Chromatograms of urine from rats exposed for 4, 5, and 7 weeks were all the same. 2-hydroxyethyl-L-cysteine, Rf 0.26-0.28, appeared to be present all 3 times.

R_f values for 2-hydroxy-2-cysteine in the chromatograms of standards were the same. In addition, the color of the respective spots was the same, reddish-purple after development with ninhydrin reagent. 2-chloroethyl-2-cysteine and 2-carboxymethyl-2-cysteine were not detected in the urine of rats exposed to vinyl chloride. Although the spots for 2-hydroxyethyl-2-cysteine and 2-carboxymethyl-2-cysteine were close, they were resolvable and the spot for 2-carboxymethyl-2-cysteine was dark purple after treatment with ninhydrin reagent.

Figure 6 depicts a representative thin-layer chromatogram showing the presence of chloroacetic acid in urine collected from rats exposed to 5,000 ppm vinyl chloride for 9 weeks. Chloroacetic acid appeared to be present as visualized under ultraviolet light; the R_f value was 0.74. This value is the same as the R_f value obtained using a chloroacetic acid standard. As expected, the chloroacetic acid spots were not developed by ninhydrin reagent.

DISCUSSION

This study shows that there is a depression of the non-protein sulfhydryl content in the liver of rats exposed to concentrations of vinyl chloride ranging from 50 to 15,000 ppm 7 hrs/day for 5 consecutive days. Within this range of concentrations, the degree of depression is not significantly influenced by the magnitude of the concentration to which rats are exposed.

CONFIDENTIAL

AP00015634

Although not definitive, the degree of depression appears to decrease with additional weeks of exposure - 7 hrs/day, 5 consecutive days/week.

The depression of the non-protein sulfhydryl content of the liver of rats exposed to vinyl chloride occurred in the absence of concurrent signs of antemortem toxicity or gross pathological changes. Absence of definitive signs of toxicity is not surprising. In a previous study, no antemortem signs of toxicity or gross pathological changes were observed in rats exposed to 500 ppm vinyl chloride 7 hrs/day, 5 days/week for 4.5 months (Torkelson et al., 1961). A mild degree of central lobular degeneration in the liver and interstitial and tubular changes in the kidney were observed. In rats exposed to 100 or 200 ppm vinyl chloride 7 hrs/day, 138 to 144 times in 204 days, the only discernible effect was a slight increase in liver weight. Increased weights of the liver did not occur in rats exposed to 50 ppm.

Whether a decrease in the non-protein sulfhydryl content of the liver constitutes a toxicologically significant parameter remains unknown. Hayes et al. report a correlation between the single dose lethality of monochloroacetic acid and depression of the non-protein sulfhydryl content of liver and kidney. Recently, Jaeger et al., 1974 reported a depression of the non-protein sulfhydryl content in rats exposed for 4 hrs to

CONFIDENTIAL

AP00015635

1,1-dichloroethylene. Also demonstrated was an increased lethality in rats whose liver non-protein sulfhydryl content had been reduced by 16 hrs of starvation and subsequently exposed for 4 hrs to 1,1-dichloroethylene. Although these studies suggest that toxicity may be related to a reduction of the non-protein sulfhydryl content of liver and perhaps other tissues, a definitive association between the two remains to be established. It is not unreasonable to expect that the non-protein sulfhydryl content of tissues provides a pathway for the detoxification of various alkylating agents and free radicals generated normally within the body.

The results of the study reported herein provide initial support for our hypothesis that the tumorigenic activity of vinyl chloride in rats may be mediated via a reduction in the non-protein sulfhydryl content of liver. As rationalized in the introduction, such a reduction may depress the natural defense of the body against tumor development. The attractiveness of this hypothesis is augmented by the finding that the degree of the depression of the sulfhydryl group concentration of the liver is not dependent on the magnitude of the concentration of vinyl chloride to which rats were exposed. This finding also provides support for the hypothesis that a dose response curve for tumor induction by vinyl chloride might be expected to have a very shallow slope.

CONFIDENTIAL

AP00015636

The absence of a dose-response depression of the non-protein sulfhydryl content of the liver is also consistent with our hypothesis that this event is preceded by the oxidation of vinyl chloride to 2-chloroethanol and subsequently to chloroacetaldehyde. The oxidation of 2-chloroethanol to chloroacetaldehyde is mediated via alcohol dehydrogenase (Johnson, 1967) which is readily saturatable. Chloroacetaldehyde but not vinyl chloride (unpublished results) or 2-chloroethanol (Johnson, 1967) readily alkylates the sulfhydryl group of cysteine and glutathione. Therefore, the absence of a dose response for the depression of the non-protein sulfhydryl content of the liver may be expected if our hypothesis is valid. That vinyl chloride is oxidized via this pathway is supported by the finding of monochloroacetic acid in the urine.

The finding of 2-hydroxyethyl-L-cysteine but not carboxymethyl-L-cysteine in the urine of rats exposed to vinyl chloride might at first appear to be inconsistent with our original hypothesis. If production of chloroacetaldehyde precedes alkylation of the sulfhydryl groups of glutathione and cysteine, the latter but not the former metabolite may be expected. Johnson (1967) reported carboxymethylglutathione but not 2-hydroxyethylglutathione in the urine of rats given 2-chloroethanol. In our studies, standards for the glutathione conjugates were not available; therefore, the urine of rats exposed to vinyl

CONFIDENTIAL

chloride was not examined for their presence. Originally we had expected the cysteine conjugates to be like the glutathione conjugates reported by Johnson (1967).

