Arch Toxicol (1986) 59: 127–134

Archives of
Toxicology

Inhalation pharmacokinetics of 1,2-dichloroethane after different dietary pretreatments of male Sprague-Dawley rats

Orisa J. Igwe[1], Shane S. Que Hee[1], and William D. Wagner[2]

[1] Kettering Laboratory, Department of Environmental Health, University of Cincinnati, Mail Location #56, Cinciannati, Ohio 45267, USA

[2] Document Development Branch, Division of Standards Development and Technology Transfer, NIOSH, 4676 Columbia Parkway, Cincinnati, OH 45226, USA

Abstract. The effect of the pretreatment of male Sprague-Dawley rats with phenobarbital (PB), butylated hydroxyanisole (BHA) and disulfiram (DSF) on the inhalation kinetics of 1,2-dichloroethane [ethylene dichloride (EDC)] was studied by the gas uptake method. A closed recirculating system was constructed and characterized. The rate curves in all the pretreatment regimens showed saturable dependence on EDC concentration. These saturable dependencies (Michalis-Menten) appeared to be associated with enzymatic metabolism. In general, a two-compartment, steady-state pharmacokinetic model described the uptake data. Data were transformed by Hanes plots to calculate the inhalational K_m, the ambient EDC concentration at which uptake proceeded at half maximum rate, and V_{max}, the maximum rate of uptake (i.e., maximum rate of metabolism). Although PB and BHA pretreatments did not affect the K_m of EDC, PB pretreatment increased the V_{max} while DSF pretreatment decreased both the K_m and V_{max}.

Key words: Inhalation pharmacokinetics – Butylated hydroxyanisole – Tetraethylthiuram disulfide (Disulfiram) – 1,2-Dichloroethane – Glutathione S-transferases – Cytochrome P_{450}

Introdcution

1,2-Dichloroethane [also called ethylene dichloride (EDC)] is a volatile organic used as an intermediate in the manufacture of vinyl chloride and as a grain fumigant (Gold 1980). EDC has a vapor pressure of 80 torr at 25 °C and a high blood/air partition coefficient compared to other chloroethanes [19.5 in man at 37 °C (Sato and Nakajima 1979a)]. Thus, inhalation is a primary means of exposure and the lung is the major route of entry into the body. Hence, the basic kinetic parameters of pulmonary absorption of EDC and its equilibration in the body may be valid for both low and high EDC concentrations.

A convenient access to pharmacokinetics of volatile xenobiotics is provided by gas uptake studies (Filser and Bolt 1979; Andersen et al. 1980). Here, the equilibration of vapor with the whole animal and its metabolic elimination can be inferred from the decline of the vapor within a closed exposure system. Gas uptake is an indirect measure of chemical metabolism. It is assumed that the uptake which shows saturation kinetics represents uptake driven by metabolic processes.

Non-linear pharmacokinetic behavior is common (Filser and Bolt 1979; Young et al. 1978; Andersen et al. 1980), especially for chemicals that require metabolic activation (Gehring et al. 1978). The toxic effects of many inhaled compounds are related to the formation of reactive intermediates through metabolism (Van Bladeren et al. 1981). Dose-response relationships for such chemicals are related to the amount of parent chemical metabolized during exposure and not to total exposure concentration. Therefore, determination of the rate of metabolism in vivo as a function of exposure concentration may be necessary, as for halothane (Gargas and Andersen 1979), methylene dichloride (McKenna et al. 1979), vinyl chloride (Gehring et al. 1978), acetone (Hallier et al. 1981), halogenated ethylenes (Filser and Bolt 1979) and others. In general, a three-compartment model has been used to describe the transfer of gaseous material in a biological system (Sato et al. 1974; Fiserova-Bergerova et al. 1980). However, Andersen et al. (1980) and Filser and Bolt (1981) utilized simplified compartmental analyses of gas uptake to obtain rate equations for disappearance of volatile chemicals from the gas phase.

Two principal pathways, involving microsomal cytochrome P_{450} and cytosolic glutathione S-transferase (GST) with reduced glutathione (GSH), respectively, have been proposed for the metabolism of EDC (Guengerich et al. 1980; Anders and Livesey 1980). The purpose of the present study was to compare the kinetic constants of EDC uptake by rats pretreated with dietary disulfiram (DSF), butylated hydroxyanisole (BHA) and phenobarbital (PB), agents which have differential effects on microsomal and/or cytosolic pathways of metabolism. DSF induces GST, increases tissue GSH and decreases cytochrome P_{450} (Hunter and Neal 1975; Sparnins et al. 1982; Cha et al. 1982; Igwe et al. 1984). BHA, on the other hand, induces GST and increases tissue GSH, but has no effect on cytochrome P_{450} (Benson et al. 1979; Cha and Bueding 1979; Cha et al. 1982) while PB induces both GST and cytochrome P_{450} (Baars et al. 1978; Kaplowitz et al. 1975; Guengerich and Watanabe 1979). The relative contributions of cytochrome P_{450} and cytosolic GST to the metabolism of EDC have been quantified in vitro (Guengerich et al.

