EFFECTS OF ETHANOL PRETREATMENT ON MORPHINE DISTRIBUTION IN RATS. D.R. Steup and R.B. Forney. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN

Previous studies have indicated that ethanol can alter the pharmacokinetics of morphine intravenously administered to rats. This study was undertaken to determine whether this change resulted from an altered distribution of morphine to the brain, the primary site of action, or the liver, the primary site of metabolism. Naive, male Sprague-Dawley rats (193-213 g) were administered a single iv dose of morphine sulfate (2.5 mg/kg) one hour after an oral gavage of either ethanol (3 g/kg) or an equivalent volume of saline. After ten minutes, blood was collected by orbital sinus puncture, the animals were sacrificed, and the forebrain, hindbrain, and liver were removed and frozen in liquid nitrogen. Blood and tissue homogenates were then assayed for free (unmetabolized) morphine. The data indicate a statistically significant ($P<0.025$) increase in the liver to blood ratio of free morphine in the ethanol-treated (0.36 ± 0.07) vs control (0.20 ± 0.05) rats. No changes were seen in either brain morphine levels or brain to blood ratios for the same animals. The observed changes may represent either an enhanced distribution of morphine to the liver or a higher level of free morphine secondary to inhibition of hepatic metabolism.

943 ABSORPTION STUDY OF DIMAC WITH SILVER SULFADIAZINE AS COMPARED TO SILVADENE® CREAM IN THE RABBIT. Kin-Kai Hwang, Jan Battor, David Drees, Joe Lacz, Marion Laboratories, Kansas City, MO.

The purpose of this study was to determine blood levels of silver and sulfadiazine when silver sulfadiazine (AgSD) is applied to artificial wounds using SILVADENE cream or DIMAC patches as vehicles. Twenty-five young, healthy, male adult rabbits were used for this study. A 2x3 inch epidermal layer was surgically removed from the back of each rabbit. Two grams of cream (1% AgSD) or a 4x3 inch DIMAC patch (0%,1%,2% and 5% AgSD) was applied directly to the wound once a day for 3 days. Blood samples were taken and assayed for Ag and SD content at 48 and 72 hour periods. The results show that samples from 2 of 5 rabbits treated with 5% DIMAC patches have an Ag blood level of 0.1360 ± 0.018 and 0.1572 ± 0.024 ug/ml at 48 and 72 hour intervals, respectively. Values obtained from the lower doses were below the lower sensitivity of 0.100 ug of silver/ml of whole blood. Assays of plasma samples at 48 and 72 hour time points demonstrated a linear relationship between the patches and their sulfadiazine content. A maximum plasma concentration of 0.4714 ± 0.227 and 0.4212 ± 0.128 ug/ml of sulfadiazine for 5% DIMAC treated groups was observed at 48 and 72 hours; whereas, 0.1548 ± 0.075 and 0.2502 ± 0.064 ug/ml of sulfadiazine for 1% DIMAC was found. Sulfadiazine blood levels obtained from animals treated with 1% cream were similar to those treated with 1% DIMAC.

944 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR VINYLIDINE CHLORIDE. R.W. D'Souza and *M.E. Andersen, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati, OH and *AAMRL/TH, Wright Patterson AFB, OH. Sponsor: G.P. Daston.

A physiologically-based pharmacokinetic (PB-PK) model has been developed for vinylidine chloride (VDC). Simulations generated by the model offer insight into the behavior of VDC that would not be detected with compartmental modeling. Because of its low blood:air partition coefficient and saturable metabolism, the fraction of VDC dose that is metabolized or exhaled unchanged is dependent both on exposure dose and rate of absorption. Intravenous bolus studies cannot be used to elucidate the pharmacokinetics/metabolism of VDC as a major portion of VDC dose will be exhaled unchanged within a few minutes. Blood VDC half-life is not representative of metabolism, but is due to redistribution from fat to blood. A glutathione (GSH) depletion model is written as part of the PB-PK description and can be used to estimate the amount of intermediate epoxide that conjugates with GSH or binds to macromolecules. Simulations generated by the PB-PK model are consistent with literature data and have also been validated with appropriate experimental observations.

945 PHARMACOKINETIC MODELS FOR INDIRECT CARCINOGENS. R.H. Reitz, R.B. Conolly and M.E. Andersen. The Dow Chemical Company, Midland, MI; Northrop Services Inc., Dayton, OH; AAMRL/TH, Wright Patterson AFB, OH.