It is conceivable that the alkylation of glutathione and cysteine with chloroacetaldehyde may not lead to the excretion of similar analogs. Perhaps, the conjugation of chloroacetaldehyde with cysteine will produce a thiazolidine ring which subsequently undergoes ring opening and hydrolysis to give 2-hydroxyethyl cysteine. The acetaldehyde glutathione conjugate cannot form such a structure because it is N,N'-disubstituted, (Figure 7).

Another way to explain the finding of 2-hydroxyethyl-L-cysteine but not carboxymethyl-L-cysteine in the urine is that vinyl chloride may alkylate cysteine and glutathione directly to form 2-chloroethyl-L-cysteine and 2-chloroethylglutathione. These products spontaneously form the hydroxyl analogs in an aqueous environment (Moppett and Martin, 1973). Direct alkylation of glutathione and cysteine by vinyl chloride occurs when vinyl chloride is bubbled through an aqueous-ethanol but not in an aqueous system (Martin and Moppett, 1973). Although unresolved, it is not expected that vinyl chloride will directly alkylate cysteine and glutathione in vivo. Furthermore, if the depression of non-protein sulfhydryl groups is mediated via this reaction, there should be a strong correlation

between the degree of depression and the magnitude of the exposure. Thus, it seems unlikely that vinyl chloride alkylates non-protein free sulfhydryl directly; however more data are needed to exclude this possibility.

Verification of our original hypothesis, as well as identification and verification of the metabolites of vinyl chloride is required, as the analytical procedures used in detecting 2-hydroxyethyl- β -cysteine and chloroacetic acid in the urine of rats exposed to vinyl chloride were only qualitative at best. Therefore, we propose a metabolism study using ^{14}C labeled vinyl chloride to further elucidate the validity of our hypothesis. Such a study would allow a definitive qualitative and quantitative identification of metabolites. Also additional studies are planned to determine what levels of exposure to vinyl chloride may be incurred without a reduction of the non-protein sulfhydryl concentration in the liver.

CONFIDENTIAL

AP00015639

LITERATURE CITED

1. Calcutt, G., Connors, T. A., Elson, L. A. and Ross, W. C. J., Reduction of the toxicity of "radiomimetic" alkylating agents in rats by thiol pretreatment part II, mechanism of protection, *Biochem. Pharmacol.*, 12:833-837, 1963.
2. Patt, H. M., Protective mechanisms in ionizing radiation injury, *Physiol. Rev.*, 33:35-76, 1953.
3. Connors, T. A., Jeney, A. and Jones, M., Reduction of the toxicity of "radiomimetic" alkylating agents in rats by thiol pretreatment - III, *Biochem. Pharmacol.*, 13:1545-1550, 1964.
4. Goldenthal, E. I., Nadkarni, M. U. and Smith, P. K., A study of comparative protection against lethality of triethylenemelamine, nitrogen mustard and x-irradiation in mice, *Rad. Res.*, 5:571-583, 1959.
5. Stacey, K. A., Cobb, M., Consens, S. F. and Alexander, P., The reactions of the "radiomimetic" alkylating agents with macromolecules in vitro, *Annals of the N.Y. Acad. of Sciences*, Vol. 68, Art. 3, 657, 1958.
6. Ball, C. R., Estimation and identification of thiols in rat spleen after cysteine or glutathione treatment: relevance to protection against nitrogen mustards, *Biochem. Pharmacol.*, 15:809-816, 1966.
7. Hayes, F. D., Short, R. D. and Gibson, J. E., Differential toxicity of monochloroacetate, monofluoroacetate and monoiodoacetate in rats, *Toxicol. Appl. Pharmacol.* (to be published).
8. Grigorescu, I. and Toba, G. H., Vinyl chloride, industrial aspects. I., *Rev. Chim.*, 17:499-501, 1966.
9. Devik, F., *Brit. J. Radiol.*, 27:463, 1954.
10. Violo, P. L., Bigotti, A. and Caputo, A., Oncogenic response of rat skin, lungs and bones to vinyl chloride, *Cancer Research*, 31:516-522, 1971.

CONFIDENTIAL

AP00015640

11. Sinclair, W. E., Protection by cysteamine against lethal x-ray damage during the cell cycle of Chinese hamster-cells, Radiation Research, 39:135-154, 1969.
12. Vergroesen, A. J. Budhe, L., Cohen, J. A., Factors influencing the radio-protection of tissue culture cells by su.phydryl compounds, Nature, 204:296-247, 1964.
13. Jaeger, R. J., Conolly, R. B., and Murphy, S. D., Diurnal variation of hepatic glutathione concentration and its correlation with 1,1-dichloroethylene inhalation toxicity in rats, (submitted for publication).
14. Shimkin, M. B., Cancer:7, 410-413, 1954.
15. Roe, F. J. C. and Salaman, Brit. J. Cancer: 9, 177-203, 1955.
16. Fahmy, O. G. and Fahmy, J. J., Cancer Research: 30, 195-205, 1970.
17. Moppett, C. E. and Martin, J., Reaction of vinyl chloride with cysteine and glutathione, Eastern Research Lab., The Dow Chemical Co., April, 1973.
18. Martin, J. and Moppett, C. E., Reaction of vinyl chloride with cysteine, cysteine and Purina Lab Chow®. The Dow Chemical Co., February 21, 1973.
19. Sedlak, J. and Lindsay, R. H. (1968). Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192-205.