Offprint requests to: Orisa J. Igwe. The Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68105, USA

1980). The pharmacokinetics of EDC have been studied in Osborne Mendel and male Sprague-Dawley CD-COBS rats (Reitz et al. 1982; Spreafico et al. 1980). The saturability (non-linear kinetics) of the metabolizing enzyme(s) involved was postulated to explain the kinetic data. The gas-uptake methodology affords the opportunity to study EDC metabolism at very high EDC concentrations which would definitely saturate its metabolizing enzyme(s) (Spreafico et al. 1980).

Materials and methods

Chemicals. Crystalline phenobarbital (PB) and mixed isomers of 2,3-*tert*-butyl-4-hydroxyanisole [butylated hydroxyanisole (BHA)] were purchased from Sigma Chemical Company, St. Louis, MO. Spectranalyzed 1,2-dichloroethane [ethylene dichloride (EDC)], and disulfiram (DSF) were purchased from Fisher Scientific Company, Pittsburgh, PA and Aldrich Chemical Company, Milwaukee, WI, respectively. Commercial DSF was further purified by recrystallization of a cold ethanol solution with double distilled water and the crystals air dried (m.p. 69–70 °C). The identity, purity and composition of EDC were confirmed by GC/MS while the identity and composition of DSF were confirmed by MS only. Commerical BHA and PB were used without further purification.

Animals and treatments. Weanling Sprague-Dawley male rats (6 weeks old, 160–174 g) were purchased from Charles River Breeding Laboratories, Portage, MI. Upon receipt, the animals were housed in groups of five and placed on Purina brand rat chow. They were quarantined and acclimated to the laboratory environment (12-h fluorescent white light-dark cycle, temperature = 21–23 °C and relative humidity = 50–69%) for 10 days and screened for viruses and mycoplasmas. At the end of the quarantine and acclimation periods, the animals were weighed, assigned randomly to individual cages and then placed on AIN'76 powdered diet (Ziegler Brothers, Gardner, PA, USA). Cages were preassigned to four treatment groups: I) Control, II) DSF in diet for 10 days, III) 0.75% BHA in the same diet for 10 days, and IV) 0.10% PB in the same diet for 7 days. Feed mixes, usually stored at 5–10 °C, were dispensed in specially constructed stainless steel cups which almost completely eliminated wastage. Animals were allowed food and water ad libitum except during gas uptake exposure periods.

Animals were started on their individual treatment diets on a sequential basis so that exposures were performed in pairs on the morning following the last day on the diet. Animals were weighed every day but the feed cups were weighed every other day to determine the quantity of feed consumed.

The rationale for the dietary pretreatments was based on our earlier DSF/EDC interaction studies (Igwe et al. 1984). The need for equivalent treatment of all groups predicated the use of dietary pretreatment for BHA and PB groups also, for which precedent also exists in the toxicological literature (Benson et al. 1979; Guengerich and Watanabe 1979).

Exposure system design (construction) and calibration. The closed recirculating system is schematically shown in Fig. 1. The components of the system include the exposure desiccator chamber, the carbon dioxide/water (CO_2/H_2O) scrubbing unit, the mixing loop with the distribution tubing and the infrared (IR) gas analyzer (Wilks Scientific Corp., South Norwalk, CT). The total system volume was estimated by summation of the volumes of the components; the volume was further confirmed by injecting known volumes of EDC into the empty recirculating system and noting the concentration from a calibration curve [concentration (ppm) versus absorbance] generated in the Foxboro Wilk's closed loop calibration system. The volume by summation was 17.15 l and by calibration, 17.10 l. Expired carbon dioxide was continuously removed from the exposure system by absorption on a column of 17 g Ascarite II (8–20 mesh, 20–30% w/w absorptive capacity). The inclusion of 17 g Ascarite II in the CO_2/H_2O absorbing unit resulted in increased volume of the exposure sys-

