Simulations of Methylene Chloride Pharmacokinetics
Using a Physiologically Based Model

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The pharmacokinetics of the solvent methylene chloride have been studied using a physiologically based model that was developed for iv administrations to mice. Subsequently, the model was expanded to simulate pharmacokinetic behavior in mice and rats following single and repeated oral exposures. Through computer simulations, how different dosing variables, such as dose vehicle and exposure route, could influence the time course of methylene chloride concentrations at potentially critical sites of toxicity was examined. With this technique, methods of quantifying tissue exposure as it relates to externally applied doses was sought. In this way pharmacokinetic models help investigators design experiments that lead to more appropriate and reliable toxicologic assessment studies. © 1984 Academic Press, Inc.

INTRODUCTION

A number of laboratories have collected data for use in the safety assessment of the solvent methylene chloride. Some of these investigations were chronic 2-year exposures in which different dosing protocols were used. For example, the chronic bioassay of the National Toxicology Program (NTP) utilized a daily gavage regimen in which the dosing solution consisted of methylene chloride solubilized in a corn oil carrier (Menneear, 1982). In contrast, the National Coffee Association (NCA) sponsored a 2-year study in which animals were exposed to methylene chloride contained in drinking water (Serota et al., 1983, 1984). Current studies by the NTP are designed to expose animals via the inhalation route.

Pharmacokinetic data are limited, however, because they have not been routinely collected as part of safety assessment programs. A comparison of specific internal tissue exposures, which are dependent upon the externally applied exposure pattern, is not readily performed due to this frequent lack of pharmacokinetic information.

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Our studies were designed to collect the necessary experimental information in order to develop a physiological model of methylene chloride disposition. This model was used to simulate the pharmacokinetics of orally administered methylene chloride, with primary emphasis being given to describing the time course of methylene chloride concentration in the liver, at that time the suspected site of toxicity. With this model, we were able to explore the influences that a variety of dosing conditions exerted on the "internal" exposure to methylene chloride at the sensitive target tissue. Additionally, the saturable metabolism of methylene chloride to its principal metabolites CO₂ and CO was examined as a function of the dosing protocol.

This discussion focuses on the rationale used to develop the structure of the global model and the estimation of the parameter values. Comparison of the model simulations with experimental data verified the performance of the model, and simulations of pharmacokinetics as a function of hypothetical dosing situations illustrates how this technique served as a means of investigating internal distribution and metabolism when data were unavailable due to cost considerations or experimental limitations.

MODEL DEVELOPMENT

Following is a brief discussion of the model development in which pharmacokinetic data obtained from iv administrations to mice were used to generate the parameter estimates. A more detailed account of this procedure (Angelo et al., 1984a) and of the experimental methods and results (Angelo et al., 1984b, c) may be found elsewhere.

Figure 1 shows the schematic representation of the physiological model for methylene chloride. The individual tissue compartments are connected to each other by the systemic blood flow (Q), and intracompartmental transport rates are represented by double arrows. Included were compartments for venous and arterial blood pools (B), major organs (liver (L), kidney (K), lungs (LG)), the GI tract (G), and lumped carcass (C) tissue which represented a distribution volume for a large fraction of methylene chloride in the body.

The schematic was translated into mathematical form using "mass-balance" equations for each compartment, and the resulting series of nonlinear ordinary differential equations were solved numerically on a CDC Cyber 170/760 computer using the method of Gear (1971a, b).

Model parameters that represented actual physiological variables such as tissue volumes and blood flow rates were available in the literature (Bischoff et al., 1971; Adolph, 1949; Dedrick et al., 1973). Parameters unavailable in the literature or through experimentation were obtained via parameter estimation techniques using a "hybrid model" for individual compartments (King and Dedrick, 1981). These constructs served to separate, or decouple, the pharmacokinetic behavior in individual compartments from the integrated structure of the global model. In doing this, the model was described by mass-balance equations of individual compartments and empirical equations that described the afferent blood concentration as a function of time (C_i(t)). The unknown parameters in each of the decoupled systems were obtained using computer software that was developed for parameter estimations in
models described by differential equations (D'Argenio and Schumitzky, 1979), i.e., open-form models, and appropriate data relating to the pharmacokinetic variables of the system. Furthermore, this analysis incorporated data from two different iv dose levels to capture the concentration dependencies of certain kinetic events. The iv data were used in this estimation phase so that the confounding factors related to GI absorption following oral dosing could be neglected initially.