CONFIDENTIAL

TABLE 1

TOTAL SULFHYDRYL CONTENT (TSH), 1×10^{-8} MOLES/MG, OF THE LIVERS OF RATS EXPOSED TO 5,000, 500, AND 50 PPM VINYL CHLORIDE FOR VARIOUS TIMES AND RESPECTIVE CONCURRENT CONTROLS

Duration of Exposure**	Concentration of Vinyl Chloride					
	5,000 ppm	Concurrent Controls	500 ppm	Concurrent Controls	50 ppm	Concurrent Controls
7 hours per day for 5 days	2.08	2.07	2.15	2.12	2.09	2.44
	2.06	2.28	2.21	2.18	2.14	2.20
	2.10	2.30	2.25	2.11	2.10	2.36
	2.08		2.09	2.08	1.98	
	2.15		2.35			
Mean±S.D.	2.09±0.04	2.22±0.13	2.21±0.10	2.12±0.04	2.08±0.07*	2.33±0.12
7 hours per day 5 days per week for 3 weeks	2.37	2.31	2.28	2.55		
	2.53	2.53	2.15	2.45		
	2.50	2.48	1.90	2.31		
	2.38		1.99			
	2.50					
Mean±S.D.	2.45±0.08	2.44±0.06	2.08±0.17*	2.44±0.12		
7 hours per day 5 days per week for 7 weeks	2.36	2.50	2.60	2.31		
	2.00	2.75	2.53	2.33		
	2.18	2.61	2.61	2.48		
	2.50		2.61			
	2.38					
Mean±S.D.	2.28±0.20*	2.62±0.13	2.59±0.04*	2.37±0.09		

*Significantly different using Students "t" test, $p < 0.05$.

**Determinations made between 1 - 2 PM after completing 5 to 6 hours of the 5th and final weekly 7 hour exposure.

CONFIDENTIAL

AP00015642

TABLE 2

PROTEIN BOUND SULFHYDRYL CONTENT (PSH), 1×10^{-8} MOLE/MG, OF THE LIVERS OF RATS EXPOSED TO 5,000, 500 AND 50 PPM VINYL CHLORIDE FOR VARIOUS TIMES AND RESPECTIVE CONCURRENT CONTROLS

Duration of Exposure**	Concentration of Vinyl Chloride					
	5,000 ppm	Concurrent Controls	500 ppm	Concurrent Controls	50 ppm	Concurrent Controls
7 hours per day for 5 days	1.71	1.63	1.75	1.62	1.75	1.96
	1.75	1.84	1.97	1.59	1.79	1.87
	1.76	1.81	1.91	1.53	1.65	1.88
	1.75		1.86	1.50		
	1.83		1.88			
Mean±S.D.	1.76±0.05	1.76±0.12	1.87±0.08	1.55±0.05*	1.75±0.06	1.90±0.05
7 hours per day 5 days per week for 3 weeks	1.95	1.58	1.81	1.87		
	2.13	1.79	1.63	1.84		
	2.09	1.79	1.47	1.78		
	2.02		1.61			
	2.06					
Mean±S.D.	2.05±0.07*	1.72±0.12	1.63±0.14	1.83±0.05		
7 hours per day 5 days per week for 7 weeks	1.78	1.81	1.95	1.76		
	1.55	2.03	1.92	1.68		
	1.53	1.93	2.06	1.76		
	1.90		2.09			
	1.73					
Mean±S.D.	1.70±0.16	1.92±0.11	2.01±0.08*	1.74±0.05		

*Significantly different using students "t" test, $p < 0.05$

**Determinations made between 1 - 2 PM after completing 5 to 6 hours of the 5th and final weekly 7 hour exposure.

CONFIDENTIAL

AP00015643

TABLE 3

NON-PROTEIN BOUND SULFHYDRYL CONTENT (NPSH), 1×10^{-8} MOLE/MG, OF THE LIVERS OF RATS EXPOSED TO 5,000, 500, AND 50 PPM VINYL CHLORIDE FOR VARIOUS TIMES AND RESPECTIVE CONCURRENT CONTROLS

Duration of Exposure**	Concentration of Vinyl Chloride					
	5,000 ppm	Concurrent Controls	500 ppm	Concurrent Controls	50 ppm	Concurrent Controls
7 hours per day for 5 days	0.37	0.44	0.40	0.50	0.34	0.48
	0.31	0.44	0.24	0.64	0.35	0.33
	0.34	0.49	0.34	0.58	0.32	0.48
	0.33		0.23	0.58	0.35	
	0.32		0.47			
Mean±S.D.	0.33±0.02*	0.46±0.03	0.35±0.09*	0.58±0.06	0.34±0.01	0.43±0.09
7 hours per day 5 days per week for 3 weeks	0.42	0.73	0.47	0.68		
	0.40	0.74	0.52	0.61		
	0.41	0.68	0.43	0.53		
	0.35		0.38			
	0.44					
Mean±S.D.	0.41±0.03*	0.72±0.03	0.45±0.06*	0.61±0.08		
7 hours per day 5 days per week for 7 weeks	0.58	0.69	0.65	0.55		
	0.45	0.72	0.60	0.64		
	0.65	0.68	0.55	0.71		
	0.60		0.52			
	0.65					
Mean±S.D.	0.59±0.08	0.70±0.02	0.58±0.06	0.64±0.08		

*Significantly different using Students "t" test, $p < 0.05$.

