PROTOCOL FOR CONTINUED STUDIES ON THE PHARMACOKINETICS/METABOLISM OF VINYL CHLORIDE MONOMER IN MAMMALS

Same is good to me
WBK 1/2/75

Prepared for the companies sponsoring
The Technical Task Group on Vinyl Chloride Research

December 16, 1974

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

I. INTRODUCTORY SUMMARY

This document was prepared in response to the need for data on the pharmacokinetic and metabolic characteristics of vinyl chloride monomer (VCM) in mammalian species. Little information is currently available on the disposition of VCM in mammals. Such data would be extremely valuable in assessing the potential health hazard of occupationally exposed individuals.

This Dow Proposal describes a series of studies to elucidate the absorption, distribution, metabolism, and elimination of VCM as a function of dose level and route of administration in laboratory animal species. Following determination of the pharmacokinetic characteristics, additional studies are proposed to define the binding of VCM to plasma/tissue, and effects of VCM on intermediary metabolism. Considering the results collectively, it may be possible to establish the carcinogenic compound and its disposition in the body.

The proposed project on the pharmacokinetics and metabolism of VCM in mammals will draw on the broad spectrum of resources of the Dow Chemical Company. Primary responsibility for the project will lie with the personnel of the Health and Environmental Research Laboratory located in Midland, Michigan. The research will be under the direction of Dr. Perry Gehring, Director of the Toxicology Research

Laboratory. Dr. P. G. Watanabe and Mr. R. E. Hefner, Jr., will be principal investigators in the program.

The Toxicology Laboratory is capable of conducting studies on acute/chronic toxicity, teratology, reproduction, inhalation toxicology, and pharmacokinetics/metabolism. This branch of the Health and Environmental Research Laboratory is staffed with 19 professional toxicologists, pathologists, and DVM's all at the doctoral level, plus 17 technicians at the masters and baccalaureate levels with the necessary hourly support personnel.

The Toxicology Laboratory has had prior experience with VCM (Hefner et al., 1974), and the present studies are an extension of the previous work.

II. TECHNICAL PROPOSAL

1. Introduction

Vinyl chloride monomer (VCM) is used extensively in the production of polyvinyl chloride and other plastics. VCM was long considered to have low toxicity when administered by inhalation, and it was once suggested as a gaseous anesthetic (Schauman 1934). Narcosis was believed to be the primary physiologic effect of high concentrations of inhaled VCM. Torkelson, et al., 1961, studied the effects of r peated inhalation exposures of VCM to laboratory animals and showed that it caused slight damage to the liver and kidney.

Male and female rats exposed to 500 ppm VCM 7 hours/day, 5 days/week, for 4.5 months showed histopathological changes in the liver and kidney. More recently, angiosarcomas, zymbal gland carcinomas, and nephroblastomas, were detected in rats exposed to concentrations of VCM ranging from 50-10,000 ppm, 4 hours/day, 5 days/week, for 12 months and subsequently maintained and observed until death (Maltoni and Lefemine, 1974). Concurrent epidemiologic evidence has suggested that angiosarcoma and portal cirrhosis of the liver is associated with industrial workers exposed to undetermined but undoubtedly high concentrations of VCM (Tabershaw, 1974). Other characteristics of the "vinyl chloride syndrome" include splenomegaly and acro-osteolysis.

Studies in our laboratory (Hefner, et al., 1974) have suggested that VCM may not be the ultimate carcinogen. The carcinogenic activity may be mediated through a metabolite of VCM formed in vivo. Preliminary evidence has indicated that VCM is metabolized by the alcohol dehydrogenase (ADH) pathway at low exposure levels (<100 ppm). Above 220 ppm ADH is saturated and other, possibly microsomal, pathways metabolize VCM. It has become increasingly apparent that over a given dose range all elimination processes do not exhibit first order kinetics. A number of drugs such as ethanol (Lundquist and Walthers, 1958) and salicylate (Levy, 1965; Levy et al., 1972) deviate from first order elimination due to saturable (rate-limiting)

