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The Uptake and Disposition of 1,1-Dichloroethylene in Rats during Inhalation Exposure[1,2]

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The Uptake and Disposition of 1,1-Dichloroethylene in Rats during Inhalation Exposure. DALLAS, C. E., WEIR, F. W., FELDMAN, S., PUTCHA, L., AND BRUCKNER, J. V. (1983). *Toxicol Appl Pharmacol* 68, 140–151. The uptake, disposition, and respiratory elimination of 1,1-dichloroethylene during inhalation exposure were evaluated to gain insight into the pharmacodynamics of this halocarbon. Anesthetized male Sprague–Dawley rats inhaled 25, 75, 150, or 300 ppm for 3 hr from an aluminized Mylar bag through a miniaturized one-way breathing valve into the trachea. Periodic air samples were taken immediately adjacent to the nares to monitor separate inhaled air and exhaled breath streams concurrently with blood samples from a cannulated femoral vein and analyzed for 1,1-DCE content by gas chromatography. 1,1-DCE was absorbed very rapidly, in that substantial levels were present in the venous blood at the first sampling time (i.e., 2 min). Percentage systemic uptake decreased over time after initiation of exposure until equilibrium was established. Percentage uptake after reaching equilibrium varied inversely with the exposure concentration. 1,1-DCE venous whole-blood levels in animals exposed to 25, 75, and 150 ppm 1,1-DCE increased rapidly to near steady state values in approximately 45 min, as did concentrations of 1,1-DCE in the exhaled breath and alveolar air. Calculation of the amount of 1,1-DCE taken up by the body over the course of the 3-hr exposures revealed that cumulative uptake of the inhaled chemical was statistically linear for the 25-, 75-, and 150-ppm exposures. Accumulation plots for 300-ppm exposed animals, however, were best fitted to a cubic curve. Although trends toward the establishment of equilibrium were initially seen in the 300-ppm exposed animals, levels of 1,1-DCE in the blood and breath rose progressively during the latter hour of the 3-hr exposure period. Thus, despite increased exhalation of 1,1-DCE, these animals could not prevent systemic accumulation of the chemical.

1,1-Dichloroethylene (1,1-DCE), also known as vinylidene chloride, is used widely as a monomer in the manufacture of a variety of plastic materials. In addition to the potential for exposure in the occupational environment, 1,1-DCE and other halocarbons are contaminants of drinking water supplies (U.S. EPA, 1975, 1977).

The pharmacokinetics and metabolic fate of 1,1-DCE are of considerable interest, in that both toxicity (Andersen and Jenkins, 1977; Andersen *et al.*, 1979a) and carcinogenicity (Maltoni *et al.*, 1977) are highly dose dependent. Maltoni *et al.* (1977), for example, saw no increase over controls in tumor incidence in male mice exposed to 10 ppm of 1,1-DCE vapor, but a significant incidence in 25-ppm exposed animals. McKenna *et al.* (1978a) reported that rats subjected to 10 ppm 1,1-DCE for 6 hr were capable of metabolizing about 98% of the systemically absorbed dose to nonvola tabolites. Rats s metabolized a s sorbed dose an as unchanged 1 posed to 200 pp creased hepatic as well as liver and Jenkins (1 and Jones and ported similar terms for 1,1-DC to the hypothe animal's metal detoxification

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dose to nonvolatile, apparently nontoxic metabolites. Rats subjected to 200 ppm for 6 hr metabolized a smaller percentage of their absorbed dose and exhaled a larger percentage as unchanged 1,1-DCE. Nevertheless, rats exposed to 200 ppm [^{14}C]1,1-DCE exhibited increased hepatic covalent binding of radiolabel as well as liver and kidney damage. Andersen and Jenkins (1977), McKenna *et al.* (1978b), and Jones and Hathway (1978) have also reported similar dose-dependent excretion patterns for 1,1-DCE, lending additional support to the hypothesis that high doses exceed the animal's metabolic capacity and associated detoxification mechanisms.

Investigators have recently dealt with the question of saturable 1,1-DCE metabolism by employing indirect measures of 1,1-DCE uptake during inhalation exposures. Filser and Bolt (1979) and Andersen *et al.* (1979b) exposed rats to 1,1-DCE in closed inhalation systems and periodically monitored the chamber concentration of 1,1-DCE as a measure of systemic uptake. The resulting time course of uptake curves were used to derive a number of kinetic constants for 1,1-DCE metabolism. Although this method of kinetic modeling has provided useful estimates of saturation kinetics, there have been no direct pharmacokinetic studies of 1,1-DCE during ongoing exposures. Direct studies have been limited to the elimination phase of exposure. Hepatotoxicity, however, has been seen as early as 2 hr after fasted rats begin to inhale 200 ppm 1,1-DCE (Reynolds *et al.*, 1975). It would appear that saturation of detoxification pathways may occur during ongoing exposure, leading to altered disposition and toxicity. Therefore, one of the primary objectives of the current investigation was to define the exposure conditions under which saturable metabolism can occur, by direct, repetitive measurement of 1,1-DCE in exhaled breath and blood samples.

