



THE DOW CHEMICAL COMPANY

June 9, 1977

BENNETT BUILDING
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Mr. Joseph Seawell
Manufacturing Chemist Assoc.
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Washington, DC 20009

*Copy to DAK
CEW
HBF
For your info: ~~indicated~~
No objection to Torkelson*
FK

IMPORTANT: RESPONSE MAY
BE REQUIRED

Dear Joe:

As indicated in my letter of April 5, 1977, the final portion of the metabolic studies have been completed and 10 copies of the final report dated May 28 are enclosed. In addition 10 copies of an overall summary report dated May 31 with abstracts are also enclosed as requested by the Task Group.

Since this completes the contract, MCA will under separate cover, be billed for the balance of the cost which is \$12,000. Again, to expedite the distribution of the report, I am sending copies of the report and the final summary to each of the Task Group members.

Since it is the wish of Drs. Watanabe and Gehring to present this information at an international Symposium on Industrial Toxicology at the University of Surrey in Surrey England on July 25-30, I ask that you and each of the Task Group members immediately inform me if they have reasons as to why these reports should not be discussed at that meeting. If we do not have your comments by July 15, I will inform Drs. Watanabe and Gehring that they may proceed with the presentation.

I apologize for the short time provided for your review and ask your cooperation. You may wish to call me (517)636-5197, Dr. Watanabe (517)636-1313 or Dr. Gehring (517)636-1089.

Sincerely yours,

T. R. Torkelson

T. R. Torkelson, ScD
Chairman, MCA Technical Task Group
on Vinyl Chloride Research

ebg

cc: MCA Technical Task Group
on Vinyl Chloride Research
(Metabolism Studies, Dow Chemical)
P. J. Gehring
P. G. Watanabe

Enclosures

CHEMICALS
RESEARCH

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MCA Technical Task Group on Vinyl Chloride Research
(Metabolism Studies, Dow Chemical)

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SUMMARY OF THE STUDIES CONDUCTED ON THE PHARAMCOKINETICS/
METABOLISM OF VINYL CHLORIDE IN RATS

By:

P. G. Watanabe, R. E. Hefner, Jr., J. A. Zempel,
D. G. Pegg, C. N. Park, and P. J. Gehring

May 31, 1977

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SUMMARY OF THE STUDIES CONDUCTED ON THE PHARMACOKINETICS/
METABOLISM OF VINYL CHLORIDE IN RATS

Preliminary studies on the fate of inhaled vinyl chloride (VC) indicated two important points: 1) VC was metabolized extensively in vivo; and 2) the metabolism of VC was saturated at high exposure concentrations. Since it appeared that VC was biotransformed to a reactive metabolite which was responsible for carcinogenesis, studies on the pharmacokinetics and metabolism of VC were pursued. These studies confirmed that following large doses via oral administration (100 mg/kg) or high exposure concentrations via inhalation (1000 ppm) the metabolism of VC approached saturation. Furthermore, urinary metabolites of VC were identified to be conjugates of cysteine indicating that the reactive metabolite(s) are detoxified primarily by conjugation with hepatic glutathione (GSH).

Since the conjugation of chemicals with hepatic GSH has been shown to be a saturable process, studies were conducted to determine the effect of increasing exposure to VC on the depression of GSH in the liver. Exposure for 7 hours to concentrations of VC ranging from 150-2000 ppm caused a dose-related depression of GSH. Exposure to 50 ppm caused an inconsistent depression and exposure to 10 ppm caused no

significant depression of hepatic GSH. These results along with the pharmacokinetic data suggested that reactive metabolites formed from exposure to low levels of VC (50 ppm) are detoxified readily by conjugation with GSH. However as the exposure concentration is increased detoxification will be impaired by the reduction of GSH. This will lead to an increased level of reactive metabolite at high exposure concentrations resulting in induction of cancer. The dose-related increase of hepatic angiosarcoma in rats exposed to VC concentrations ranging from 50-500 ppm (Maltoni data) correlate well with the dose-related depression of GSH.