enzymic biotransformation processes. It is noteworthy that while the composition of excretory products are independent of dose in first order processes, they are dependent on dose for the elimination of compounds which are capacity limited (saturable). These principles becom exceedingly important when evaluating the potential toxicologic effects of a compound such as VCM. Therefore, the rationale for conducting pharmacokinetic/metabolism studies of VCM in mammals is based on the theory that the kinetics and metabolism of VCM are dose dependent (Hefner et al., 1974). If such dose dependency exists, carcinogensis may be a result of different routes of metabolism above a certain concentration. This would suggest that a threshold concentration exists below which carcinogensis would be less than that predicted from studies employing exposure concentrations above the threshold.

In assessing the potential hazard of VCM, it becomes imperative that the test species responds in a manner similar to man. In interpretation of a preliminary teratology study, it was noted that mice were more sensitive than rats and rabbits to the acute toxicity of VCM using death as the measured parameter (Schwetz, 1974). Conversely, hamsters may be more resistant to carcinogenesis than rats (industrial Bio-Test Laboratories, 1974). Metabolic differences between species may be responsible for the species susceptibility or resistance to the toxicologic effects of VCM.

The hepatic pathology induced by VCM is extremely similar to that caused by inorganic arsenicals (Popper, 1974). The toxicity of arsenic has been associated with its ability to complex essential sulfhydryl groups required for cellular respiration (Grunert and Rohdenburg, 1960). VCM has been shown to depress non-protein sulfhydryl levels in the liver of rats exposed by inhalation (Hefner, et al., 1974). Similarities may exist between VCM and arsenic with r spect to their role in intermediary metabolism.

The proposed studies will be divided into two sections. The primary phase will include determining the absorption, distribution, metabolism, and elimination of VCM after inhalation (10, 100, 1000 ppm) and oral (1, 20, 100 mg/kg) administrations. Both routes of exposure will be evaluated separately. Inhalation exposure is primarily an industrial hazard while oral ingestion is concerned with small quantities of monomer that may migrate from packaging material into food. Since it is generally accepted that extrapolating inhalation data to oral ingestion is unsatisfactory, each route of administration will be evaluated separately.

Various inhibitors and inducers of drug metabolism may be used *in vivo*, as well as *in vitro* in the elucidation of the metabolism of VCM. Jaeger *et al*. (1974 a) reported that exposure of phenobarbital induced rats to approximately 40,000 ppm VCM caused mid-zonal hepatic changes which were not evident for non-induced animals.

The secondary phase of the study will include plasma and tissue protein binding of VCM and/or its metabolites, and effects of VCM on intermediary metabolism.

2. Obsectives

a) Determine the distribution, rate of absorption, and excretion, of VCM in rats after inhalation exposure to 10, 100, 1000 ppm for a fixed time interval.

b) Determine the distribution, rate of absorption, and excretion, of VCM in rats after oral administration of 1, 20, and 100 mg/kg.

Also, determine metabolites?

c) Determine qualitatively and quantitatively the metabolites of VCM in rats exposed to varying concentrations of VCM in rats by inhalation and oral administration.

d) Compare the metabolism of VCM in rats to at least one other species (i.e. mouse, hamster, dog, or monkey) for indications of species related differences in metabolism.

e) Determine the binding of VCM and/or its major metabolites to plasma and subcellular components.

f) Evaluate the effects of VCM on intermediary metabolism.

The technical problems inherent in the proposed study are formidible since VCM is a gas at room temperature. Therefore, the development of methodology will play a primary role in fulfilling the proposed objectives.