Figure 2 shows the hybrid model used for the liver compartment. In this example, the important compartments were the liver and GI tract with concentration ($C$), blood flow ($Q$), and tissue volume ($V$) specified accordingly. The GI compartment was a simplified version of the multicompartment representation shown in Fig. 1 since the amount of detail needed to describe oral absorption of methylene chloride was not required for the case of iv administration. The liver compartment included a hidden, or deep, subcompartment into which methylene chloride distributed according to an intracompartmental partition rate $P_{L}$. This feature was necessary in order to describe the disproportionate ratios between the concentrations in liver and blood that occurred over time. The kidney, lung, and carcass tissue were modeled similarly since they also displayed a disproportionality in tissue/blood concentration ratios. As a first approximation, the fraction of the tissue volume corresponding to the deep compartment ($V_{L,2}$) was assumed equal to the lipid fraction of the specific tissue since “solubilization” of lipophilic compounds in lipid components was hypothesized as a mechanism for localization of these compounds in tissues (Mintun et al., 1980). The metabolic conversion of methylene
chloride to CO₂ and CO was described by the rates \( r_{CO₂} \) and \( r_{CO} \), respectively, and was assumed to occur in the region of the liver, \( V_{L1} \), which was accessible to the blood supply. These rates were modeled by typical Michaelis–Menten saturation kinetics since the metabolism was known to be dose dependent (McKenna and Zempel, 1980).

Following the parameter estimation phase, model predictions for the pharmacokinetic variables were generated simultaneously from the entire recoupled system of equations. Each blood pool in the global model was explicitly represented by the mass-balance equations with no empirically based forcing functions used.

The ability of the global model to predict the data following iv administrations was first verified before subsequently attaching the detailed version of the GI compartment to accommodate gut absorption of methylene chloride following oral dosing. The absorption parameters were estimated from separate sequential kill experiments which quantified the rate of disappearance of methylene chloride from the two lumped portions of the GI tract into the portal blood as shown in Fig. 1. The solubility differences of methylene chloride in corn oil versus water were included in these parameters since the vehicle was found to have a substantial influence on the absorption rate of methylene chloride into the systemic blood.

Following this, pertinent parameters were “scaled,” i.e., calculated according to body weight correlations or reestimated for the rat, so that the performance of the physiological model in describing pharmacokinetic data in a larger species could be tested.

RESULTS AND DISCUSSION

Figure 3 illustrates the performance of the model in describing venous blood and liver concentrations in mice for iv dose levels of 10 and 50 mg/kg administered via a carrier solution of 25% v/v polyethylene glycol 400 in water. Note that blood levels, if analyzed without the benefit of additional tissue level data, would have misrepresented the elimination pattern that existed in the liver. Figure 4 shows an elimination pattern for the lung which was similar to that displayed by the liver.
FIG. 3. Model simulations and experimental data (mean ± SEM, N = 6) showing concentrations of methylene chloride in liver and venous blood of BrC₃F₃ mice following single iv doses of 10 (---) or 50 mg/kg (——).

FIG. 4. Model simulations and experimental data (mean ± SEM, N = 6) showing methylene chloride concentration in the lung of BrC₃F₃ mice following single iv doses of 10 (---) or 50 mg/kg (——).
The lung and liver tissues are typically "lumped" into a central compartment, which includes the blood, in familiar forms of "classical" compartmental models (Gibaldi and Perrier, 1982). However, Figs. 3 and 4 show that blood concentrations did not reflect tissue levels in the case for methylene chloride. The distribution kinetics in the well-perfused tissues did not correspond to the expected distribution phenomena since Withey (1984), using a carrier solution of pure water, reported a rapid elimination from rat tissues following iv dosing. The physiological model accommodated the individual differences in tissue distribution albeit at the expense of a larger data set and more sophisticated mathematics. However, accurate information at a specific target tissue was available only with this approach. This point is important in toxicological investigations where the time course of toxicant concentrations at a suspected site of activity is potentially powerful information when evaluating the degree of specific tissue exposure.

Performance of the model, having been verified via comparison to data following iv administrations, then was tested for its ability to predict selected aspects of the pharmacokinetics following oral exposures.

Figure 5 shows the model predictions and experimental data for liver concentration in mice during a repeated oral-dosing regimen. In this protocol, animals received a daily gavage dose of methylene chloride for fourteen consecutive days. The dose of 1000 mg/kg, using a corn oil vehicle, was the same experimental procedure as that used in the NTP’s chronic bioassay for the highest exposure level. The model performed well in describing these data over the 14-day period.

Figure 6 shows the prediction of expired gas levels, as percentages of administered dose, that resulted from the same dosing pattern. The model somewhat overpredicted the rate of expiration of unchanged methylene chloride and of the volatile metabolites CO₂ and CO although the total amounts of each species excreted following the transient phase was well described.