Since attention in pharmacokinetic studies of 1,1-DCE has been focused primarily on elimination of the chemical, information on its uptake and disposition is lacking. One might predict that uptake and disposition of 1,1-DCE will be dependent upon the compound's basic physical/chemical properties and upon physiological processes which govern the kinetics of anesthetic gases (Goldstein *et al.*, 1974). The onset of cytotoxicity and alterations in metabolism during the course of exposures to 1,1-DCE, however, would be anticipated to alter the disposition and ultimately the uptake of the chemical. Thus, a second major objective of the project was to characterize the uptake and disposition of inhaled 1,1-DCE and to determine whether the pharmacodynamics of the halocarbon changes during ongoing exposures.

METHODS

Animals. Adult, male Sprague–Dawley rats were obtained from Timco Breeding Laboratories (Houston, Tex.). The animals were maintained on a constant reverse light–dark cycle, with light from 2200 to 1000 hr and darkness from 1000 to 2200 hr. They were housed in solid-bottom polypropylene cages fitted with vented stainless-steel lids. Tap water and Ralston Purina Formulab chow were provided *ad libitum*. The rats were used after a 14-day acclimation period, at which time their body weight ranged from 350 to 400 g. Solvent exposures were initiated at the same time for each animal (at 0100 hr or 3 hr after the beginning of the dark cycle).

Test material. 1,1-Dichloroethylene (1,1-DCE) (98% minimum purity) was obtained from Matheson, Coleman and Bell (Norwood, Ohio.)

Animal preparation. The rats were anesthetized by im injection of 0.2 ml of a mixture consisting of ketamine HCl:acepromazine maleate:xylazine HCl (3:2:1, v:v:v). A tracheostomy was then performed and a miniaturized one-way breathing valve inserted directly into the trachea. Since such valves were not commercially available, a valve was designed and fabricated for the study. It consisted of a T-shaped polypropylene tube (6.4 × 0.95 cm i.d.), with a rubber septum set on either side of a centrally positioned 1-cm side arm. A tapered plastic pipet was attached to the side arm, and the pipet tip inserted into the trachea. The septa were positioned so that as the animal inhaled, the inlet septum opened, and the outlet septum closed. Upon exhalation the flow of air forced the outlet septum open and the inlet septum closed. Thereby, separate airways were established for the inhaled and exhaled breath streams. The septa were made of flexible latex rubber, in order that they offer minimal resistance to air flow. The valve's dead space (0.75 ml) was comparable to that of

rats of the weight range utilized. The femoral vein of each animal was cannulated so that samples of venous blood could be obtained for 1,1-DCE analysis. A temperature probe was inserted into the rectum so that core temperature of the anesthetized animal could be monitored and regulated by means of variable temperature heating pad. The cardiac rate and blood pressure were monitored in four subjects over a 3-hr period and found to be stable. The mean values ± SD were: cardiac rate, 390 ± 20; systolic pressure, 115 ± 8.7; and diastolic pressure, 90 ± 6.4. These values are similar to those measured in harness-trained (Hunt and Kimeldorf, 1960) and in unanesthetized, unrestrained (Popovic and Kent, 1964) rats.

Inhalation system. A known concentration of 1,1-DCE was generated in a 70-liter aluminized Mylar bag, which was connected in series by Teflon tubing with a pneumotachograph, the breathing valve, and an empty 70-liter Mylar bag. The empty bag served as a reservoir to accumulate exhaled gas. Thereby a closed system was created to prevent release of the agent into the laboratory. Samples of the exhaled breath and inhaled air were taken with a gas-tight syringe from sampling ports immediately adjacent to the breathing valve. The animal and the area between the two Mylar bags, including all valve and tubing connections, were located under a ventilation hood with an exhaust fan to further minimize potential contamination of the laboratory.

Experimental procedure. After the breathing valve was inserted into its trachea, the animal was allowed to adjust to breathing through the valve for approximately 10 min (without solvent exposure) until consistent, stable breathing patterns were established. For individual exposures, a concentration of 25, 75, 150, or 300 ppm of 1,1-DCE was generated in the influent Mylar bag. Once breathing was stabilized, the Mylar bag with the 1,1-DCE was connected by Teflon tubing to the valve and thus to the animal for a 3-hr exposure period. Blood samples were periodically withdrawn from the femoral vein over the course of the exposure. Samples of exhaled breath were taken at 2- to 8-min intervals during this period, while samples of the inhaled air were monitored at 30-min intervals. After 3 hr, the bag containing 1,1-DCE was disconnected, and the animal was allowed to breathe room air for 30 min while postexposure blood samples and exhaled breath samples were taken.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored. This procedure was accomplished by measuring the airflow, created by respiration of the animal, with a size No. 0 Fleish No. 7318 pneumotachograph connected to a Narco No. 7172 strain gauge coupler of a Narco physiograph. The magnitude of each respiration was recorded, and each respiratory rate signal was accumulated for a 1-min interval with a Narco GPA-10 integrator unit. The resulting integrated signal was then continuously recorded as volume of respiration per minute ($\dot{V}_E$) with a Narco No. 7173 transducer in a second channel of the physiograph. Mean values ± SD over the course of the 3-hr exposures for the 16 rats utilized in the study were: $\dot{V}_E$ = 221 ± 28 ml/min; respirations per minute (f) = 145 ± 21/min; tidal volume (V_T) = 1.5 ± 0.2 ml. These values are quite similar to values for unanesthetized rats (Guyton, 1947) and for tracheostomized rats anesthetized with thiamylal (Johanson and Pierce, 1971).