3. Test Material

It is anticipated that labeled 1,2-^{14}C-VCM will be used in the majority of the proposed studies. The methodology for synthesis of ^{14}C-VCM directly from 1,2-^{14}C dichloroethane (New England Nuclear, Corp., Boston, Mass.) has been previously established (Wagner and Muelder, 1974). The ^{14}C-VCM will be synthesized directly prior to use and maintained in the gaseous state to prevent polymerization and degradation. The chemical purity of the labeled VCM will be compared to authentic vinyl chloride monomer (Matheson Gas Products, Joliet, Ill., minimum purity 99.9%) by at least two different gas chromatographic

systems and infrared spectrophotometric analysis. The radio-chemical purity will be established by liquid scintillation counting of trapped eluent fractions from the gas chromatograph.

4. 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 3 days before initiation of the experiment. Since alterations in hepatic glutathione levels may change the metabolic pattern of VCM (Hefner, et al., 1974; Jaeger, et al., 1974b) experiments will be initiated at a fixed time of day and food and water will be provided ad libitum. A second species used for comparative metabolism may be either mouse, hamster, beagle dogs or Rhesus monkeys.

5. Housing

Rats used for the pharmacokinetic studies will be housed in modified Roth glass metabolism cages. The second species will also be appropriately maintained for the separate collection of urine, feces, and volatile organics in expired air. All animals will be kept in rooms in which a constant temperature, humidity, and a 12 hour light-dark cycle are maintained. Noise and activity in the animal rooms are minimal and constant

from day to day. The animal facility is approved by the American Association for Laboratory Animal Science and the American Association for Accreditation of Laboratory Animal Care.

6. Administration Procedure

Rats will be exposed to ^{14}C-VCM in a closed, recirculating 4.7 liter inhalation chamber. The plexiglas chamber can accomodate 4 rats at one time. Only the nares of the rats protrude into the chamber through a rubber membrane in order to minimize contact with fur and skin. Expired CO_2 will be continuously removed from the chamber by absorption on an ASCARITE® column in the recirculating system. Oxygen will be metered into the chamber with a syringe pump to replace the expired CO_2. Oxygen consumption for each experiment will be determined by measuring the additional oxygen metered into the system. The chamber atmosphere will be continuously monitored for VCM concentration with an inline Miran I infrared analyzer set at 10.9 μ (Wilks).

^{14}C-VCM will be bubbled through USP corn oil for oral administration. The concentration and purity of the corn oil ^{14}C-VCM dosing solution will be determined directly prior to administration by comparison to VCM gas standards using gas chromatographic analysis. The radioactivity will be determined by counting an aliquot of the dosing solution

by liquid scintillation techniques. The volume administered by oral gavage to rats will not exceed 10 ml/kg.

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

7. Pharmacokinetics/Metabolism Studies

a. Pilot Study

The purpose of this preliminary study is to determine the optimum methodology for sample collection/analysis of plasma VCM and excreted ^{14}C activity in the urine, feces, and expired air. Due to the volatility and low solubility of VCM in aqueous media, it may be necessary to use a gas chromatographic analysis for plasma VCM. It is anticipated that VCM will be expired rapidly after both oral and inhalation exposure. Schaumann (1934) reported that 82% was eliminated from the blood within 10 minutes after discontinuation of narcosis. Therefore, gas chromatographic analysis of air sampled from the metabolism cage may be the preferred technique to determine expired VCM. Other specific methods for quantitating VCM including methods for solvent trapping, cold trapping, or derivatization, will also be screened.

Five male rats will be dosed orally with 20 mg/kg ^{14}C-VCM in corn oil. Plasma samples will be taken from 2 rats while the remaining 3 will be used for a balance study of excreted ^{14}C-activity. Urine, feces, and CO_2 samples will be taken at 12 hour intervals for 72 hours. At 72 hours all the rats will be decapitated and selected tissues (plasma, brain, heart, lung, liver, spleen, stomach, duodenum, kidney, perirenal fat, muscle, skin) and the remaining carcass analyzed for ^{14}C-activity by liquid scintillation techniques (details given in the following sections). Samples will be analyzed during the course of the pilot study and methodology and sample times will be optimized.