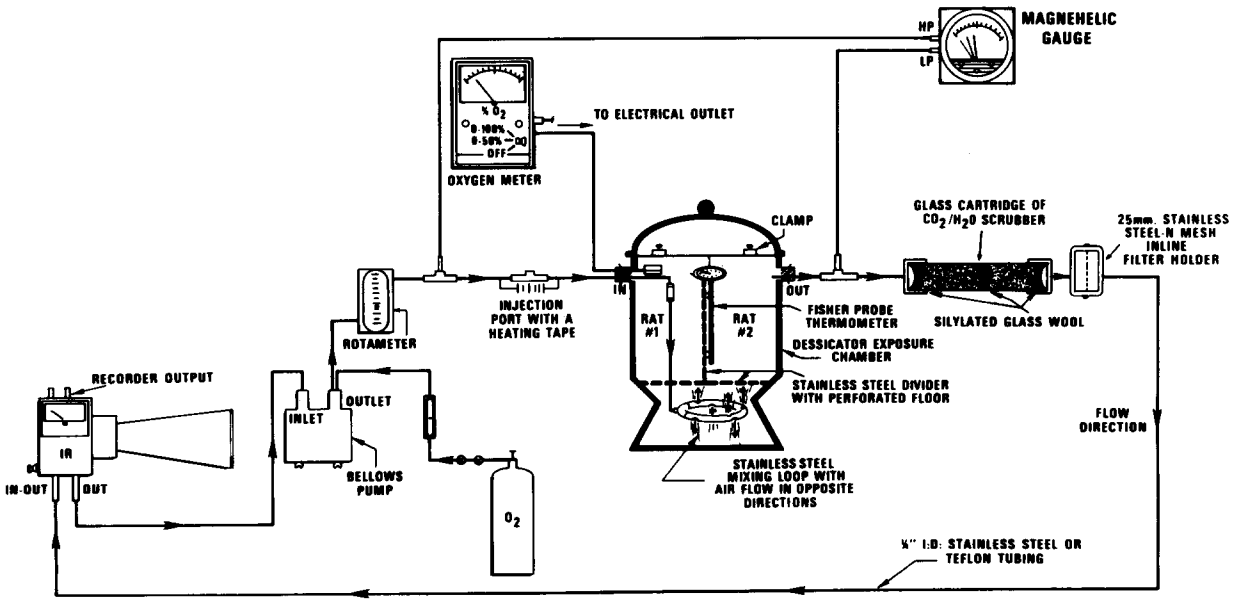

Fig. 1. Schematic of the closed recirculating system used in the gas uptake study

tem from 17.10 l to 18.66 l, i.e., a change in volume by a factor of 1.091. The virtual gas volume of the system also depended on the weight of animals in the chamber and was calculated by substracting the volume of 17 g Ascarite II and animal volume (assuming 1 kg = 1 l) from 17.10 l and multiplying by 1.091. The use of Drierite alone for water absorption in different weight combinations caused unacceptable depletion of injected EDC. Ascarite II alone did not cause depletion.

Six holes each of 0.249 cm diameter and equal to one-sixth of the inner circumference (0.476 cm i.d.) of the stainless steel tubing, were drilled into the mixing loop [circumference (51.44 cm)]. The holes were interspersed at equal distances from one another and were alternated at the bottom and at the top, to achieve complete mixing in the chamber. The size and placement of the holes prevented a pressure drop which can affect the smooth operation and calibration of the in-line rotameter, thus leading to pressure fluctuations and IR sampling errors. The system pressure drop was detected by a magnehelic gauge with the high pressure (HP) end connected to the inlet and the low pressure (LP) end connected to the outlet.

The oxygen concentration within the system was monitored and maintained between 19 and 23% with USP grade oxygen. A bellows pump with a stainless steel/teflon diaphragm was used to recirculate the system atmosphere at 6 lpm. The system was operated between -1 and -2 inch water gauge relative to the atmosphere, thus minimizing system leakage. To check for leaks the system was pressurized regularly to 5 in water gauge and all fittings examined for outward leaks with detergent solution.

The chamber atmosphere was continuously monitored using an in-line Miran 1A-CVF (Wilks Scientific Corp., South Norwalk, CT) infrared gas analyzer (IRGA) at wavelength and pathlength of 8.2 μm and 20.25 m, respectively. During exposures, routine checks for CO_2 in the system were made at 4.26 μm. Except for the difference in total volume (17.10 l), the closed recirculating system was equivalent to the closed-loop calibration system developed by Foxboro Wilks Analytical.

The exposure system was calibrated as a unit by injection of predetermined increments of liquid EDC via the injection port septum with the pump turned on. The observed absorbance values versus the corresponding concentrations delineated the calibration curve. For high EDC concentrations, heating tape was used for 5 min to aid in rapid vaporization of the liquid EDC. The concentration of EDC vapor in ppm after injection of liquid EDC was calculated assuming the ideal gas law. Where non-linearity occurred, concentrations were read directly from the calibration curve. Concentrations of EDC generated ranged from 18 to 2980 ppm.