While exhaled breath levels of 1,1-DCE were measured from air samples taken at the sampling port, alveolar levels of the solvent could be calculated from the exhaled breath data and the respiratory rate and $\dot{V}_E$. A certain amount of the inhaled gas containing 1,1-DCE resides in the dead space of the valve and is exhaled without participating in alveolar gas exchange. By correcting for the contribution of this dead space volume to the exhaled breath 1,1-DCE concentration, an approximation of the alveolar levels of 1,1-DCE could be calculated (Kelman, 1982).

Since the $\dot{V}_E$ and the alveolar concentration of 1,1-DCE at each sampling point were known, subtraction of the concentration of 1,1-DCE in the alveolar air from the inhaled concentration yielded an approximation of the quantity of compound which remained in the body for each sampling period. Thereby it was possible to monitor percentage systemic uptake and to determine cumulative uptake of 1,1-DCE over the course of the 3-hr exposure.

Analysis of 1,1-DCE in air and blood. The concentrations of 1,1-DCE in the inhaled and exhaled air were measured with a Perkin–Elmer 3920 gas chromatograph equipped with a flame ionization detector. Air samples of 1.0 ml were taken from the sampling ports with a gas-tight syringe and injected directly onto a 6-ft × 1/8-in. stainless-steel column packed with 10% FFAP on 80/100 mesh Chromasorb. Operating temperatures were: 160°C, injection port; 220°C, detector; 109°C, isothermal column operation. Chromatographic grade nitrogen was used as the carrier gas with a flow of 30 ml/min. Maximum sensitivity of this detector was maintained with settings of 16 psi for hydrogen and 60 psi for the zero air.

1,1-DCE levels in the blood were measured by gas chromatographic headspace analysis. A 0.1-ml blood specimen was withdrawn through a stopcock, connected to an indwelling femoral cannula, into a 1-ml tuberculin syringe, and injected into chilled 1-ml air-tight vials. An equal volume (0.1 ml) of heparinized saline was then injected back through the stopcock to replace the lost volume. The blood samples were kept under refrigeration overnight, then placed into a 55°C water bath to volatilize the 1,1-DCE (boiling point, 32°C). After allowing 30 min for equilibration of the 1,1-DCE between the blood and head space in the vial, a 0.1-ml sample of the head space was taken with a gas-tight syringe and injected directly onto a 6-ft × 1/8-in. stainless steel column packed with Durapak in a Hewlett–Packard gas chromatograph equipped with a ^{63}Ni electron capture detector. Operating temperatures were: 150°C, injection port; 250°C, detector; 80°C, column oven. The flow rate for nitrogen, the

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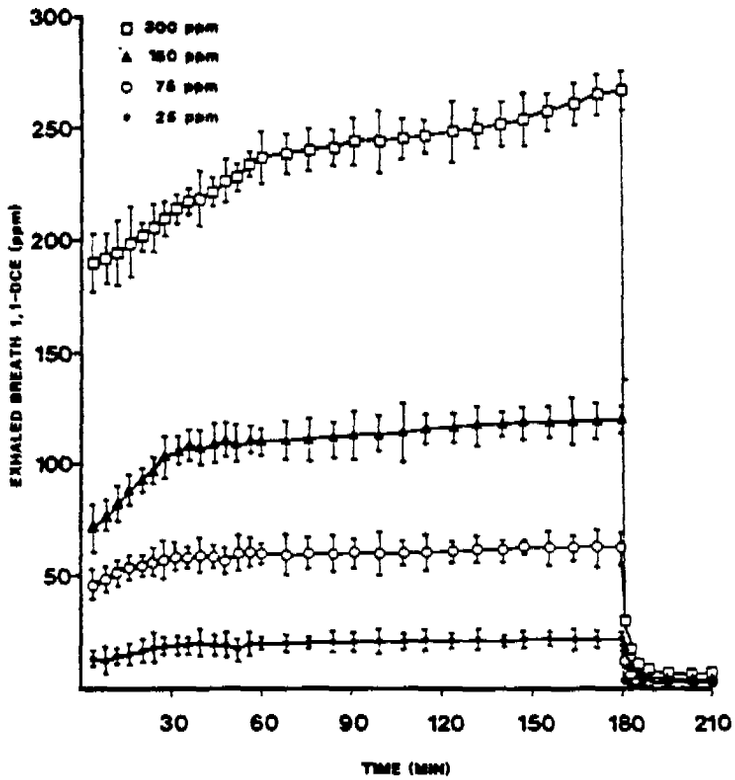

FIG. 1. Levels of 1,1-DCE in the exhaled breath during and after inhalation exposures. Rats were exposed to 25, 75, 150, or 300 ppm 1,1-DCE for 3 hr. Concentrations of 1,1-DCE in the exhaled breath were measured at 2- to 8-min intervals during the exposure and for 30 min thereafter. Brackets encase $\bar{x} \pm SD$ for groups of four animals.

carrier gas, was 25 to 28 ml/min. The concentration of 1,1-DCE in the blood was determined from a standard curve generated daily from blood samples containing a known concentration of 1,1-DCE.