A number of "animal carcinogens" apparently act through indirect (nongenotoxic) mechanisms. Chemically-induced cell death followed by compensatory cellular regeneration (CCR) may be involved in the activity of these agents. To better understand this process, we have combined a two-stage carcinogenesis model (Moolgavkar and Knudson, JNCI 66:1037, [1981] with a physiologically-based pharmacokinetic (PB-PK) model for chemical disposition (Ramsey and Andersen, TAP 73:159 [1984]. The model describes the uptake, distribution, and metabolic activation of chemical(s). Depletion of a hepatic macromolecule (MM) is correlated with bioactivation, and depletion of MM increases the probability of cell death. The population of liver cells is described dynamically, with spontaneous and chemically-induced rates of cell death and CCR. Simulation indicates that the population of cells with two mutations increases disproportionately with increasing chemical exposure and time. This work suggests that PB-PK models may be useful in exploring the relationship between chemical exposure, cytotoxicity, and tumor development.

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR VINYLIDINE CHLORIDE

R. W. D'Souza and *M. E. Andersen
Miami Valley Laboratories
Procter & Gamble Company
Cincinnati, OH and *AAMRL/TH,
Wright Patterson AFB, OH
Sponsor: G. P. Daston

ABSTRACT

A physiologically-based pharmacokinetic (PB-PK) model has been developed for vinylidine chloride (VDC). Simulations generated by the model offer insight into the behavior of VDC that would not be detected with compartmental modeling. Because of its low blood:air partition coefficient and saturable metabolism, the fraction of VDC dose that is metabolized or exhaled unchanged is dependent both on exposure dose and rate of absorption. Intravenous bolus studies cannot be used to elucidate the pharmacokinetics/metabolism of VDC as a major portion of VDC dose will be exhaled unchanged within a few minutes. Blood VDC half-life is not representative of metabolism, but is due to redistribution from fat to blood. A glutathione (GSH) depletion model is written as part of the PB-PK description and can be used to estimate the amount of intermediate epoxide that conjugates with GSH or binds to macromolecules. Simulations generated by the PB-PK model are consistent with literature data and have also been validated with appropriate experimental observations.

INTRODUCTION

VDC is a large volume chemical used extensively as a solvent and as an intermediate in the manufacture of plastics. VDC has been shown to be hepatotoxic and possibly carcinogenic in laboratory animals.

Studies in the literature have demonstrated that VDC is rapidly metabolized, and it is the metabolite(s) and not parent VDC that is responsible for its toxicity and possible carcinogenicity. The metabolite(s) is detoxified by gluthathione (GSH), and therefore liver GSH status is an important determinant in VDC's toxicity.

OBJECTIVES

To develop a physiologically-based pharmacokinetic (PB-PK) model for VDC, for extrapolation of metabolism and toxicity data between different dose levels, dosing vehicles and routes-of-exposure.

METHODS

For modeling purposes a lumped compartment approach was used for distribution of VDC. Organs were grouped together based on their blood flow and ability to slowly accumulate VDC. The well-perfused compartment represented such organs as kidney and spleen and the perfused compartment included muscle and skin. The liver was considered the only metabolising organ.

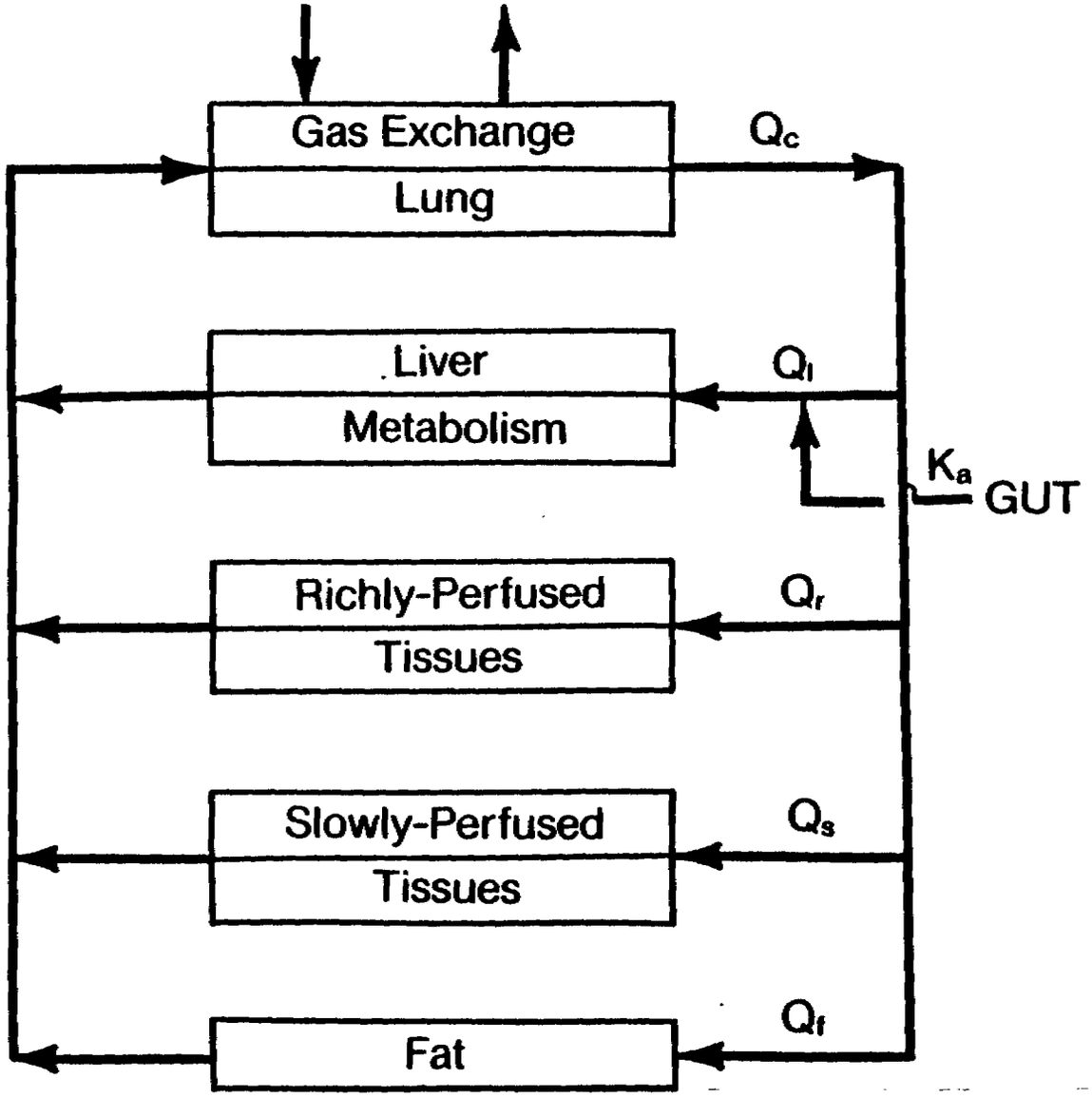
Parameters used in constructing the PB-PK model were:

Partition Coefficients were determined in vitro by a modification of the method of Sato and Nakajima (1979).

Metabolism Rate Constants were estimated in vivo by Gas Uptake Measurements as described by Gargas et al., (1986).

A Glutathione Depletion Model (D'Souza et al., 1986) was included as part of the modeling description of VDC in order to predict liver GSH status and therefore VDC toxicity.

Physiologic Parameters like blood flow and organ volume were obtained from the literature.


Gas Exchange
Lung
Q_c
Liver
Metabolism
Q_l
K_a
GUT
Richly-Perfused
Tissues
Q_r
Slowly-Perfused
Tissues
Q_s
Fat
Q_f

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Parameters Used in PB-PK Model

Partition Coefficients

$P_{lu} = 1.1$
$P_l = 1.1$
$P_r = 1.1$
$P_s = 0.6$
$P_f = 18.4$
$P_b = 5.0$

Metabolism Rate Constants

$V_{max} = 7.5$ mg $hr^{-1}kg^{-1}$
$K_m = 0.25$ mg $L^{-1}kg^{-1}$
$K_{gsm} = 0.1$ $hr^{-1}kg^{-1}$
$K_{fee} = 300$ $hr^{-1}kg^{-1}$
$K_{CO_2} = 0.2$ $hr^{-1}kg^{-1}$
$K_{mac} = 0.8$ $hr^{-1}kg^{-1}$

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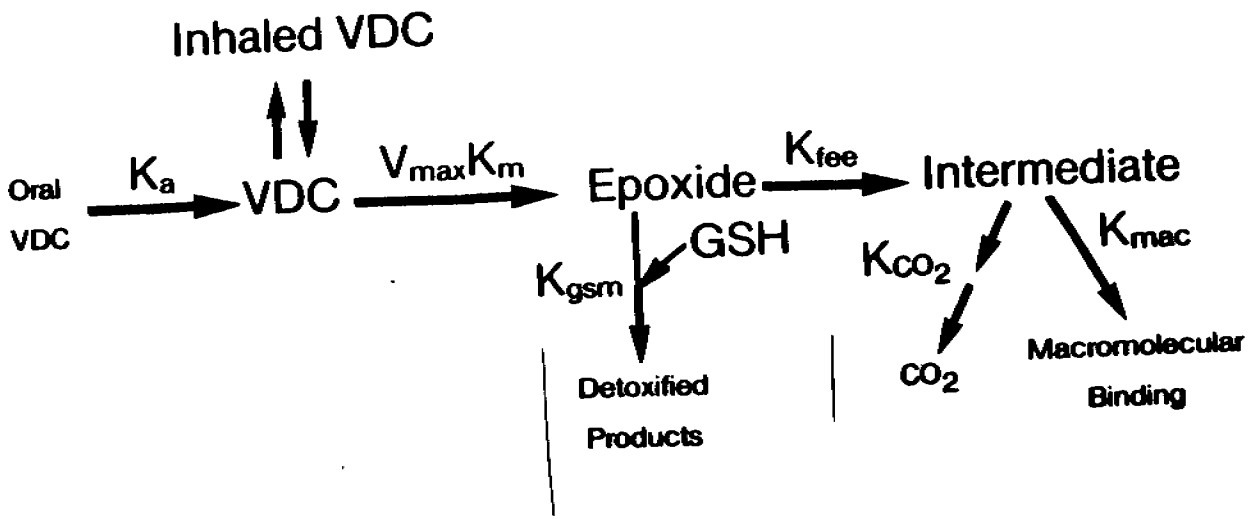
Modeling for VDC Metabolism