The urine will be pooled and various analytical techniques (thin layer, ion exchange, gas liquid, liquid-liquid chromatography) screened for separation, quantitation, and identification of urinary metabolites.

The methodology developed in the pilot experiment will be used in the following pharmacokinetic studies to elucidate the absorption, distribution, metabolism, and excretion of VCM and metabolites after inhalation and oral exposure.

b. Excretion of VCM and metabolit s after inhalation exposure

Rats (4) will be exposed to a constant concentration of ^{14}C-VCM in the inhalation chamber as previously described. The exposure concentration of VCM for a given group of 4 rats will be 10 ppm, 100 ppm, or 1000 ppm with a fixed exposure time. Immediately following the exposure the animals will be placed in Roth-type glass metabolism cages. Urine and feces will be collected at appropriate intervals and maintained at dry ice temperature. Room air will be pulled through the cages at 500 ml/minute. For a specified time (about 4 hours) this air will be collected in a saran bag and analyzed for expired VCM. After the initial period of air collection, the air exiting the cage will be bubbled through a trap containing 5M ethanolamine in 2-methoxyethanol to trap expired CO_2.

Upon termination of the experiment (72 hours) the rats will be sacrificed by decapitation and the brain, heart, lung, liver, stomach, duodenum, spleen, muscle, fat, skin and plasma will be sampled. All samples of excreta collected during the course of the experiment, as well as the selected tissues and the remaining carcass will be frozen (-20°C) until analyzed.

c. Excretion of VCM and metabolites following oral administration

^{14}C-VCM will be administered (1, 20, or 100 mg/kg body weight) by oral gavage to 5 rats per group. Following administration, the rats will be placed in Roth-type glass metabolism cages and samples of excreta and tissue collected as in the previous study. Plasma levels of ^{14}C-VCM will be determined using separate groups of rats for each specified dose level.

Blood samples will be obtained from the caudal vein by incision. After centrifugation the plasma VCM concentration will be determined by directly counting the radioactivity or by gas chromatographic analysis.

Carbon 14-Assay. The concentration of ^{14}C in urine and trapped CO_2 will be determined directly by liquid scintillation counting in a Nuclear Chicago Mark II liquid scintillation spectrometer 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. The amount of ^{14}C in feces, tissues, and selected organs, will be determined by counting $^{14}CO_2$ released upon oxidation of appropriate aliquots of these samples (Harvy Biological Material Oxidizer). Overall recovery of administered ^{14}C will be determined.

d. 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. It is expected for the non-volatile metabolites that extraction and various types of preparative chromatography followed by derivitization, gas chromatography-mass spectrometry (LKB 9005 or Finnigan Quad 3000) will be the major tool utilized in metabolite identification.

Urinary metabolites will be elucidated first. An attempt will be made to identify significant levels of VCM/metabolites in various target organs (i.e. liver). This will require modification of the urinary metabolite procedure. If extensive efforts become necessary in order to identify a metabolite, further consultation may be necessary to justify the added cost of such experiments.

e. Data Analysis and Interpretation

The kinetics of elimination of VCM and metabolites from rats following inhalation and oral exposure will be determined by analyzing the time course of concentration of ^{14}C and VCM in plasma and excreted ^{14}C labeled metabolites. The rates of elimination by the various routes will be fit to the simplest pharmacokinetic model that is consistent with the experimental data. Preliminary estimates of pharmacokinetic parameters will determine if the kinetics

are linear. Further refinement of parameters will be obtained if necessary with a non-linear program on a digital computer (IBM 360/70). Data analysis of the pharmacokinetic patterns will determine differences in routes of administration of VCM in rats and dose dependent kinetics by inhalation or oral administration.