Statistical analyses of accumulation plots. Data were analyzed by regression analysis and tested for linearity by examination of plots for individual animals and for groups of animals. By examination of individual plots, it was possible to determine the duration of exposure and the cumulative uptake of 1,1-DCE beyond which uptake was no longer linear. The appropriateness of a linear model to describe cumulative uptake at each exposure concentration was determined by testing for lack of fit of an assumed straight-line model (Kleinbaum and Kupper, 1978) of the pooled data of test animals at each exposure level. By use of the residual sum of squares from the appropriate regression analysis, pure error estimates from repeated observations for each concentration could be determined. The level of significance was set at $p < 0.05$.

RESULTS

The 1,1-DCE exposure concentrations in the inhalation airway immediately adjacent to the breathing valve were determined at 30-min intervals during the exposures. The actual values, following each desired concentration, represent the mean ± SE for 24 determinations per exposure level: 25 ppm, 23.7 ± 1.4 ppm; 75 ppm, 77.1 ± 1.8 ppm; 150 ppm, 145.9 ± 0.6 ppm; and 300 ppm, 295.1 ± 3.8 ppm.

Concentrations of 1,1-DCE in the exhaled breath over the course of the 3-hr exposure are shown in Fig. 1. The exhaled breath concentrations during the initial min of exposure

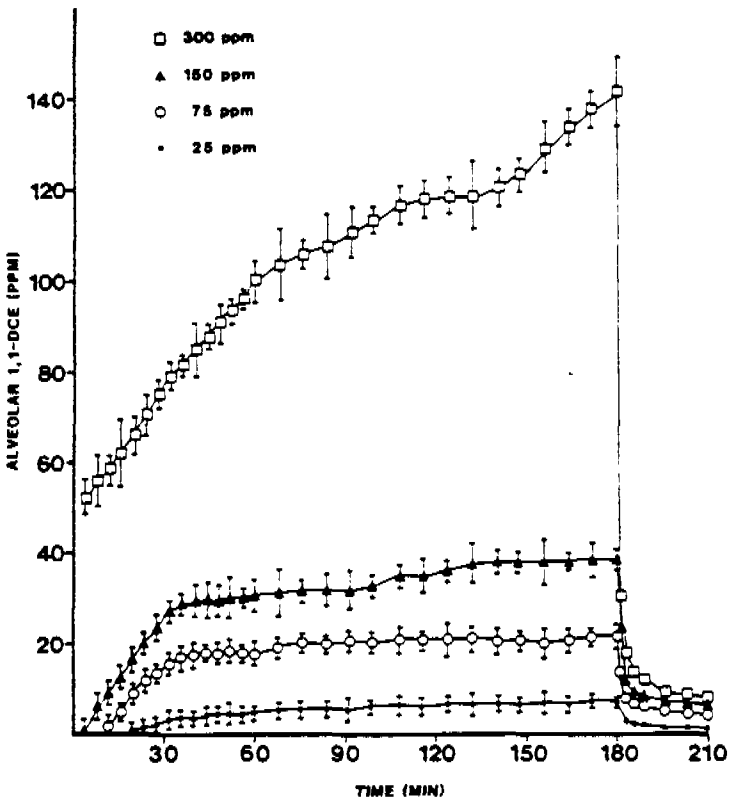

FIG 2. Alveolar concentrations of 1,1-DCE during and after inhalation exposures. Rats were exposed to 25, 75, 150, or 300 ppm 1,1-DCE for 3 hr. Concentrations of 1,1-DCE in the alveolar air were determined at 2- to 8-min intervals during the exposure and for 30 min thereafter. Brackets encase $\bar{x} \pm SD$ for groups of four animals.

were about 60% of the inhaled concentrations. Levels of 1,1-DCE in the exhaled breath of animals inhaling 25, 75, and 150 ppm rose until near steady-state levels were achieved within about 30 min. The curves for these exposure groups were asymptotic, in that they continued to gradually increase throughout the 3-hr exposure period. Although exhaled breath levels in rats inhaling 300 ppm suggested that a near-equilibrium state was established between hours 1 and 2, the levels increased slightly during the last hour of the experiment. Upon cessation of 1,1-DCE inhalation, the concentration of 1,1-DCE fell very rapidly in the expired air of all exposure groups.

Conversion of the exhaled breath data to alveolar concentrations yielded more definitive information on the nature of 1,1-DCE uptake. As can be seen in Fig 2, the pattern of 1,1-DCE uptake was distinctly dose dependent. At the 25-ppm exposure level, apparently all the chemical presented to the animal was retained during the first 20 min. Thereafter, the chemical appeared in the alveolar air in increasing amounts until near steady state was reached after about 45 min. Animals presented with 75 ppm 1,1-DCE appeared to