Chemical carcinogenesis has been attributed to the reaction of electrophilic metabolites with intracellular macromolecules. The saturation of GSH dependent detoxification of VC with increasing exposure suggested that this would result in a disproportionate increase in the formation of reactive metabolite and subsequent reaction with intracellular macromolecules. Therefore, studies were conducted to assess the interaction of VC with intracellular macromolecules including nucleic acids.

The results showed that the total amount of radioactivity bound to macromolecules in the liver did not increase proportionately with the increase in the exposure concentration of

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VC. A disproportionate decrease in macromolecular binding was observed as the concentration of VC increased. The covalent binding to hepatic macromolecules was related to the amount of VC metabolized, and the amount of VC metabolized indicated evidence of saturation as the exposure concentration increased. There was no indication of a threshold for the reaction of VC metabolites with total intracellular macromolecules. However, at exposure concentrations exceeding 50 ppm the covalent binding of VC metabolites to macromolecules correlated well with the percent incidence of hepatic angiosarcoma in rats. The toxicologic significance of the covalent binding of VC to cellular components below 50 ppm was not clear. Deviation in covalent binding from the log-linear relationship at higher levels suggested that the carcinogenic response of the population may be changed at lower level exposures.

The majority of studies on the fate of VC have been conducted following single exposure. Since cancer is induced by repeated exposure a study was conducted to determine if the fate of VC in rats is altered with repeated exposure. The results indicated that repeated exposure to VC (6 hours/day, 5 days/week for 7-8 weeks) does not induce its biotransformation or alter the major routes or rates of elimination.

However, covalent binding of VC metabolites to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. This increase in binding indicated that repeated exposure augments the reaction of electrophilic metabolites of VC with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

The results of all of the studies conducted by our laboratory thus far indicate that: 1) the metabolism of VC to a reactive metabolite which is ultimately responsible for carcinogenicity is a saturable process; and 2) the detoxification of VC at exposure levels below 10 ppm is more efficient than at higher levels and this diminished ability to detoxify VC at higher levels correlates with the induction of hepatic angiosarcoma. However, it has not been possible to associate definitive evidence for a threshold in the reaction of VC with hepatic macromolecules which can subsequently be correlated with induction of cancer in rats. More and more evidence is accumulating which suggests that carcinogenesis is associated with reaction of chemicals at specific sites on DNA rather than total reaction with DNA and other macromolecules. Since our studies to date have measured reaction of VC with total intracellular macromolecules or nucleic acids, this may be an explanation why it has not been possible to observe definitive evidence for a threshold. Studying the dose-

response relationship between interaction of specific sites of DNA with VC may be a worthwhile endeavor for future studies.

The final report submitted with this summary illustrates the concept of relating the carcinogenicity of VC to the amount of VC metabolized rather than the exposure concentration. This concept is exceedingly important for chemicals such as VC where the metabolism to a toxic species is a saturable process. In such cases the increase in toxicity becomes diminishingly smaller with increasing dose or exposure because activation of the chemical follows apparent Michaelis-Menten (saturable) kinetics. Extrapolation of the VC data in this manner (assuming no threshold for carcinogenesis) indicated that an incidence of .01% hepatic angiosarcoma in rats may be expected from a daily exposure to 4.6 ppm VC. Failure to consider this concept leads to unrealistic estimates of risk.

Individual abstracts from all of the studies conducted in our laboratory on the pharmacokinetic/metabolism of VC are attached.

The secondary objective of the protocol for continued studies on the metabolism of vinyl chloride (Feb. 5, 1976) involving in vitro metabolism and identification of reactive metabolites was not completed. Problems were encountered on devising an

in vitro system to metabolize satisfactorily sufficient amounts of VC, therefore it was not possible to pursue this aspect.

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ABSTRACTS

VVC 000004605

PRELIMINARY STUDIES ON THE FATE OF INHALED VINYL CHLORIDE
MONOMER (VCM) IN RATS.

R. E. Hefner, Jr., P. G. Watanabe, and P. J. Gehring

ABSTRACT

Rats were exposed to vinyl chloride monomer gas (VCM) in a closed recirculating system. The rate at which VCM was removed from the system via metabolism was determined for rats exposed to initial concentrations of VCM ranging from 50 to 1167 ppm. Upon exposure to initial concentrations of 50 to 105 ppm, the rate of metabolism was $8.04 \pm 3.04 \times 10^{-3} \text{ min}^{-1}$. Upon exposure to initial concentrations ranging from 220 to 1167 ppm, the rate constants were less; the mean value being $2.65 \pm 1.35 \times 10^{-3} \text{ min}^{-1}$. Regardless of concentration, the disappearance followed apparent first order kinetics.