**Determinations made between 1 - 2 PM after completing 5 to 6 hours of the 5th and final weekly 7 hr exposure.

TABLE 4

NON-PROTEIN BOUND SULFHYDRYL CONTENT (NPSH), 1×10^{-8} MOLE/MG, OF THE
LIVERS OF RATS EXPOSED TO 50 PPM VINYL CHLORIDE FOR 1 WEEK
AND CONCURRENT CONTROLS ***

<u>Duration of Exposure**</u>	<u>Concentration of Vinyl Chloride</u>	
	<u>50 ppm</u>	<u>Concurrent Controls</u>
7 hours per day for 5 days	0.759	1.296
	0.717	1.060
	0.770	1.300
	0.579	1.250
	0.484	.991
	0.732	
	0.747	
	0.671	
Mean $\pm$ S.D.	.682 $\pm$.101*	1.179 $\pm$ 0.144

*Significantly different using Students "t" test, $p < 0.05$.

**Determinations made between 1-2pm after completing 5 hours of the
5th and final weekly 7 hour exposure.

***The relative NPSH values for both exposed and control rats are
higher than those found in previous assays. This is most likely
due to homogenizing each liver sample for 2 minutes, rather than
1 minute as in the previous assays.

CONFIDENTIAL

AP00015645

AS 9113 91

10 2 10 10 THE HALF CENT

10000

10000

10000

10000

10000

Absorbance

1.6
1.4
1.2
1.0
.8
.6
.4
.2
0

1×10^{-5} 2 3 4 5 6 7 8 9 1×10^{-4} 2 3 4 5

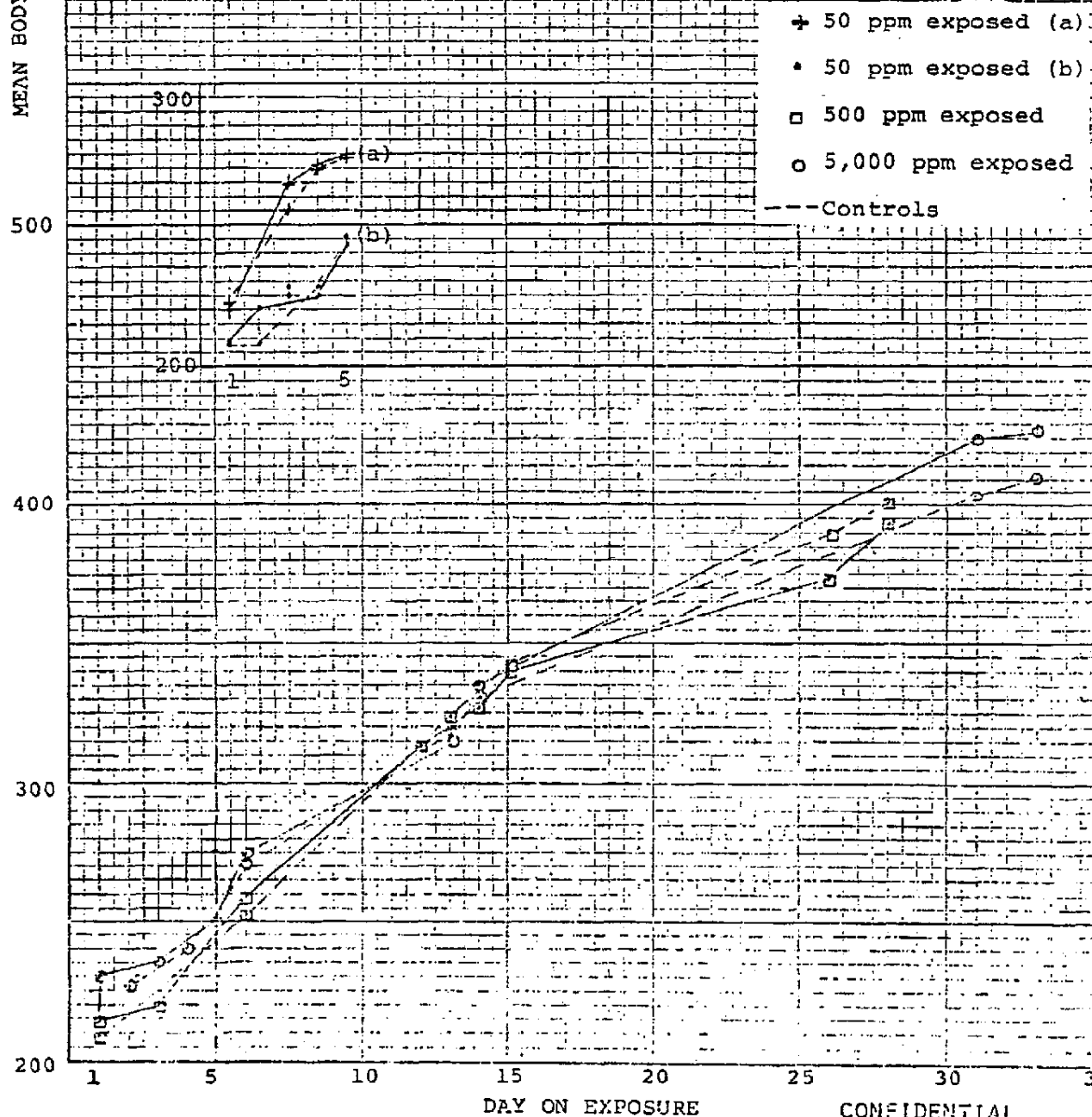
Figure 1,
BEER'S LAW PLOT - CYSTEINE AND
GLUTATHIONE - DTNB COMPLEXES