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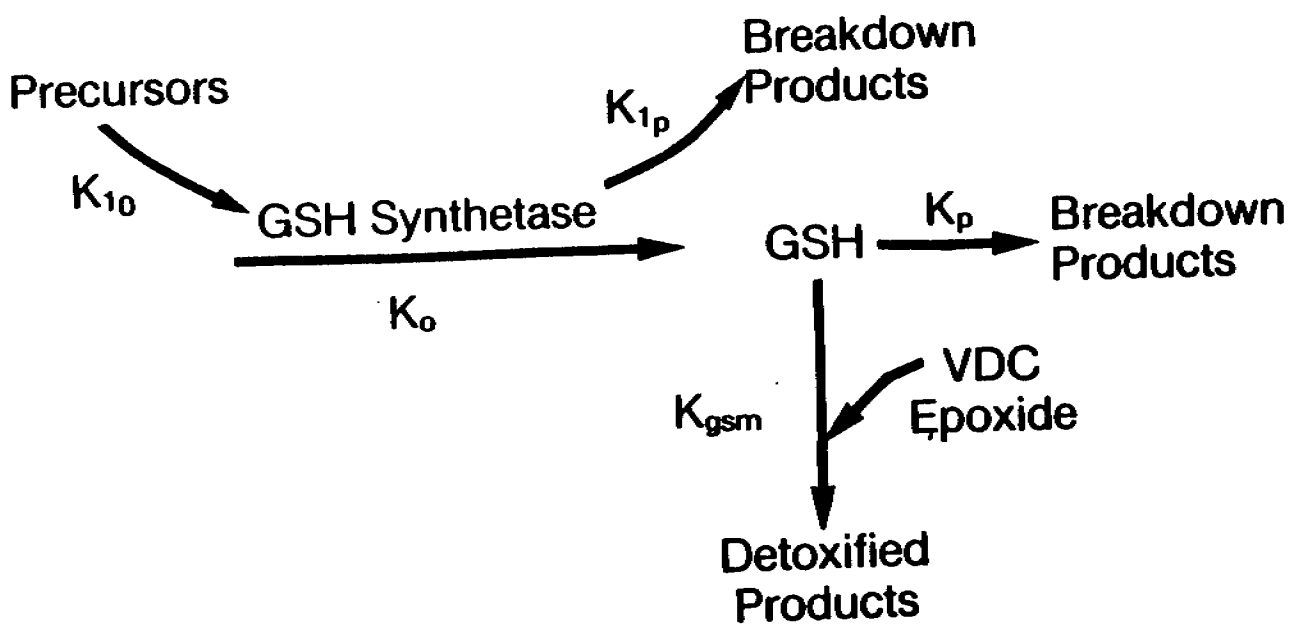
Modeling for GSH Depletion