Prior to the inclusion of rats, the desired concentration was generated in the empty recirculating system with and without the 17 g Ascarite II column in place, and the decline in concentration was followed over a time (5.5 h) exceeding that intended for the experiment (5 h). The decline in concentration was in accordance with first-order kinetics as described by:

$$dC/dt = -kC \quad \text{(1)},$$

where C (ppm) is the concentration of EDC at the time t (min) and k (min^{-1}) is the rate constant for the decline in EDC concentration from the recirculating system. Regression analysis of the logarithm of the concentration of EDC versus time yielded the value of k. Values for k were also determined with the inclusion of two dead rats [total weight (0.725 kg) killed by lethal injection of sodium pentobarbital (180 mg/kg body wt.] to determine the effect of animals on each generated concentration. Whether animals exhaled any materials that might absorb light in the 8.1–8.2 μm range, which could be mistaken for EDC absorbance, was investigated with two live rats without the injection of EDC in the system; they did not exhale any such substance. Before initiation of animal exposures, the value of k was determined at each target concentration using four replicates with different units of 17 g Ascarite II.

After the determination of k, two rats chemically pretreated with BHA, PB or DSF in their diets as described earlier, or two control rats, were placed in the chamber. The system was charged with the desired concentrations of EDC and the declining concentration recorded over 5 h. Between experiments, the system was taken apart, cleaned, and reassembled with a new Ascarite II column. Each observation during the exposure of live animals was corrected on a point-to-point basis for non-specific loss of EDC from the system determined earlier with dead animals. The corrected data were plotted semilogarithmically with respect to time.

Analysis of corrected EDC uptake curves. Uptake curves after point-by-point corrections for non-specific losses at each concentration, are shown (Figs. 2A and B) for control and DSF groups only. The corrected uptake curves were biphasic, containing a rapid equilibration phase complete in 90–100 min in each case and a subsequent slow phase thereafter. The rapid phase was calculated by subtracting the extrapolated line for the slow phase at the same time. This assumed that both fast and slow processes, described by first-order rate constants, occurred simultaneously initially. Regression analysis of the data points gave the slopes for calculation of the rate constants of the rapid phase.

The slow phase rate constants in all groups decreased with increasing chamber concentration, thus further demonstrating that the uptake of EDC is dose-dependent.

The experimental curves after the initial equilibration period (90–100 min) were divided into small segments and by tangential fitting for each segment, rates of disappearance of EDC, in $ppm.kg^{-1}.h^{-1}$, from the gas phase in this closed system were estimated. Plots of the corrected rate of uptake versus concentration were constructed (Figs. 3A and B). The points were represented accurately by a rectangular hyperbola in each treatment group, again supporting the assumption that the rate was due to metabolism with saturable uptake dependence (Andersen et al. 1980). By linearizing the data using Hanes plots and fitting the lines by unweighted least squares, the maximum rate of metabolism (V_{max}) and the apparent inhalation Michaelis constant (K_m) were estimated for each treatment group (Table 1).

Determination of the Ostwald's partition coefficient. The distribution of EDC between air and various liquid phases was studied using the headspace technique.

Forty headspace vials (each 13 ml volume) were divided into four groups of equal number; 0.4 ml deionized water, 0.4 ml freshly drawn rat whole blood containing