Sulfhydryl Concentration

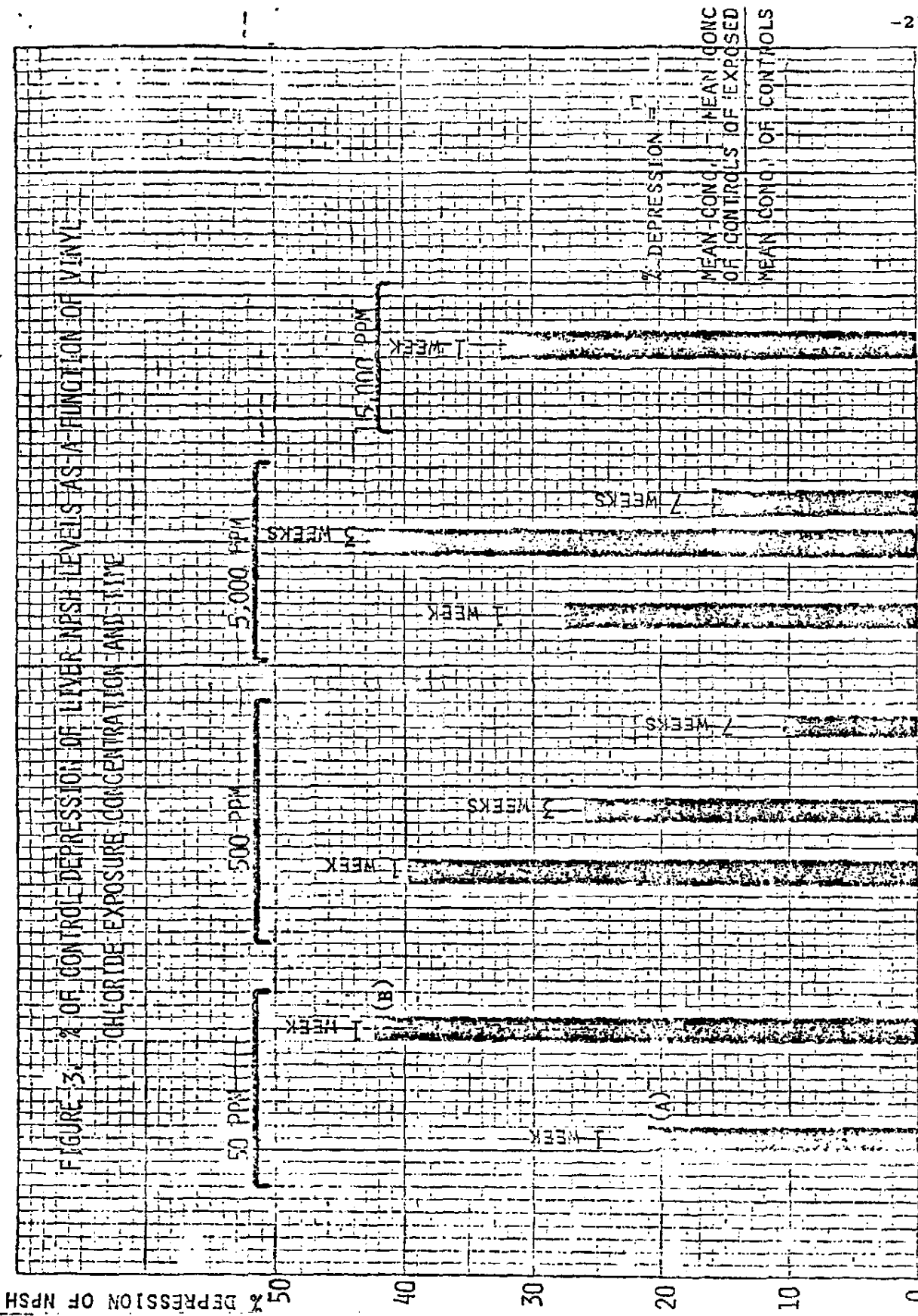
CONTINUED

FIGURE 2. MEAN BODY WEIGHTS OF RATS EXPOSED TO 50, 500, AND 5,000 PPM VINYL CHLORIDE

MEAN BODY WEIGHT

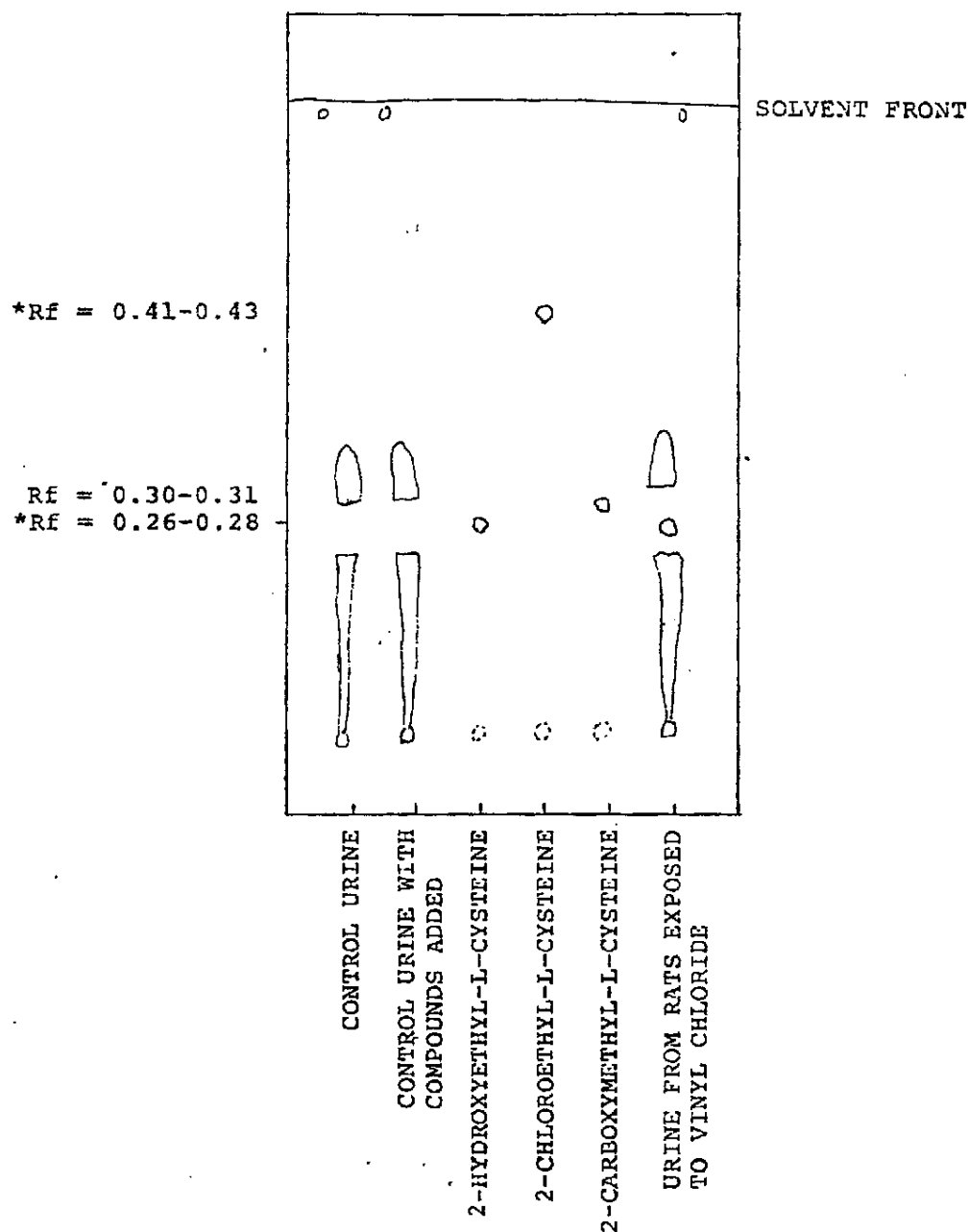


CONFIDENTIAL



VINYL CHLORIDE EXPOSURE CONCENTRATION

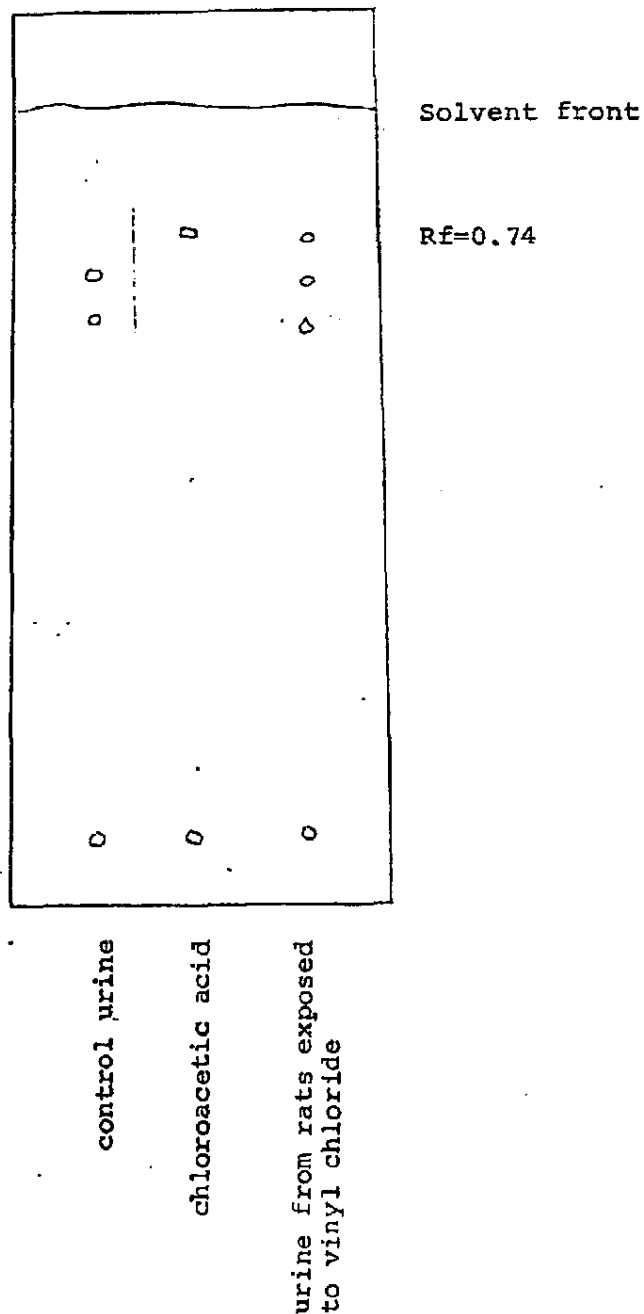
FIGURE 1. REPRESENTATIVE THIN-LAYER CHROMATOGRAM OF URINE FROM RATS EXPOSED TO 5,000 PPM VINYL CHLORIDE FOR 4, 5, AND 7 WEEKS



*Value for 80, 10, 10 n-butanol, acetic acid and water. 2-hydroxyethyl-L-cysteine has a 0.13-0.14 Rf and 2-chloroethyl-L-cysteine has a .27-.28 Rf in 80, 20, 20.