Pretreatment of rats with pyrazole prior to exposure to initial concentrations of 65 and 1234 ppm VCM caused 71 and 87% reductions in the rate of metabolism. Ethanol caused 96% and 83% reductions in the rate of VCM metabolism by rats exposed to 56 and 97 ppm VCM, respectively. Ethanol was less effective in blocking the rate of metabolism by rats exposed to high concentrations of VCM; 46 and 36% in rats exposed to 1025 and 1034 ppm VCM. In rats exposed to an initial concentration of 65 ppm VCM, SKF-525-A administration caused no inhibition of the rate of VCM metabolism; however, a 19% inhibition was seen in rats exposed to 1038 ppm.

The nonprotein sulfhydryl content of the liver (glutathione and cysteine) of rats exposed to VCM concentrations ranging from 50 to 15,000 ppm VCM is reduced without a relationship to dose. With repeated daily exposure the degree of reduction is reduced. Preliminary results indicate that the primary metabolites of VCM

react with the nonprotein sulfhydryls. Final metabolic products excreted in the urine appear to be S-(2-hydroxyethyl) cysteine and S-(2-carboxymethyl) cysteine and the respective N-acetyl derivatives. Monochloroacetic acid was identified as another potential metabolite.

Considering the results in toto, it is hypothesized that VCM is readily and extensively metabolized. Metabolism via the primary pathway, postulated to involve alcohol dehydrogenase, is swamped by exposures to concentrations exceeding 220 ppm. In rats exposed to concentrations at and exceeding this level, metabolism occurs via a secondary pathway(s), postulated to be epoxidation and/or peroxidation. These results are considered pertinent in assessing the potential hazard at low level exposures to VCM. (Environmental Health Perspectives, (1975), 11, 85-95.

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FATE OF ^{14}C -VINYL CHLORIDE AFTER SINGLE ORAL ADMINISTRATION IN RATS

P. G. Watanabe, G. R. McGowan, and P. J. Gehring

ABSTRACT

Male rats were given single oral doses of 0.05, 1, and 100 mg/kg of ^{14}C -vinyl chloride (VC), and the routes and rates of elimination of ^{14}C activity followed for 72 hours. Following 0.05 and 1 mg/kg excretion in the urine as nonvolatile metabolites and as $^{14}\text{CO}_2$ in expired air accounted for 59-68% and 9-13%, respectively of the administered dose. Only 1-2% of the dose was expired by the lungs as VC. Conversely, after 100 mg/kg, 67% of the dose was eliminated by the lungs as VC, while urinary non-volatile metabolites and $^{14}\text{CO}_2$ comprised 11 and 3%, respectively. Pulmonary elimination after 100 mg/kg showed an apparent biphasic clearance with half-times ($t_{1/2}$) of 14.4 and 40.8 min for the respective fast and slow phases. Following 0.05 and 1 mg/kg the pulmonary clearance of VC was monophasic with $t_{1/2}$ of 53.3 and 57.8 min. The percentage of the dose remaining in the carcass after 72 hr was 10, 11 and 2% for the 0.05-, 1- and 100-mg/kg doses, respectively. The urinary radioactivity was separated by high pressure liquid chromatography into three major metabolites. Two of the three major urinary metabolites have been identified as N-acetyl-S(2-hydroxyethyl)-cysteine and thiodiglycolic acid by gas chromatography-mass spectrometry. The proportions of the urinary metabolites were not influenced by the dose. The fate of VC following an oral dose between 1 and 100 mg/kg was clearly dose-dependent. Consistent with our previous studies on the fate of VC following inhalation exposure in rats, the metabolism of VC appears to be a saturable process.

(Toxicology and Applied Pharmacology, (1976), 36, 339-352).