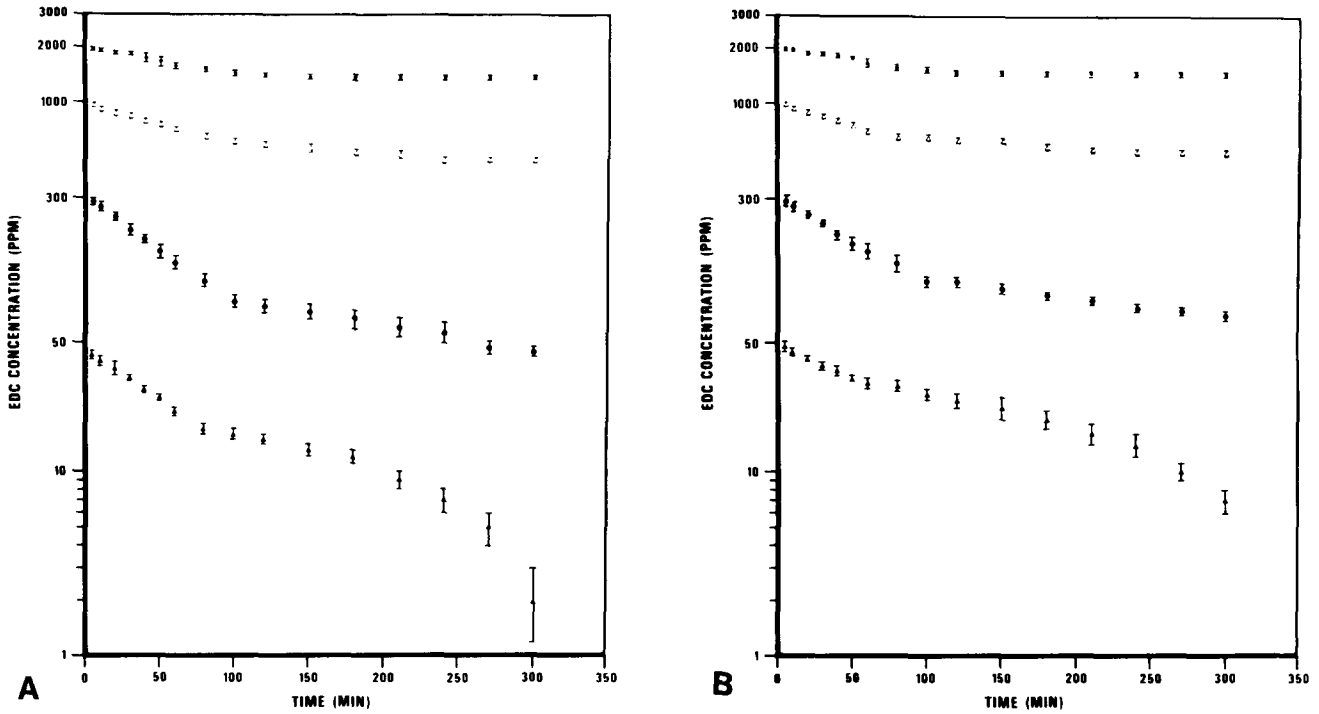

Fig. 2A, B. Uptake of EDC by control and DSF-pretreated male Sprague-Dawley rats, respectively, at four initial concentrations. Plotted data (mean ±SD) were derived from uptake curves corrected on a point-to-point basis for a non-specific loss rate of 0.0327 h^{-1}. Animals were exposed in pairs and three exposures were conducted per initial concentration. The plot is semilogarithmic

disodium EDTA as anticoagulant and 0.1 ml medium chain triglyceride (MCT) oil were put into the vials of the first, second and third groups, respectively. The vials of the fourth group (reference group) were left empty.

An aliquot (0.1 ml) containing 9.96 mg EDC/ml of deionized water was added to each vial. The vials were capped with teflon-lined screw caps and maintained in Fisher Isotemp dry bath at 37 °C for 2 h to establish equilibrium as shown by Sato and Nakajima (1979b). After equilibration, 5 ml of the headspace from each vial was withdrawn with a 10 ml airtight Hamilton gas syringe and injected into the closed loop/IR GA recirculating system.

The absorbance values obtained were linear up to 0.08 (50 ppm) and concentrations were confirmed by gas chromatography.

The partition coefficients (water/air, blood/air and MCT oil/air) were calculated from the IR absorbances of the air in the sample and reference vessels using the principles developed by Sato and Nakajima (1979b).

Analysis of uptake data. Kinetically, the exposure system consists of two chambers in series with each other, the 7.81 l IR cell with the Ascarite II column (V_3) and then the 10.85 l desiccator jar (chamber) (V_1) with animals in it (Fig. 4). The total system volume is 18.66 l with CO_2/H_2O scrubbing column charged with 17.0 g Ascarite II and the flow rate through it is 6 lpm. The chamber and the IR cell equilibrate by first-order processes represented by the rate constants k_{13} and k_{31}.

This signifies compartmental clearances, $k_{13}V_1$ and $k_{31}V_3$, at 6 lpm. With the known volumes V_1 and V_3, k_{13} was calculated to be (6*60/10.85) = 33.2 h^{-1}, and k_{31} to be

Table 1. Comparison of the effects of different chemical (dietary) pretreatments on the inhalational kinetic constants of EDC in vivo by the gas uptake method

Treatment Group[a]	Apparent K_m[a] (ppm)	V_{max}[a] (ppm·h^{-1} kg^{-1})	V_{max}[a] (μmol·h^{-1} kg^{-1})	r^2
I. Control (on AIN'76 Feed)	214±25	54±4	41±3	0.997
II. DSF (0.15%) in the feed for 10 days	90±10[b]	34±1[b]	26±1	0.998
III. BHA (0.75%) in the feed for 10 days	226±27	61±2	47±2	0.994
IV. PB (0.1%) in the feed for 7 days	206± 7	79±2[c]	60±2	0.991

[a] The apparent K_m and V_{max} values (mean±SD, 3 experiments/concentration/group) are derived from unweighted least squares of best fit Hanes plot based on ten data points; the mean correlation coefficient, γ^2, of the fitted lines are indicated. The calculation used to convert V_{max} from ppm/h/kg to μmol/h/kg is: 40.9 μmol EDC/m^3/ppm × ppm/h/kg × 0.0187 m^3 since 1 ppm EDC = 40.9 μmol/m^3 assuming ideal gas conditions. These kinetic parameters are only valid for uptake of EDC from this closed exposure system