f. *In vivo* and *in vitro* inhibitors and inducers of drug metabolism

It has been shown that ethanol, pyrazole, SKF-525A, 3-amino-1,2,4,-triazole, inhibit the metabolism of VCM to varying degrees after short term inhalation exposure (Hefner, *et al.*, 1974). Characteristics of such inhibitory patterns led to the hypothesis that alcohol dehydrogenase (ADH) was primarily involved in the metabolism of VCM at low concentrations. At high VCM exposure levels, ADH is saturated and alternate pathways (possibly microsomal) may play the primary role in VCM catabolism. An alternate route of metabolism resulting in formation of such reactive compounds as chloroethylene oxide may be responsible for the untoward toxicologic activity of VCM. Effects of selected inhibitors; such as ethanol, pyrazole, SKF-525A, 3-amino-1,2,4-triazole, 1,1,1-trichloropropene 2,3-oxide, and inducers phenobarbital, 3 methyl cholanthrene, on metabolism of VCM may be tested *in vivo* and *in vitro*. It is not possible to predict before hand which inhibitors or inducers will be the most helpful; therefore, these types

of compounds will be appropriately screened. The qualitative and quantitative comparison of normal and drug altered metabolism may prove to be a promising avenue in elucidating the pathway responsible for carcinogenic activity.

In vitro systems (liver homogenates, microsomal preparations) are well suited for studying metabolic intermediates. Such systems will be screened and utilized in conjunction with the studies on drug altered metabolism of VCM. An additional advantage to in vitro drug systems is the capability of obtaining data on species differences in metabolism. This concept may be extended to using liver preparations from various species in order to predict with greater accuracy the metabolic picture in man. Sullivan et al. (1972) has reported comparative studies on the metabolism of carbaryl with in vitro hepatic preparations from man and lower animals.

8. Tissue Binding of VCM

Since VCM has been associated with portal cirrhosis and angiosarcoma in the liver of occupationally exposed workers, (Popper, 1974), it becomes relevant to study the binding properties of VCM in various tissues. Brodie et al., (1971) associated the hepatic binding of bromobenzene with

concommitant liver necrosis. Studies of the reaction of chloromethane with in vitro blood preparations demonstrat d that intracellular GSH in RBC's was depleted and may account for the anemia seen in poisoned individuals. Chloromethane was also shown to bind GSH in liver, kidney, and brain homogenates (Redford-Ellis and Gowenlock, 1974 a,b). In vitro incubation of ^{14}C-VCM with normal and enzyme denatured (heat treated) rat liver homogenates should yield information on the binding characteristics of parent VCM and its metabolites to the target organ. Subsequent differential centrifugation and separation of subcellular particles will further localize the bound fraction. It must be realized that results from centrifugation studies are tenuous unless the compound is strongly bound to a particular fraction. Ultrafiltration (Amicon ultrafiltration-diafiltration apparatus) coupled with solvent extraction techniques of the bound fraction may be used to characterize the type of binding involved. Microautoradiographic technique (Roth and Stumpf, 1969; Rogers, 1969; Feinendegen, 1969) with ^{3}H-VCM would be an additional means to confirm the inter- and intracellular association of VCM with hepatic tissue.

9. Effects of VCM on Intermediary Metabolism

Inorganic arsenicals have been reported to cause hepatic portal cirrhosis and angiosarcoma like those seen with VCM (Popper, 1974). The mechanism of toxicity for arsenic

occurs by chelation with 6,8-dithiooctanoic acid (α-lipoic acid). The resultant disruption of the tricarboxylic-acid cycle causes inhibition of pyruvate oxidation (Grunert, 1960). A postulated metabolite of VCM, chloroethylene oxide, would react readily with SH groups of lipoic acid. If VCM reacts in a manner analagous to arsenic, intermediary metabolism would be altered. Therefore, in vitro assays of hepatic pyruvate oxidase activity would be monitored in VCM treated and control rats. If such interaction between VCM and α-lipoic acid is suspected, additional studies will be conducted to confirm the proposed hypothesis.