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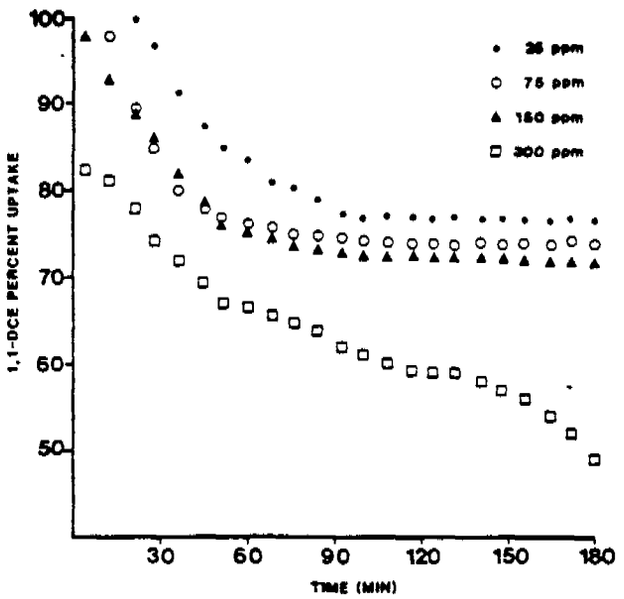

FIG. 3 Percentage systemic uptake of 1,1-DCE during inhalation exposures. Rats were exposed to 25, 75, 150, or 300 ppm 1,1-DCE for 3 hr. Percentage uptake was determined at 8-min intervals. Each point represents the mean percentage uptake in four animals per group. Standard deviation brackets are omitted for sake of clarity.

absorb all the chemical for the first 10 min, while the 150-ppm exposed animals initially retained all but a fraction of the inhaled 1,1-DCE. The time course of equilibration at these two intermediate dose levels very closely resembled that of the 25-ppm exposed animals. At equilibrium the alveolar concentrations in these groups were proportional to the exposure level. This was not true for the 300-ppm exposed animals. Alveolar concentrations in this group rose to a disproportionately high level over the 3-hr period. Though there was a trend toward establishment of equilibrium during the second hour of exposure, a substantial increase in alveolar concentrations in these animals occurred during the final 30 to 45 min. The alveolar concentrations fell very rapidly in all exposure groups upon discontinuation of 1,1-DCE inhalation.

Percentage systemic uptake of 1,1-DCE was both dose and time dependent (Fig. 3). Percentage uptake in each exposure group decreased markedly over the first 45 min of exposure and approached near steady state during the following hour. Uptake varied inversely with exposure level, though the magnitude of difference among the 25-, 75-, and 150-ppm groups was quite modest. Average percentage uptake (±SD) in these groups over the last 90 min of the 3-hr exposure was as follows: 77.2 ± 0.9%, 25 ppm; 75.7 ± 1.1%, 75 ppm; and 72.5 ± 1.9%, 150 ppm. Approximately 60% of inhaled 1,1-DCE was retained by the 300-ppm exposed animals at 2 hr. Thereafter, pulmonary retention in the 300-ppm exposed animals decreased progressively.

Plots of the cumulative uptake of 1,1-DCE during the 3-hr exposures revealed the rates and patterns of accumulation to be concentration dependent. Figure 4 is a plot of mean cumulative uptake over time for each of the four exposure groups. Cumulative uptake appeared to be relatively linear during the 3-hr

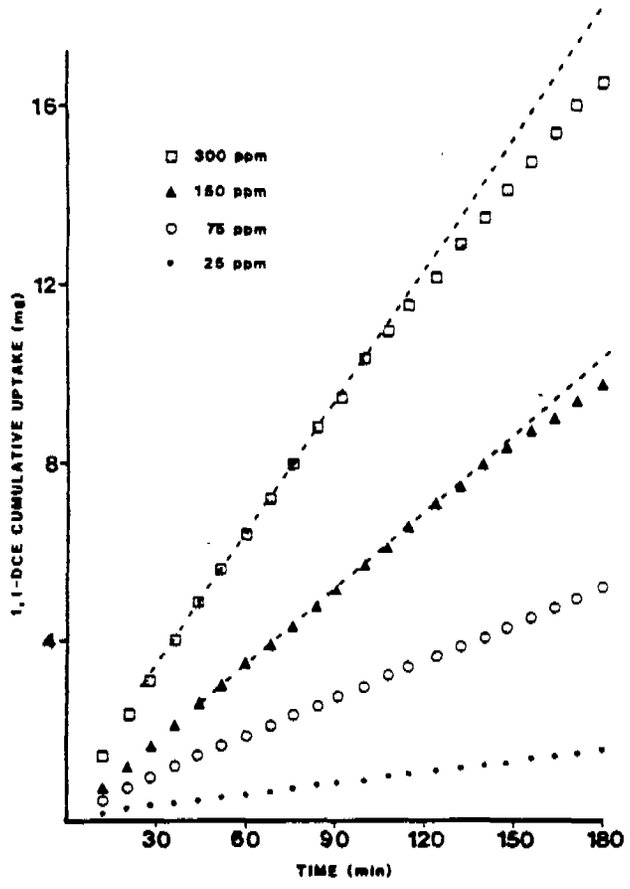

FIG. 4. Cumulative uptake of 1,1-DCE during inhalation exposures. Rats were exposed to 25, 75, 150, or 300 ppm 1,1-DCE for 3 hr. The quantity of 1,1-DCE retained during successive 8-min intervals was calculated on the basis of the minute volume and difference between inhaled and alveolar 1,1-DCE concentrations. Each point represents the mean value of four animals per group. Standard deviation brackets are omitted for sake of clarity. The pooled data of the test animals at each exposure level were tested for lack of fit to an assumed straight-line model of uptake.