[b] Significantly different from I, III an IV, $p \leq 0.05$

[c] Significantly different from I, III, $p \leq 0.05$

(6*60/7.81) = 46.1 h^{-1}. The theoretical rate constant for attainment of the final chamber concentration was given by the sum of k_{13} and k_{31} which was 79.3 h^{-1}. Using this value the half-life for the equilibration period between compartments 1 and 3 was calculated to be 0.52 min. Thus, chamber equilibration should be 99% complete within 3.4 min. For that reason, since the equilibration period between the two compartments 1 and 3 was very short compared to the equilibration period between compartments 1 and 2 (the animals), compartments 1 and 3 were treated as unity. In addition, the rate constant of EDC loss from the system with dead animals was very small (0.03 h^{-1}) compared to the rate constant for the attainment of equilibrium. This means that compartments 1 and 3 are well mixed with the steady state generally maintained in the exposure system without live rats. Hence, the use of a two-compartment model in calculating the kinetic parameters in this exposure system is justified. In this model, compartment 1 is represented by the IR-cell together with the dessicator exposure chamber, and V_1' is therefore the sum of V_1 and V_3, (V_1' = 18.66 l) as depicted in Fig. 4. Using the general

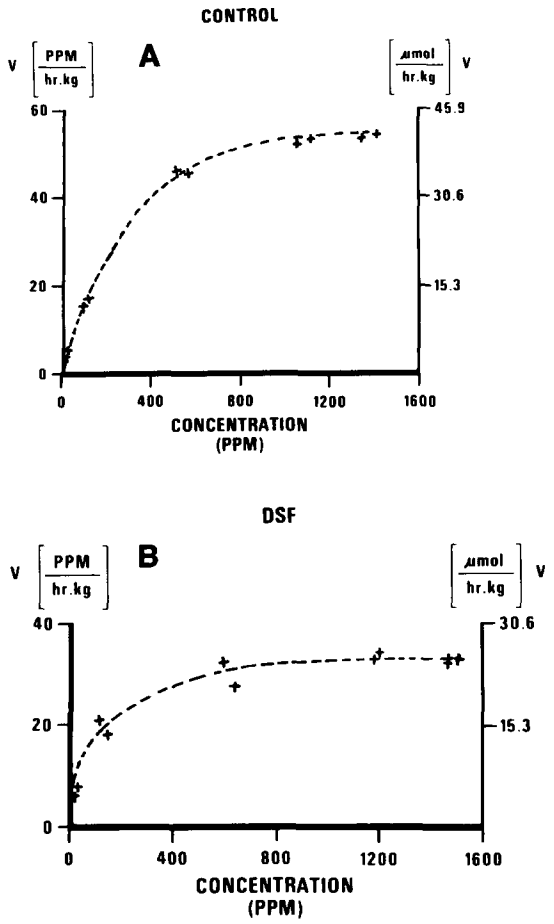

Fig. 3A, B. Dependence of corrected slow phase rate of uptake of EDC by control and DSF pretreated male Sprague-Dawley rats, respectively. Standard deviation about each point varied from 1%–18% of the mean. The concentrations (x-axis) were those at which the rates were calculated following tissue equilibration (90–100 min) and not the initial EDC concentration of the exposure. The smooth curves are the best-fit Michaelis-Menten rectangular hyperbola. These curves are only valid for uptake of EDC from this special closed exposure system

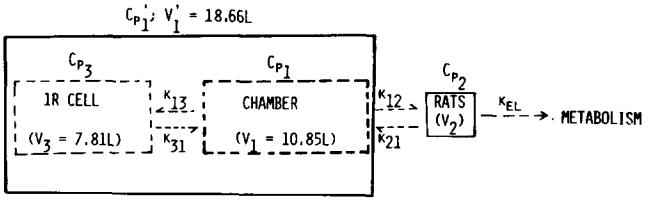

Fig. 4. Schematic for the compartmental analysis. Note that with no animals in the chamber $k_{13}V_1 = k_{31}V_3$ = 6 lpm

approach of Bolt et al. (1981) and Filser and Bolt (1983), the exposure system is regarded as compartment I and it is accessible for analysis and the animal, regarded as a single homogeneous unit, is compartment II. Because the volume of distribution of the vapor includes both the exposure system and the rats in the chamber, calculations of the rate accounted for loss in both compartments (Filser and Bolt 1979, 1981).