An oversimplified description of respiratory dynamics was most likely responsible for the discrepancies in the period following dose administration, especially with regard to the release of methylene chloride. The slow release of the metabolites may have been due to mechanisms which were not included as part of the model. These

![Graph](image)

**Fig. 5.** Concentrations of methylene chloride in liver of mice following daily oral gavage doses of methylene chloride in corn oil at 1000 mg/kg. Data are the means ± SEM of six mice.
Fig. 6. Percentages of total dose expired as $^{14}$CO$_2$, $^{14}$CO, or unchanged [$^{14}$C]methylene chloride following daily gavage doses of $^{14}$CH$_2$Cl$_2$ in corn oil at 1000 mg/kg. Data are the means ± SEM of six mice.
included the formation of carboxyhemoglobin and the involvement of CO₂ in the physiological buffer system. Additionally, the amount of metabolite available for release via respiration was assumed to equal the amount formed via hepatic metabolism at any given point in time. This assumption may not have been entirely accurate considering that "hold-up" mechanisms may have influenced the elimination pattern at intermediate steps in the sequence. In general, however, the model performed adequately in describing gas levels and the extent of metabolism considering that approximations were required to reduce the overall model to one of a computationally manageable size.

The ability of the model to predict pharmacokinetics in a larger species was tested following "scale up" (Dedrick, 1973; Boxenbaum, 1982; Tuey and Matthews, 1980) of parameter values that were not available in the literature. The evaluation of rate parameters for metabolism were not scalable between species (Schmidt-Nielsen, 1970), so the hybrid-modeling technique previously described was used to estimate these values for the rat.

Figure 7 illustrates the performance of the scaled model in predicting the liver concentration during a 14-day dosing regimen in which rats received daily gavage doses of methylene chloride in water at a level of 200 mg/kg.

The acceptable performance of the model in describing selected data following oral administrations established a basis for simulating the pharmacokinetics in exposure situations which were difficult to achieve experimentally. As an example of this, Fig. 8 shows the simulations of liver and venous blood concentrations when methylene chloride was administered in a gavage dose of 1000 mg/kg in different dosing solutions. A slower elimination phase of methylene chloride from the liver was apparent with the corn oil vehicle when compared to an aqueous-based gavage. The water vehicle led to a rapid elimination profile in both liver and venous blood. The corn oil did affect the blood elimination pattern although the difference was not as great as that exhibited by the liver. The significant features of these simulations was that a dose level of 1000 mg/kg administered in water, which was not experimentally available because of solubility limitations, was examined in terms of

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**Fig. 7.** Experimental data (mean ± SEM, N = 6) and model predictions of methylene chloride concentration in the liver of Fisher 344 rats following daily oral gavage administrations of methylene chloride in water at a dose level of 200 mg/kg.
the resulting pharmacokinetics. The behavior was compared to an equivalent dose administered in corn oil to ascertain the effects that this dosing variable had on the internal distribution of methylene chloride at the target organ.

The dose–vehicle effect on metabolism was investigated in similar fashion. Figure 9 shows the levels of expired gases as they were influenced by vehicle at the 1000-mg/kg dose level. Administration with water led to a rapid rate of methylene chloride expiration while corn oil caused a more gradual elimination pattern. The differences in the elimination patterns of CO₂ and CO between dose vehicles was not as pronounced although corn oil did lead to increased metabolite production.

Studies designed to study dose–vehicle-induced differences in pharmacokinetic patterns have not been extensive. Withey et al. (1983) studied the uptake of four different aliphatic, chlorinated hydrocarbons, including methylene chloride, after oral administration at the same dose level as corn oil or aqueous solutions. They found that corn oil significantly reduced the rate and extent of uptake in rats. Their conclusion was that the nature of the carrier vehicle substantially influenced the degree of systemic bioavailability of lipophilic compounds when they were administered at the same nominal dose level. Clearly, this implied that the “internal dosca” were not simply related to externally applied doscs.

CONCLUSIONS

Our simulation studies confirmed that corn oil cannot only affect uptake, but can have an influence on the distribution of methylene chloride to target tissues and on metabolism profiles. Any biological effects, therefore, may not simply be related to exposure at a given nominal dose level, but may include effects induced by the experimental protocol, such as the choice of dosing vehicle.

Pharmacokinetic models, such as the one discussed herein, can offer valuable insight into anticipated “internal” distribution behavior, or can aid in the interpretation of results that are generated by different experimental procedures from the various laboratories involved with data collection.

We should continue to take advantage of the information that pharmacokinetic models can provide as a powerful supplementary research tool. These models can
Fig. 9. Model simulations showing the effect of dose-vehicle on respiratory elimination of methylene chloride and its metabolites CO₂ and CO in mice following a single gavage dose of 1000 mg/kg in a corn oil vehicle (---) and a hypothetical dose in a water-based vehicle (——).

be used to increase our understanding of how the body reacts toward absorbed xenobiotic compounds, and they may help us design experiments which may be more appropriate for addressing pertinent questions in safety assessment studies.

REFERENCES