exposure in the exposure range of 25 to 150 ppm. Statistical analysis revealed no significant deviation from linearity at the 25- and 75-ppm exposure levels. There appeared to be some departure from linearity, however, during the final hour in the 150-ppm exposed animals. Examination of the cumulative data plots of each of the 4 animals in the group revealed that the rats responded somewhat differently to 150 ppm 1,1-DCE. Plots of two of the animals displayed departure from linearity after about 2 hr of exposure, one rat, after 2 1/2 hr and one rat not at all (data not shown). Despite this finding, testing of the pooled data (for the 3 hr of 150-ppm exposure) for lack of fit of a straight-line model showed there to be no significant deviation from the linear model. In contrast, the pooled 300-ppm data did show deviation from the linear model. Indeed, regression analysis determined that a

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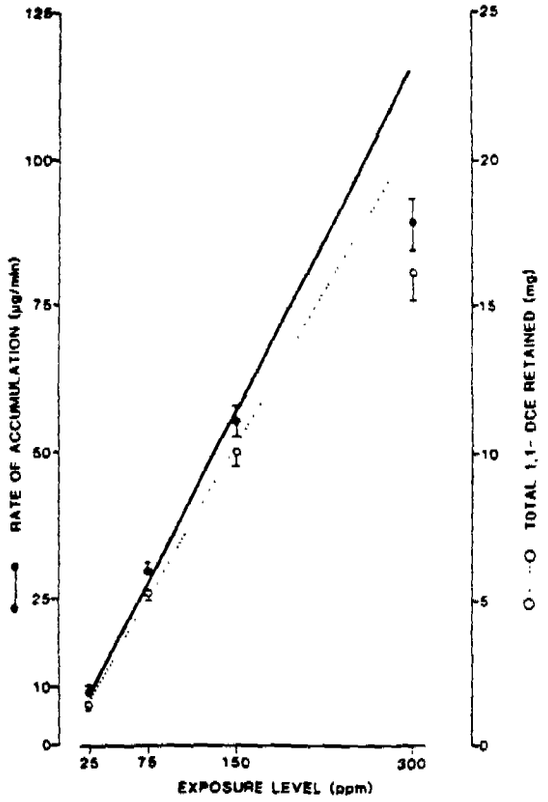

FIG. 5. Average rate of accumulation and total quantity of 1,1-DCE retained over 3 hr of inhalation exposure to 25, 75, 150, or 300 ppm 1,1-DCE. The straight lines represent the magnitude of each parameter that is directly proportional to dose. The 25-ppm values are used as the point of origin for derivation of the lines. Brackets encase $\bar{x} \pm SD$ for groups of four animals.

cubic model was the best description of cumulative uptake in the 300-ppm exposed animals.

The rate of accumulation and total quantity of 1,1-DCE retained during the 3 hr of exposure are plotted against exposure level in Fig. 5. It can be seen that both accumulation rate and total 1,1-DCE retained were directly proportional to inhaled concentration in the exposure range 25 to 150 ppm. This was not true for the 300-ppm exposed animals, in that the values for both parameters were lower than would be predicted on the basis of proportionality to dose.

The time course of venous whole-blood levels of 1,1-DCE in the animals is shown in Fig. 6. Substantial concentrations of 1,1-DCE were found in the blood of all animals at the first sampling time (2 min). Concentrations of 1,1-DCE in the blood of animals inhaling 25, 75, and 150 ppm increased rapidly, reaching near steady-state levels after about 45 min. The mean (±SD) blood levels during the final 30 min of exposure were 180 ± 28 µg/ml for 25 ppm, 348 ± 45 µg/ml for 75 ppm, and 472 ± 36 µg/ml for 150 ppm. Near steady state or equilibrium was never established in the 300-ppm exposed animals. The concentra-