A relationship between the thermodynamic blood/air (N) and the effective steady-state blood/air (N_{eff}) partition coefficients based on physiological variables were derived using the equation of Andersen (1981).

$$N_{eff} = \frac{V_{alv} \times N}{V_{alv} + E_t Q_t N} \quad (-----(2))$$

where N is the thermodynamic blood/air partition coefficient, V_{alv} is the alveolar ventilation rate (21 l/h/kg in rats), Q_t is the cardiac output and is the same as V_{alv} and E_t is the systemic extraction ratio (for predominance of hepatic metabolism, $E_t = 0.25$, because liver blood flow is 25% cardiac output). The value of N_{eff} calculated by substitution in equation (2) is 3.57.

The microconstants, k_{12}, k_{21} and k_{el} were calculated using equations developed by Bolt et al. (1981). The apparent maximum first-order rate constants were calculated from the slope of the corrected rate curves (i.e., slow phase) at 50 ppm EDC. These rate curves showed extended first-order portions at low EDC concentrations.

Statistical analysis of data. The kinetic data for the treatment groups (three experiments each/kinetic constant) were subjected to statistical analysis using a one-way analysis of variance (ANOVA) of the statistical analysis system (SAS) program (SAS User's Guide: Statistics, 1982). Means were compared by Scheffe's tests of multiple comparison at $P \leq 0.05$.

Results

Doses of pretreatment chemicals

The doses of DSF, BHA and PB received by individual rats were estimated from the quantity of feed consumed and the body weights of individual animals recorded over the feeding period. These values, expressed as mean ±SD in mg/kg body wt./day, were: DSF (75±5), BHA (344±21), and PB (58±3). The incorporation of DSF, BHA or PB at the levels stipulated did not affect food consumption.

Exposure system characteristics

The disappearance of EDC from an unoccupied chamber was represented by a single exponential function whose

rate constant varied between 0.010 h^{-1} and 0.017 h^{-1} with no Ascarite II (Fig. 1) and 0.028 h^{-1} and 0.037 h^{-1} with 17 g Ascarite II. The loss rate constant was EDC concentration independent and did not change signficantly after the system was dismantled, cleaned and reassembled. The rate constants determined with two dead rats in the chamber were not different from those deterned without the rats.

The exposure chamber environmental temperature and the oxygen concentration ranged from 22 to 27 °C and 19 to 23% (v/v) throughout the exposure period.

Effects of various pretreatments on EDC kinetic constants

DSF pretreatment resulted in the lowest apparent K_m and V_{max} values for EDC when compared to the members of all the other groups. The K_m values for groups I, III and IV are not statistically different from one another. However, the V_{max} for group IV differs significantly (30–46% higher) from those of groups I and III (Table 1).

The inhalational kinetic microconstant of EDC in vivo after different chemical (dietary) pretreatments of rats are indicated in Table 2. The inspirational constants, k_{12}, are the same in all groups. The expirational (off-gassing) rate constant, k_{21}, is highest in members of group II (DSF pretreated), but remains the same in all the other groups. The first-order metabolic rate constant, k_{el}, is also the same in all groups.

Partition coefficient of EDC

The thermodynamic (Ostwald's) partition coefficient values for EDC (distribution between water/air, blood/air and oil/air) using the head-space method at 37 °C are 11.8±1.2 for water/air, 33.0±3.7 for rat whole blood/air and 972±8 for medium chain triglycerides (MCT) oil/air. The values reported are mean ±SD with $n=10$ in each case.

The distribution between MCT oil/blood in this study is 29, indicative of moderate solubility of EDC in adipose tissues However, an earlier determination of the oil/air partition coefficient for EDC using olive oil by Sato and Nakajima (1979b) gave a much smaller value of 447, probably because of the different oils used in the two studies.

Discussion

This study has demonstrated the pharmacokinetic dose-dependence of EDC metabolism in naive and DSF-, BHA- and PB-pretreated male Sprague-Dawley rats, using gas uptake methodology. Spreafico et al. (1980) have also shown that the blood levels of EDC rose 22-fold when the exposure concentration was changed from 50 to 250 ppm. The results also imply that at low EDC concentration range, uptake and/or transport to the metabolizing enzymes limit the rate of metabolism but not the substrate affinity to the enzymes. The influence of dose on inhalation kinetic parameters has been recognized for halogenated ethylenos (Gehring et al. 1978; Filser and Bolt 1979; Reichert and Henschler 1978). This kinetic behavior concerning EDC is further supported by the N value of 33 compared with an N_{eff} of only 3.57. Andersen (1981) has shown that when perfusion limits the rate of metabolism, an induction of the metabolizing enzymes may not have any effect on the rate of metabolism in the low concentration range.

The effect of DSF, PB and BHA on the basic inhalational kinetic constants (apparent K_m, V_{max}, k_{12}, and k_{21}) may be used to predict the time-course of equilibration and metabolism of any EDC concentration.