VVC 000004608

FATE OF ^{14}C -VINYL CHLORIDE FOLLOWING INHALATION EXPOSURE IN RATS

P. G. Watanabe, G. R. McGowan, E. O. Madrid, and P. J. Gehring

ABSTRACT

Inhalation exposure to vinyl chloride (VC) has been shown to be carcinogenic in rats and man. It is important in assessing the toxicological potential of inhaled VC to understand the disposition of VC in the body. Therefore, the objective of the present study was to determine the fate of inhaled ^{14}C -VC at different exposure concentrations in rats. Male rats were exposed to 10 or 1000 ppm ^{14}C -VC for 6 hr and the routes and rates of elimination of ^{14}C -activity were followed for 72 hr after termination of exposure. Following exposure to 10 ppm of VC, urinary ^{14}C activity and expired VC comprised 68 and 2%, respectively, of the recovered radioactivity. After exposure to 1000 ppm of VC, the proportion of the radioactivity in the urine decreased while that expired as VC increased representing 56 and 12%, respectively. The pattern of pulmonary elimination of VC per se was described by similar apparent first-order kinetics following 10 or 1000 ppm with respective half-lives of 20.4 and 22.4 min. The elimination of ^{14}C activity in the urine occurred in accordance with a two-exponential equation; the half-lives for the initial phase of excretion were 4.6 and 4.1 hr following 10 and 1000 ppm, respectively. The percent of the recovered ^{14}C activity remaining in the carcass after 72 hr was 14 and 15% at the respective low and high exposure level. VC per se was not found in tissues. The urinary ^{14}C activity was separated by high pressure liquid chromatography into three major metabolites corresponding to N-acetyl-S-(2-hydroxyethyl)cysteine, thiodiglycolic acid, and a third unidentified metabolite. The proportions of the urinary metabolites were not markedly influenced by the exposure magnitude. The fate of inhaled ^{14}C -VC was shown to be dose-dependent; this is consistent with previous studies on the fate of VC following ingestion as well as inhalation.

(Toxicology and Applied Pharmacology, (1976), 37, 49-50.)

COMPARISON OF THE FATE OF VINYL CHLORIDE FOLLOWING SINGLE AND
REPEATED EXPOSURE IN RATS

P. G. Watanabe, J. A. Zempel, and P. J. Gehring

ABSTRACT

Rats were exposed by inhalation to 5000 ppm nonlabeled vinyl chloride (VC) 6 hours/day, 5 days/week for 7 weeks. On the last day of repeated exposure ^{14}C -labeled VC was used. The fate of the ^{14}C -VC was compared in the group of rats exposed repeatedly to a group exposed simultaneously for a single 6 hour period to 5000 ppm ^{14}C -VC. The routes and rates of excretion of ^{14}C -activity were the same for the two experimental groups. The activity of microsomal enzymes, as reflected by aniline hydroxylase and p-nitroanisole-O-demethylase of 9000 x g liver supernatants was essentially the same in rats exposed once, repeatedly or in nonexposed control rats. Covalent binding to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. These results indicate that repeated exposure to VC does not induce its biotransformation. However, the increase in hepatic macromolecular binding indicates that repeated exposure augments the reaction of electrophilic metabolites with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

VINYL CHLORIDE-INDUCED DEPRESSION OF HEPATIC NON-PROTEIN
SULFHYDRYL CONTENT AND EFFECTS ON BROMOSULPHALEIN (BSP)
CLEARANCE IN RATS

P. G. Watanabe, R. E. Hefner, Jr., and P. J. Gehring

ABSTRACT

Rats were exposed to atmospheres of 2000, 250, 150, 50 and 10 ppm vinyl chloride (VC) for 1-7 hr to determine the effect of VC on the hepatic non-protein sulfhydryl content. Exposure to 2000, 1000, 250 and 150 ppm VC caused a progressive depression of the hepatic non-protein sulfhydryl content. Following exposure to 50 ppm VC for 7 hr the depression was inconsistent, and no depression was observed after 10 ppm VC for 7 hr. Also, exposure to 1000 ppm VC did not alter the serum clearance of bromosulphalein (BSP).