CONFIDENTIAL

REPRESENTATIVE FLUORESCENT THIN-LAYER CHROMATOGRAM (AS VISUALIZED UNDER U.V. LIGHT) OF URINE FROM RATS EXPOSED TO 5,000 PPM VINYL CHLORIDE FOR 9 WEEKS



PROPOSED STUDIES ON THE METABOLISM OF VINYL CHLORIDE

R. E. Hefner and P. J. Gehring
Toxicology Research Laboratory
The Dow Chemical Company
Midland, Michigan 48640

INTRODUCTION AND RATIONALE

The rationale for conducting studies on the metabolism of vinyl chloride is elucidated in the attached report. Briefly, vinyl chloride has been shown to be tumorigenic in rats exposed via inhalation. There is suggestive evidence that vinyl chloride is not the proximate carcinogen but rather the tumorigenic effect is mediated via its metabolism. Preliminary evidence indicates that vinyl chloride is oxidized in the body sequentially to 2-chloroethanol, chloroacetaldehyde and monochloroacetic acid. Except at low exposure concentrations the overall kinetics of this reaction is expected to be "zero" order. This explains the apparent lack of dependence of the incidence of tumors on the exposure concentration in rats exposed to 250 ppm and greater of vinyl chloride. If our hypothesis concerning the association between tumor production and metabolism of vinyl chloride is correct, it is expected that at some exposure concentration yet to be elucidated no tumors would be induced.

Aside from the rationale described briefly above and more extensively in the attached report, it is highly desirable to characterize both the pharmacokinetics and metabolism of vinyl chloride in rats as well as other species including

CONFIDENTIAL

man. With acquisition of this information, the results of studies in rats and other laboratory animals can be used more reliably to assess the hazard incurred by humans exposed to vinyl chloride.

OBJECTIVES OF PLANNED WORK

1. Determine the rate of absorption, distribution and excretion of vinyl chloride in rats exposed to various concentrations via inhalation and perhaps via oral gavage.
2. Determine qualitatively and quantitatively the metabolism of vinyl chloride in rats exposed to various concentrations via inhalation. Particular attention will be given to characterizing the metabolites formed by oxidation of vinyl chloride (2-chloroethanol, chloroacetaldehyde and mono-chloroacetic acid) and the cysteine and glutathione conjugates of metabolites of vinyl chloride.
3. Determine whether significant differences in the metabolism of vinyl chloride exist in rats and dogs or monkeys. The intent is to use at least one other species to shed some light on the degree of differences in metabolism between species.
4. Determine how various agents and procedures affect the metabolism of vinyl chloride. For example, a few hours of starvation should decrease the availability of

cysteine and glutathione for conjugation with metabolites of vinyl chloride. Also, the administration of pyrazole, which inhibits alcohol dehydrogenase should block the metabolism of vinyl chloride.

5. Obtain samples of urine from individuals exposed to vinyl chloride and determine whether similar metabolites are excreted by man and animals.

METHODS

It is anticipated that there will be only one significant deviation in the methodology used in these experiments and the methodology used in other metabolism experiments. In these experiments, it is intended that vinyl chloride will be administered via inhalation. Some work has been done to synthesize an apparatus that will allow exposures to be conducted in a recycled closed system. Since CO_2 must be removed from the system and O_2 added, our one remaining problem is to develop a method to remove CO_2 without affecting the concentration of vinyl chloride. Usually, an alkaline trapping solution is used; however, it is predicted that an alkaline trapping solution will degrade vinyl chloride. Currently, we are considering the use of carbonic anhydrase to convert CO_2 and H_2O to H_2CO_3 .

In order to follow the metabolism of vinyl chloride, ^{14}C vinyl chloride will be used. Bids have been obtained

for its synthesis. If ^{14}C labelled cysteine and glutathione are needed, they will be purchased directly as they are commercially available.

Synthetic schemes have been formulated for preparation of glutathione and cysteine conjugates of potential metabolites of vinyl chloride. Some of the potential metabolites have been synthesized.

COST

It is requested that \$50,000 be appropriated for this work. This amount will cover one year "best effort" work. Since it is impossible to predict the difficulties encountered in a study of this type, an absolute commitment cannot be made to completing all the work indicated above. The work will be done at cost and any money not spent will be returned. One year after being granted this request, a complete detailed report will be issued for review. Supplemental informal reports will be issued when sufficient data are gathered to justify issuance.

