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

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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

VC. A disporportionate 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 pharamcokinetic/metabolism of VC are attached.

The secondary objective of the protocol for continued studies on the metabolism of vinyl chloride (Feb. 5, 1976) involving <u>in vitro</u> 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.