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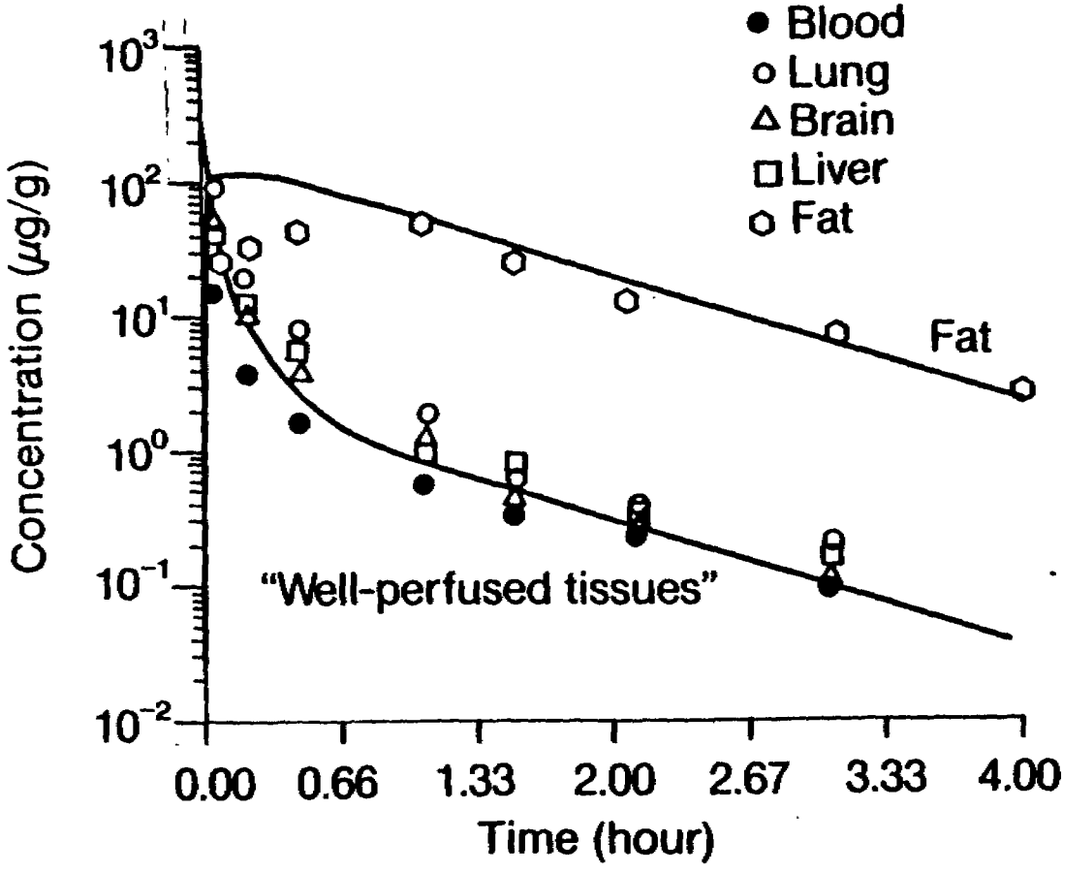
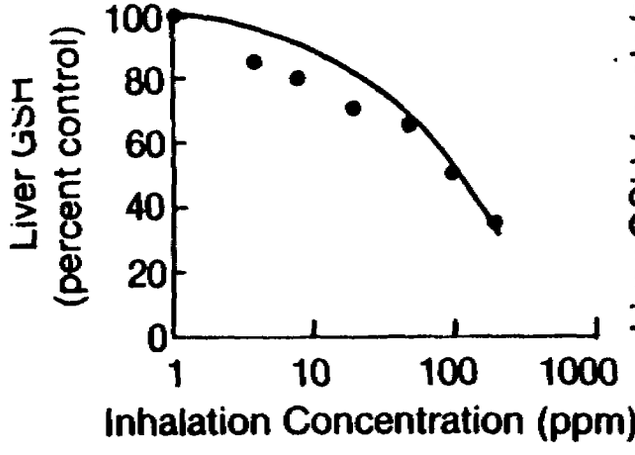
Predicted (——) and observed (symbols) VDC concentrations in different tissues of the rat following a single 50 mg/kg intravenous dose. Predicted VDC concentrations for blood, liver, lung and brain are shown as a single curve, as the tissue:blood partition coefficients for these "well-perfused" tissues is approximately one.



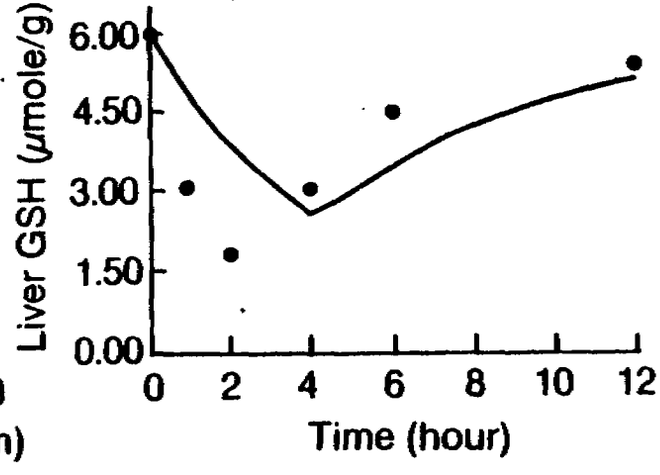
SL 061731

Predicted and observed liver glutathione concentrations following inhalation and oral exposures to VDC. Glutathione is depleted due to conjugation with VDC epoxide. Since only that amount of metabolite that is not detoxified by glutathione has toxic potential, therefore, by predicting the amount of glutathione depletion the PB-PK model can be used to predict toxic potential as a function of dose and route-of-exposure.

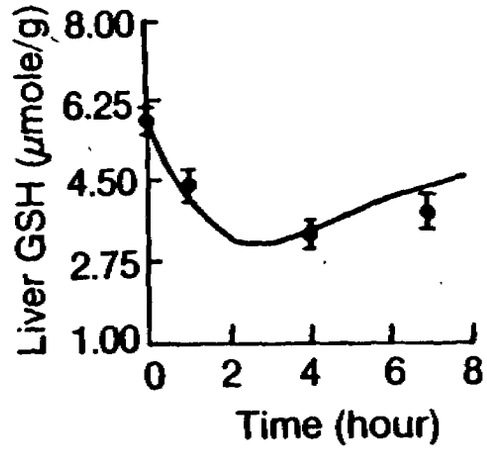
6-Hour Inhalation Exposures at Different Concentrations[1]