CONFIDENTIAL

PROTOCOL FOR A STUDY OF THE EFFECTS OF MATERNALLY INHALED
VINYL CHLORIDE ON RAT AND RABBIT EMBRYONAL AND FETAL
DEVELOPMENT

B. A. Schwetz
Toxicology Research Laboratory
The Dow Chemical Company
Midland, Michigan 48640

INTRODUCTION

Vinyl chloride is widely used in the preparation of poly-vinyl chloride resin, as a co-polymer in Saran and other plastics, as a solvent and as a chemical intermediate. A report of the effect of single exposures of mice, rats and guinea pigs to vinyl chloride by Mastromatteo et al, (1960) indicates that this compound has very low acute toxicity. Anesthesia is the primary significant effect of acute exposure to high concentrations (75,000-100,000 ppm). Use of vinyl chloride as a surgical anesthetic has been discouraged because of its undesirable effect on the circulatory system and its high flammability. The effect of repeated exposure of laboratory animals to vinyl chloride has been reported by Torkelson et al, (1961). Groups of animals were exposed 7 hours/day, 5 days/week for up to six months to either 500, 200, 100 or 50 ppm vinyl chloride in air. Detectable changes occurred at all but the lowest concentration. Repeated exposure for six months to 200 ppm resulted in histologic changes in the centrilobular area of the livers of rabbits but not in rats, guinea pigs or dogs. At 100 ppm, only slight

CONFIDENTIAL

liver enlargement was observed. In a study reported by Viola et al, (1971), rats were exposed to 30,000 ppm vinyl chloride vapor for 4 hours daily, 5 days/week for 12 months. Findings on these rats at or before the end of 12 months exposure included severe chronic hepatitis, interstitial pneumonia, as well as tumors of the skin, lungs and bones.

Reports of studies of the potential of vinyl chloride to have a deleterious effect on the developing embryo and fetus have not been found in the literature. The study described in this protocol has been designed to determine whether or not exposure of pregnant rats and rabbits has a deleterious effect on embryonal and fetal development.

EXPERIMENTAL PROCEDURES

A) Design

In an initial study, bred rats and rabbits will be exposed to twice the maximum excursion limit of vinyl chloride (TLV = 200 ppm). Twice the maximum excursion limit of vinyl chloride is 500 ppm ($200 \text{ ppm} \times 1.25 \times 2$). Rabbits will be exposed on days 6 through 18 and rats on days 6 through 15 of gestation for 7 hours on each day. Groups of 30 rats and 15-20 rabbits will be exposed. A group of control rats and rabbits will be exposed in a chamber to filtered room air. If exposure to 500 ppm causes no evidence of maternal toxicity, embryotoxicity or teratogenicity, additional groups of rats and rabbits will be

CONFIDENTIAL

exposed to 5,000 ppm on the same days of gestation. If maternal toxicity is observed at 500 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 500 ppm, additional lower concentrations will be studied at half-fold decrements until no embryotoxicity or fetal toxicity is evident. Thus, concentrations of 250, 125 or 62.5 ppm, etc, will be studied if a significant embryotoxic effect is observed at 500 ppm in the initial experiment.

B) Exposure Procedure

Exposure of pregnant animals will be carried out in stainless steel dynamic chambers of 3.7 cubic meter volume. The chamber atmosphere will be generated by metering gaseous vinyl chloride at a known rate into a metered stream of air into the chamber. The concentration of vinyl chloride in the chamber atmosphere will be calculated from the ratio of material delivery rate and the total chamber air-flow rate. The analytical concentration will be determined by infrared spectrometry (Beckman IRL0). The wave lengths for analysis will be 10.6 and 11.2 μ . The chamber concentration will be analyzed periodically during exposure. Combustion conductivity analysis will also be used to continuously monitor the exposure concentration.

C) Animals

Adult New Zealand white rabbits and Sprague-Dawley rats

will be used. The day of natural mating will be considered day zero of pregnancy. Animals will be housed individually in wire-bottom cages and maintained on commercially available laboratory animal chow ad libitum. Animals will not have access to water or food in the inhalation chamber during the exposure period. Food consumption will be measured at 3-day intervals during gestation.

D) 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 18 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.

E) 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.

CONFIDENTIAL

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 sexed and placed in 95% ethanol, cleared and stained with Alizarin Red S for subsequent examination for skeletal anomalies.

F) Statistics

Statistical evaluation of the frequency of anomalies and resorptions among litters will be made by the Fisher Exact Probability test (Siegel, 1956). Analyses of maternal and fetal body weights and body measurements and liver weights will be made by an analysis of variance. Group means will be compared to controls using Dunnatt's test (Steel and Torrie, 1960). The level of significance chosen for all cases is $P < 0.05$. The litter is considered the experimental unit of treatment and observation.

G) Estimated Cost

The estimated cost of this study for both species is \$8,000 $\pm$ 10% for each concentration to be studied.

H) References

Mastromatteo, E., Fisher, A. M., Christie, H. and Danziger, D. Acute inhalation toxicity of vinyl chloride to laboratory animals. Amer. Ind. Hyg. Assoc. J. 21, 394, 1960.

Siegel, S. Non-parametric Statistics for the Behavioral Sciences. McGraw-Hill Book Co., Inc. New York, 1956.

Steel, R. G. D. and Torrie, H. H. Principles and Procedures of Statistics, McGraw-Hill Book Co., Inc. New York, 1960.

Torkelson, T. R., Oyen, F. and Rowe, V. K. The toxicity of vinyl chloride as determined by repeated exposure of laboratory animals. Amer. Ind. Hyg. Assoc. J. 22, 354-361, 1961.

Viola, P. L. Bigotti, A. and Caputo, A. Oncogenic response of rat skin, lungs and bones to vinyl chloride. Cancer Res. 31, 516, 1971.