10. Conclusion

The primary objective of the proposed study is to define the pharmacokinetic characteristics of varying levels of VCM by inhalation and oral exposure in mammals. This phase of the research will be initiated first. Since it is not possible to predict the difficulties that will be encount red in further elucidating VCM metabolism, binding properties, or carcinogenic mechanism, an absolute commitment can not be made to complete that portion of the proposed work. Therefore, the proposed studies will be done on a "best effort" cost plus fixed fee basis. It is anticipated that an equivalent of 2 man years plus necessary technicians and hourly support personnel will be required for the proposed studies.

SUMMARY OF PROPOSED PHARMACOKINETIC STUDIES IN RATS ADMINISTERED ^{14}C-VCM

Study	Route of Administration	No. Animals	Dose (Exposure) Level	Excreta Samples[a]	Plasma Samples	Tissue Samples[b]
Pilot	oral gavage	3	20 mg/kg	X		X
		2	20 mg/kg		X	X
Inhalation Excretion	Inhalation[c]	4	10 ppm	X		X
		4	100 ppm	X		X
		4	1000 ppm	X		X
Oral Dose Excretion	oral gavage	5	1 mg/kg	X		X
		5	20 mg/kg	X		X
		5	100 mg/kg	X		X
		5	1 mg/kg		X	X
		5	20 mg/kg		X	X
		5	100 mg/kg		X	X

[a] urine, feces, expired CO_2 and VCM.

[b] brain, heart, lung, liver, kidney, duodenum, stomach, spleen, fat, muscle, skin, plasma.

[c] fixed time of exposure to be determined.

REFERENCES

1) Feinendegen, L. E., Tritium-Labeled Molecules in Biology and Medicine, Academic Press, (1967).

2) Grunert, R. R. and Rohdenburg, E. L. Archives Biochem. Biophs. 86:185, (1960).

3) Hefner, R. E. Jr., Watanabe, P. G., Gehring, P. J., Ann. N. Y. Acad. Sci., in press, (1974).

4) Jaeger, R. J., Harvard University, personal communication, (1974).

5) Jaeger, R. J., Conolly, R. B., Murphy, S. D., Exp. Mol. Path. 20:187, (1974b).

6) Levy, G., J. Pharm. Sci., 54:959, (1965).

7) Levy, G., Tsuchiya, T., Amsel, L. P., Clin. Pharmacol. Therap. 13:258, (1972).

8) Lundquist, F., Wolthers, H., Acta Pharmacol. Toxicol. 14:265, (1958).

9) Maltoni, C., Lefemine, G., Lincei-Rendiconte Delle Classe di Science, Tesiche, Mathmatische Naturalo, 56, 1,(1974).

10) Popper, H., National Institute of Health, personal communication,(1974).

11) Redford-Ellis, M., Gowenlock, A. H., Acta Pharmacol. et. Toxicol. 30:36, (1971).

12) Redford-Ellis, M., Gowenlock, A. H., Acta Pharmacol. et. Toxicol., 30:49, (1971).

13) Rogers, A. W., Techniques of Autoradiography, Elsevier Publishing Co., (1969).

14) Roth, L. J., Stumpf, W. E., Autoradiography of Diffusable Substances, Academic Press, (1969).

15) Schauman, O., Medizin, U. Chemie, 2:139, (1934).

16) Schwetz, B., Leong, B.K.J., Smith, F. A., Balmer, M., Gehring, P.J., (Dow Chemical, unpublished data).

17) Sullivan, L. J., Chin, B. H., Carpenter, C. P., Toxicol. Appl. Pharmacol., 22:161, (1972).

18) Tabershaw/Cooper Associates, Report to Manufacturing Chemists Association, May 3, (1974).

19) Torkelson, T. R., Oyen, R., Rowe, V. K., Amer. Ind. Hyg. Assoc. J. 22:354, (1961).

20) Wagner, G., Muelder, W., Ann. N. Y. Acad. Sci. in press, (1974).

21) Industrial Bio-Test Laboratories (interim communications, 1974).