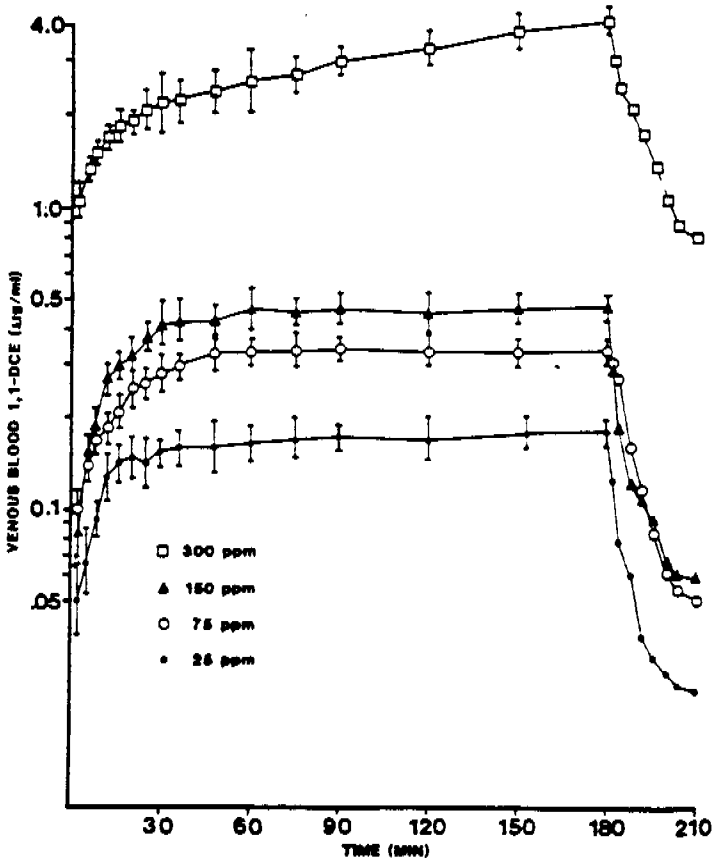

FIG. 6. Venous blood levels of 1,1-DCE during and after inhalation exposures. Rats were exposed to 25, 75, 150, or 300 ppm 1,1-DCE for 3 hr. Concentrations of 1,1-DCE in whole venous blood samples were measured at 2- to 30-min intervals during the exposures and for 30 min thereafter. Brackets encase $\bar{x} \pm SD$ for groups of four animals.

tion of 1,1-DCE in the blood of these animals progressively increased over the course of the 3-hr exposure, reaching levels eightfold higher than levels found in the 150-ppm exposed animals. Although 1,1-DCE blood levels in all groups fell rapidly upon cessation of 1,1-DCE inhalation, the decreases were not so dramatic as the falls in exhaled breath and alveolar concentrations.

DISCUSSION

Pharmacokinetic data based upon direct measurements of 1,1-DCE and other halocarbons during exposures are limited. Most pharmacokinetic studies of halocarbons pertain to metabolism and elimination of the chemicals. Investigators typically utilize ^{14}C-labeled halocarbons because of technical difficulties in quantifying the volatile parent compounds. In such instances, the investigator is largely unable to distinguish among parent compound, metabolite(s), and ^{14}C which enters the metabolite pool. Astrand (1975) summarizes the results of several studies in which levels of parent halocarbons were monitored in human subjects' alveolar air and

blood during and after exposures. These studies provide useful information on uptake and elimination characteristics of halocarbons, though emphasis is not placed on pharmacokinetic interpretation of the data. Such human studies are limited by ethical considerations, in that one normally cannot administer levels high enough to produce saturation kinetics nor study toxic halocarbons. An appropriate animal model, such as that employed in the current investigation, can be used to advantage under these circumstances.

In the absence of knowledge of the uptake and disposition of inhaled 1,1-DCE, one might speculate that the chemical will behave as a water-insoluble anesthetic gas. Findings in the current investigation support this hypothesis. Since 1,1-DCE is a small, uncharged, lipid-soluble molecule, it should be absorbed readily across membranes in the pulmonary capillary bed into the systemic circulation. Indeed, substantial levels of 1,1-DCE were found in the venous circulation at the initial sampling point (2 min after initiation of exposures). As with other gaseous anesthetics (Goldstein *et al.*, 1974), the net transfer rate of 1,1-DCE from alveoli to blood should initially be very rapid but become progressively slower as the agent accumulates in the blood and tissues. This pattern was reflected by the time course of systemic uptake of 1,1-DCE, where percentage uptake of 25, 75, and 150 ppm 1,1-DCE decreased over time from virtually 100% to a relatively constant steady-state level. Approach to equilibrium in blood and in alveolar air was rapid, indicative of the relatively low solubility of 1,1-DCE in blood and poor perfusion of adipose tissue, the major site of deposition of lipophilic compounds. The limited solubility of 1,1-DCE in blood was also demonstrated by the dose dependency of the alveolar concentrations at the initiation of the exposures. Equilibration of the blood was asymptotic. The delay in attainment of equilibrium can be attributed largely to the metabolism of 1,1-DCE and its deposition in tissues. Deposition in lipid depots very likely played a more important role during deequilibration. Lipids should release 1,1-DCE more slowly than other tissues, thereby prolonging the presence of the compound in the bloodstream. Although we did not monitor blood levels long enough after the exposures to accurately define the terminal elimination phase, we did observe that 1,1-DCE was present in venous blood longer than in exhaled air.

Findings in the current study indicate that the rat's capacity to metabolize and eliminate 1,1-DCE can be exceeded during the course of inhalation of 150 ppm or more of 1,1-DCE. Inhalation of 300 ppm definitely exceeds the animal's capability to assimilate the chemical. Despite evidence after the initial hour of inhalation of 300 ppm of the approach of steady-state conditions, 1,1-DCE levels in the blood and alveolar air increased substantially during the final hour of the 3-hr exposures. Significant departures from linearity during the final hour in the plots of cumulative uptake of 1,1-DCE also indicate saturation of the animals' elimination mechanisms at 300 ppm. Although deviation from linearity in three of four 150-ppm exposed animals is not sufficient to be statistically significant, this finding nevertheless suggests that inhalation of 150 ppm 1,1-DCE for some 2 hr can also result in saturation kinetics in the rat. This level is in agreement with the 150-ppm saturation point Filser and Bolt (1979) determined by indirect measurement of 1,1-DCE uptake in rats. Thus, it would be expected that higher vapor concentrations of 1,1-DCE exceed the rat's capacity to metabolize 1,1-DCE. Filser and Bolt (1979) note that 1,1-DCE exhibits a high V_{max} relative to most other halocarbons they tested. On the basis of a similarly designed study, Andersen *et al.* (1979b) calculated the chamber V_{max} for 1,1-DCE to be 132 ppm/kg/hr, corresponding to an *in vivo* V_{max} of 15.87 mg 1,1-DCE metabolized/kg/hr. The values are in general agreement with findings in the present study. In plots of cumulative uptake in the 150-ppm exposed animals, we observed departure from linearity after about