4-Hour Inhalation Exposure at 200 ppm Concentration[2]



200 mg/kg Oral Exposure in Corn Oil Vehicle



[1] Data Source: Mckenna et al., 1977

[2] Data Source: Reynolds et al., 1980

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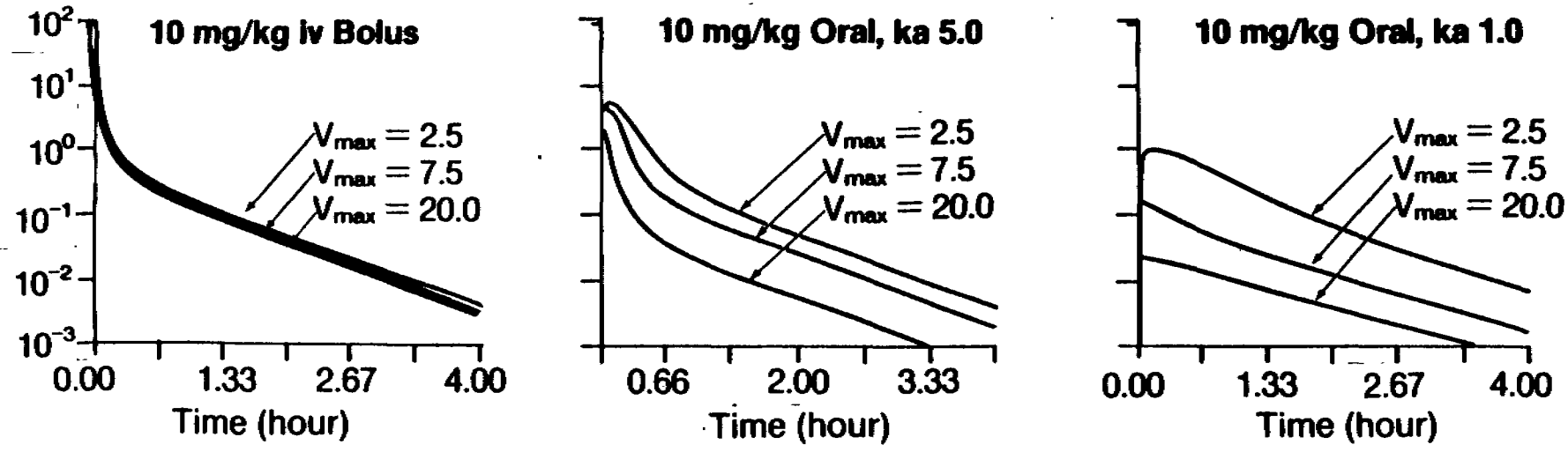
Simulations below show that because of the low blood:air partition coefficient on VDC and its saturable metabolism, the rate of VDC absorption can have profound effects on the potential for VDC to be metabolized or exhaled unchanged. At very rapid absorption Rates (intravenous bolus) almost all of the VDC dose is rapidly exhaled in the breath. Changes in metabolism, therefore, cannot be differentiated from studying blood VDC kinetics after this route of exposure. As the absorption rate is decreased more VDC is metabolized and changes in V_{max} result in changes in VDC blood kinetics. The change in blood kinetics becomes evident with an absorption rate constant (ka) of 5.0 (typically seen with water as a dosing vehicle) and is even more pronounced when ka is decreased to 1.0 (typically seen with corn oil as the dosing vehicle).