(Toxicology, (1976), 6, 1-8)

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EFFECT OF ETHANOL ON THE FATE OF VINYL CHLORIDE IN RATS

P. G. Watanabe, J. A. Zempel and P. J. Gehring

ABSTRACT

Ethanol pretreatment is known to alter the metabolism of many chemicals. Since it has been demonstrated previously that a single dose of ethanol inhibits the biotransformation of vinyl chloride (VC), the objective of this study was to investigate the effect of repeated and acute administration of ethanol on the fate of VC in rats. One group of rats was given 3.2 g/kg ethanol 0.5 hr prior to exposure of VC and another group was maintained on drinking water providing a daily dose of 11.4 g/kg ethanol for 22 days before exposure to VC. Subsequently, these rats and an untreated control group were exposed to an atmosphere containing 100 ppm ^{14}C -VC for 6 hours.

The rats pretreated repeatedly with ethanol showed a slight reduction in the total amount of VC metabolized (6%) and the degree of binding to hepatic macromolecules (26%) when compared to the group receiving no ethanol. In contrast, those pretreated acutely with ethanol showed a marked reduction in total metabolism (72%) and hepatic macromolecular binding (81%) when compared to controls. Similarly, repeated ethanol treatment did not affect markedly the routes or rates of excretion of ^{14}C -activity. However, associated with the reduction in overall metabolism of VC the acute ethanol treated rat excreted a larger proportion of the recovered radioactivity as exposed VC than the VC exposed control (13 versus 3%). It was concluded that repeated administration of ethanol for 22 consecutive days has little effect on the fate of VC in rats. In contrast, acute administration of ethanol markedly inhibits the metabolism of VC and subsequent covalent binding to hepatic macromolecules.

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HEPATIC MACROMOLECULAR BINDING FOLLOWING EXPOSURE TO VINYL CHLORIDE

P. G. Watanabe, J. A. Zempel, D. G. Pegg and P. J. Gehring

ABSTRACT

Covalent binding of radioactivity to hepatic macromolecules in rats exposed to ^{14}C -labeled vinyl chloride (VC) was studied to determine if VC induced carcinogenesis may be related to electrophilic alkylation of macromolecules in vivo. Male Sprague-Dawley rats were exposed to 1, 10, 25, 50, 100, 250, 500, 1000 or 5000 ppm ^{14}C -VC for 6 hours. Following exposure radioactivity covalently bound to hepatic macromolecules and purified nucleic acids (RNA, DNA) were determined. The total amount of ^{14}C -VC metabolized and hepatic glutathione (GSH) content was also determined. The total amount of radioactivity bound to macromolecules in the liver did not increase proportionately with the increase in the exposure concentration of VC. A disproportionate decrease in macromolecular binding was observed as the concentration of VC increased. The covalent binding to hepatic macromolecules was related to the amount of VC metabolized. At exposures greater than 50 ppm, the amount of ^{14}C bound to macromolecules in the liver correlates with induction of hepatic angiosarcoma. There was no preferential binding of radioactivity to either DNA or RNA in the liver. Hepatic glutathione content was significantly depressed only at exposure concentrations greater than 100 ppm.

RESOLUTION OF DOSE-RESPONSE TOXICITY DATA FOR CHEMICLAS
REQUIRING METABOLIC ACTIVATION: EXAMPLE - VINYL CHLORIDE

P. J. Gehring, P. G. Watanabe, and C. N. Park

ABSTRACT

The toxicity of many chemicals result from exposure to biotransformation products formed from the chemical rather than to the chemical per se. In such cases, the incremental response may become diminishingly smaller with increasing dose or exposure because activation of the chemical to the toxic form follows apparent Michaelis-Menten rather than apparent first-order kinetics.

To illustrate this concept, rats were exposed to concentrations ranging from 1.4 to 4600 ppm vinyl chloride for 6 hours and the amount metabolized determined. The amount metabolized followed apparent Michaelis-Menten kinetics. Subsequently, it was found that the tumorigenic response to vinyl chloride was linear with respect to the amount of vinyl chloride metabolized rather than the concentration of vinyl chloride to which rats were exposed. Extrapolation of the data analyzed in this manner indicated that an incidence of 0.01% hepatic angiosarcoma may be expected from an exposure to 4.6 ppm vinyl chloride. The concepts presented herein are exceedingly important in designing and interpreting experiments for which the objective is to determine the dose-response to chemicals requiring metabolic activation to a toxic form.