2 hr, at which time there was an average total uptake of 1,1-DCE of approximately 23 mg/kg. As would be anticipated, departure from linearity occurred sooner (after 1 to 1 1/2 hr) in animals inhaling 300 ppm. Average total uptake of 1,1-DCE at this time was approximately 26 mg/kg. Thus, metabolic saturation seemed to occur during the course of an exposure when there had been systemic uptake of a finite, limited quantity of 1,1-DCE.

It is possible that factors other than metabolic saturation may play a role in the saturation kinetics observed here. Inhalation of 200 ppm 1,1-DCE for 1 to 2 hr caused a marked reduction in glutathione (GSH) levels in the livers of fasted rats (Reynolds *et al.*, 1980). Although GSH is required in the major 1,1-DCE metabolic pathways (Jones and Hathway, 1978; Reichert *et al.*, 1979), it is not entirely clear whether a decrease in hepatic GSH, per se, will result in a net reduction in 1,1-DCE metabolism. Although overnight fasting is known to deplete hepatic GSH in rats (Jaeger *et al.*, 1973), Andersen *et al.* (1979b) reported no difference in the 1,1-DCE V_{max} between fed and fasted rats. Despite a 60% decrease in GSH content, isolated perfused livers from fasted rats are able to metabolize 1,1-DCE as rapidly as livers from fed rats (Reichert *et al.*, 1978). These investigators did see a modest decrease in 1,1-DCE metabolism in the *in vitro* system after a diethylmaleate pretreatment which reduced the GSH content by almost 90%. Andersen *et al.* (1980) also find diethylmaleate and certain other agents which substantially depress hepatic GSH to inhibit 1,1-DCE metabolism by rats. Although these findings suggest that very marked GSH depletion may result in some decrease in net 1,1-DCE metabolism, it appears more likely that saturation of the primary "activating" enzyme system is responsible for the altered kinetics seen in high-dose animals in the present study.

Hepatotoxicity may also play a role in the saturation kinetics phenomenon. Evidence of cytotoxicity has been seen in livers of fasted rats after 2 hr of inhalation of 200 ppm 1,1-DCE (Reynolds *et al.*, 1975, 1980). Frank hemorrhagic centrilobular necrosis was present after 4 hr. Although fasted rats were not utilized in the current study, the reverse light–dark cycle animals we used were exposed to 1,1-DCE during the period when liver GSH levels are lowest (Jaeger *et al.*, 1973). Therefore, it was quite possible that some degree of hepatic injury occurred in the high-dose animals during the latter part of the 3-hr exposures. Damage of the endoplasmic reticulum of hepatocytes would diminish their capacity to metabolize 1,1-DCE. Although Andersen *et al.* (1979a) saw evidence of this condition within 30 min in immature rats subjected to 200 ppm 1,1-DCE, relatively little effect was seen in mature rats. Reynolds *et al.* (1980) observed only slight effects on hepatic microsomal parameters in mature rats after 2 hr of inhalation of 200 ppm 1,1-DCE. Not until hepatotoxicity was fully manifest 6 hr after the beginning of the 4-hr, 200-ppm exposure were significant decreases in the microsomal parameters manifest. Thus, it would appear that 1,1-DCE-induced deactivation of functional components of the mixed-function oxidase system may not contribute significantly within the time frame of the current study to the observed saturation kinetics.

Findings in the present study indicate that the rat has a limited capacity to metabolize and eliminate 1,1-DCE. Since 1,1-DCE is volatile and relatively insoluble in blood, increased amounts of the chemical can be readily eliminated via the lungs upon the onset of metabolic saturation. However, despite increased exhalation of 1,1-DCE, we observed a progressive rise in blood levels in the 300-ppm exposed animals. The high-dose animals may be at risk from toxic injury, while those inhaling the lower concentrations may tolerate their exposures. Indeed, other investigators (Jaeger *et al.*, 1974; Andersen *et al.*, 1979a) have reported the threshold for hepatotoxicity in fasted rats to be in this same exposure range. Although the dose–response

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curves for hepatotoxicity (Andersen *et al.*, 1979a) and for lethality (Andersen and Jenkins, 1977) are initially quite steep, they soon plateau. The absence of a further increase in toxicity despite increased exposure is attributed to saturation of the metabolic activation of 1,1-DCE to toxic metabolites. In the present study metabolic saturation occurred during the course of exposure to 150 ppm or more of 1,1-DCE. It remains to be determined whether these animals were thereby afforded protection from toxicity, or whether they will experience injury of the liver, kidney, or other organs.

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