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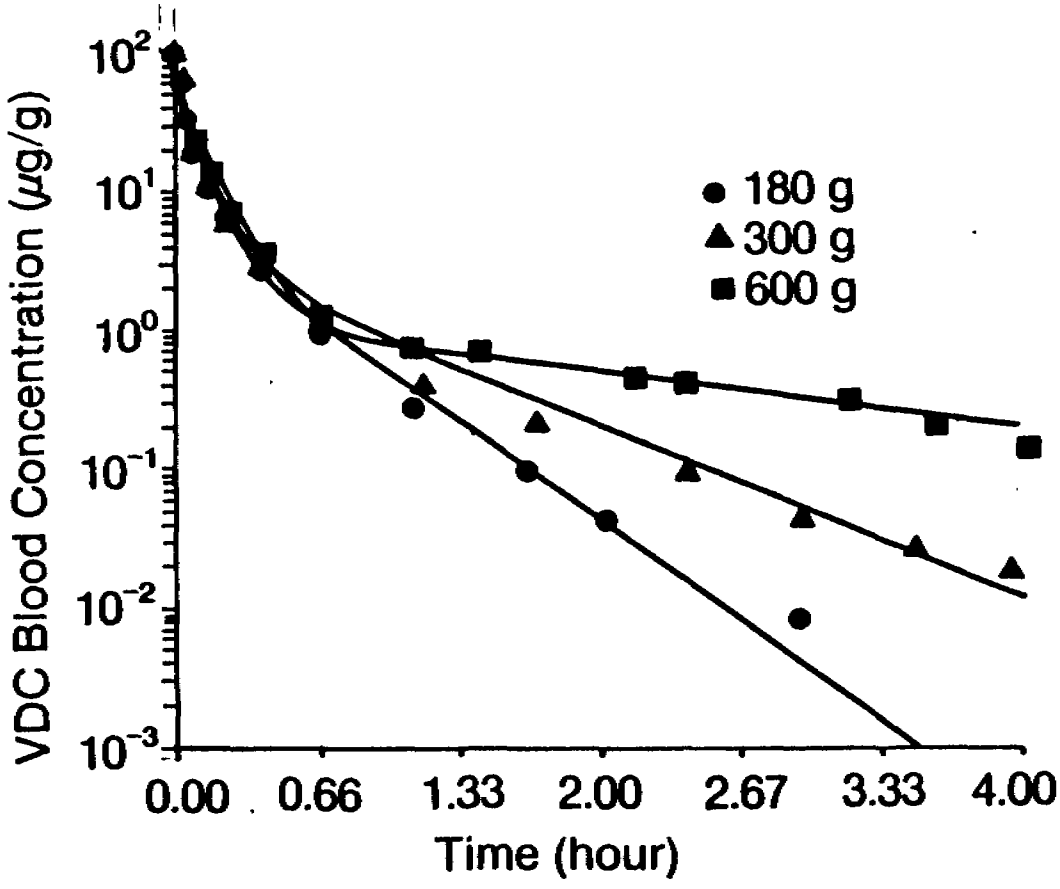
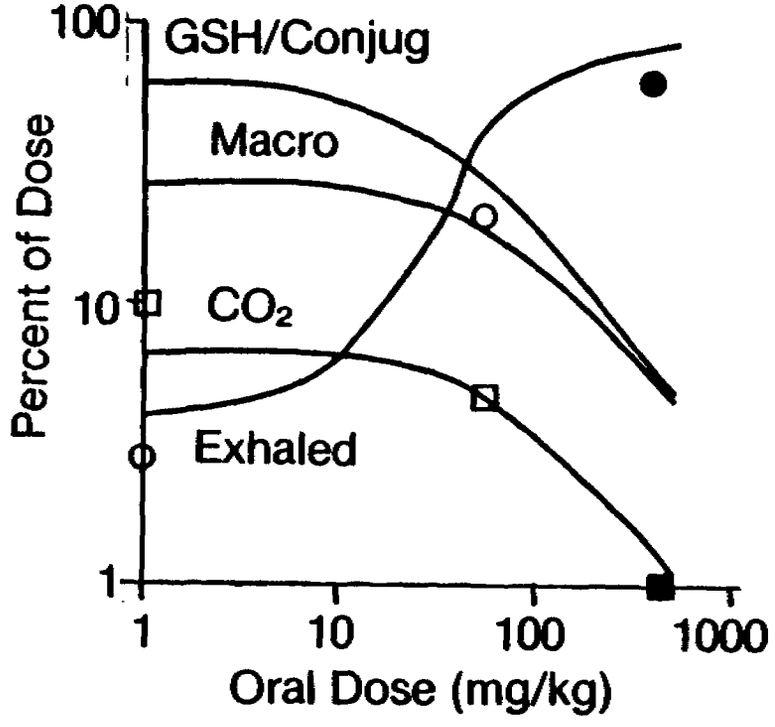
Predicted (——) and observed (symbols) blood VDC concentrations in rats of different body weights. These Sprague Dawley rats were allowed free access to food and gained weight rapidly. The approximate age of the animals were 6 weeks (180 g), 8 weeks (300 g) and 14 weeks (600 g) with fat content of 7, 12 and 20% respectively. Because of the small age difference between these groups of rats, large differences in metabolizing enzymes are not expected among these animals. The computer predictions were accomplished by changing only one parameter in th PB-PK model, that is, the amount of fat in the animal.



SL 061734

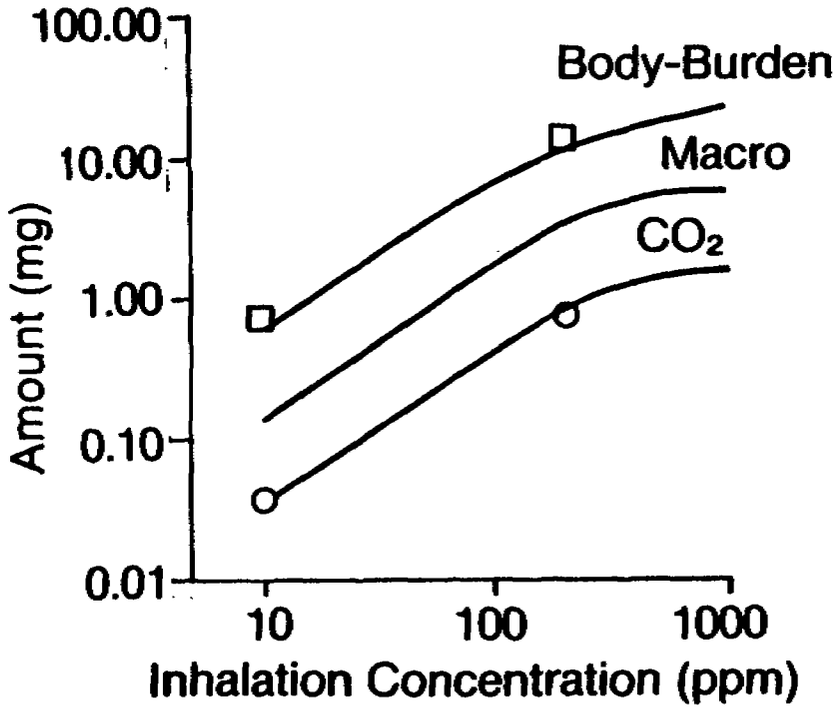
PB-PK model simulations describing the effect of oral dose level on the percent of VDC dose that will be exhaled unchanged, exhaled as CO_2, bind to macromolecules and conjugate with glutathione. Symbols shown are experimentally observed data reported in the literature for VDC (circles) and CO_2 (squares) exhalation.



○ VDC exhaled at 1 and 50 mg/kg oral dose (McKenna et al., 1977)
VDC exhaled at 350 mg/kg oral dose (Jones and Hathway, 1978)
□ CO_2 exhaled at 1 and 50 mg/kg oral dose (McKenna et al., 1977)
■ VDC exhaled at 350 mg/kg oral dose (Jones and Hathway, 1978)

SL 061735

PB-PK model simulations describing the effect of inhalation dose level (6-hour exposures) on the body-burden and the amounts of dose that will be exhaled as CO_2 or will bind to macromolecules. Data from the literature[1] for body-burden and amount of CO_2 exhaled following inhalation doses of 10 and 200 ppm are also shown.



[1] Data source: McKenna et al., 1978

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CONCLUSIONS

A PB-PK model for VDC has been developed and validated. The model demonstrates the complex interrelationship between absorption, saturable metabolism and glutathione status in determining the toxic potential of VDC.

Effects of dose level, absorption rate, and route-of-exposure on the toxic potential of VDC has been demonstrated by model simulations. Where possible, these simulations have been validated by experimental data from this laboratory or from data reported in the literature.

NOMENCLATURE

K_a	First-order rate constant for oral absorption
K_{CO_2}	First-order rate constant for CO_2 formation
K_{fee}	First-order rate constant for non-GSH route
K_{gsm}	First-order rate constant for GSH conjugation
K_m	Michaelis-Menten constant for VDC oxidation
K_{mac}	First-order rate constant for macromolecular binding potential
K_o	Zero-order GSH formation
K_p	First-order rate constant for GSH loss
K_{10}	GSH synthetase formation
K_{1P}	First-order rate constant for GSH synthetase loss
P_b	Blood:air partition coefficient
P_f	Fat:blood partition coefficient
P_l	Liver:blood partition coefficient
P_{lu}	Lung:blood partition coefficient
P_r	Richly-perfused: Blood partition coefficient
P_s	Slowly-perfused: Blood partition coefficient
Q_c	Cardiac output
Q_f	Fat blood flow
Q_l	Liver blood flow
Q_r	Richly-perfused blood flow
Q_s	Slowly-perfused blood flow
V_{max}	Maximum velocity of VDC oxidation

REFERENCES

D'Souza, R. W., Francis, W. R., and Andersen, M. E. (1986). Mathematical model for glutathione depletion and increased resynthesis following ethylene dichloride exposure. Pharmaceutical Research. 3(5), 137.

Gargas, M. L., Clewell, H. J., and Andersen, M. E. (1986). Metabolism of inhaled dihalomethanes in vivo: differentiation of kinetic constants for two different pathways. Toxicol. Appl. Pharmacol. 82, 211-223.

Jones, B. K. and Hathway, D. E. (1978). The biological fate of vinylidine chloride in the rat. Chem. Biol. Interactions. 20, 27-41.

McKenna, M. J., Watanabe, P. G., and Gehring, P. J. (1977). Pharmacokinetics of vinylidine chloride in the rat. Environ. Health Perspec. 21, 99-105.

McKenna, M. J., Zempel, J. A., Madrid, E. O., Braun, W. H., and Gehring, P. J. (1978). Metabolism and pharmacokinetic profile of vinylidine chloride in rats following oral administration. Toxicol. Appl. Pharmacol. 45, 821-835.

Reynolds, E. S., Moslen, M. T., Boor, P. J., and Jeager, R. J. (1980). 1,1-dichloroethylene hepatotoxicity. Am. J. Pathol. 101(2), 331-342.

Sato, A. and Nakajima, T. (1979). Partition Coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Brit. J. Ind. Med. 36. 231-234.