Pretreatment did not appear to affect whole body equilibration time (90–100 min in all cases), whereas the metabolic parameters for EDC were affected. The most significant effect (Tables 1 and 2) was shown by DSF (lowered K_m and V_{max} and increased k_{21}).

On the basis of the exponential increase in the EDC blood levels with increase in EDC dose, Reitz et al. (1982) suggested that the K_m of EDC lies between 150 and 250 ppm. Exposure to 150 ppm EDC was predicted to produce blood levels in this region. In the present study, the K_m for control (214±25 ppm), BHA (226±27 ppm) and PB (206±7 ppm) pretreated groups are not significantly different from each other and they lie between 150 and 250 ppm. Thus, the K_m values obtained in this study for rats with either full (control and BHA groups) or enhanced complement of cytochrome P_{450} (PB group) are in good agreement with the predictions of Reitz et al. (1982). This further supports the fact that systemic metabolism is responsible for the vast majority of EDC vapor uptake from the exposure system.

Changes in a metabolizing enzyme do not necessarily translate into toxicologically significant changes in xenobiotic disposition (Gillette and Pang 1977). The inability of PB induction to alter the hepatic clearance of benzo(a)pyrene and vinyl chloride has been shown (Roth and Wirsma 1979; Watanabe et al. 1978). Pretreatment of rats with PB did not affect the uptake of low levels of vinyl chloride in vivo (Bolt et al. 1976), a phenomenon also observed in this study for EDC.

Table 2. The inhalational kinetic microconstants of EDC in vivo after different chemical (dietary) pretreatment of male Sprague-Dawley rats

Treatment group	k_{12}[a] (h^{-1})	k_{21}[a] (h^{-1})	k_{el}[a] (h^{-1})
I. Control (on AIN'76 Feed)	1.11±0.03	0.36±0.03	0.37±0.02
II. DSF (0.15%) in the feed for 10 days	1.05±0.04	0.80±0.05[b]	0.42±0.03
III. BHA (0.75%) in the feed for 10 days	1.13±0.03	0.33±0.01	0.40±0.02
IV. PB (0.1%) in the feed for 7 days	1.16±0.08	0.32±0.01	0.40±0.06

[a] k_{12} and k_{21} are the rate constants for inspiration and expiration (offgassing), respectively, and k_{el} is the elimination rate constant (at 50 ppm EDC in each group). They were all estimated by the equations of Bolt et al. (1981) as indicated in the text; the number of experiments used to determine each mean±SD microconstant value was three. The microconstants k_{12} and k_{21} are dependent on the volume of the gas phase ($V_1' = 18.66$ l) and of the animal V_2 (see Filser and Bolt 1983)

[b] Significantly different from I, III and IV, $p<0.05$

The increase in V_{max} of EDC by PB does not appear to be proportionally related to the quantity of the PB-inducible species of cytochrome P_{450} and cytosolic GST isoenzymes involved in halocarbon metabolism, a capacity which is substantial when compared with other inducing agents (Kaplowitz et al. 1975; Pohl et al. 1980). The increase in the EDC metabolic rate by PB may be due to either the induction of cytochrome P_{450} and/or GSTs. Induction of cytochrome P_{450} enzymes can alter the steady-state concentrations of a parent compound and its metabolites in the blood (Gillette 1979, 1980).

Although BHA induces GST and increases hepatic and extrahepatic GSH (Benson et al. 1979), two conditions which would predispose EDC to increased metabolism in vivo, no increased EDC metabolism was observed. The V_{max} in BHA-pretreated rats was not significantly higher than control.

DSF possesses antiarrhythmic properties in vitro (Fossa et al. 1983; Fossa and Carlson 1983). Thus, DSF has the potential to decrease cardiac output which is related to organ perfusion and consequently hepatic blood flow, which is rate limiting in inhalant metabolism (Andersen 1981). Therefore, the quantity of EDC that reaches the liver and subsequently the critical enzymic sites (probably 1,2-dichloroethane GST isoenzyme) is conceivably lower than in all the other groups especially at high EDC concentrations. The amount metabolized would then be dependent upon the quantity of the GST isoenzyme induced by DSF, the effective EDC uptake and its intracellular transport in the organism. Pretreatment with DSF lowers V_{max}; this inhibitory effect would not be dramatic in the low concentrations because, under normal conditions, metabolism is limited by perfusion. At steady state, the inhibition of the metabolizing enzymes (cytochrome P_{450}) by DSF could result in EDC enrichment of the blood and of exhaled vapor in the pretreated animals compared to the controls. This enrichment would be opposed to the smaller EDC metabolic capacity due to inhibition. Since the rate of metabolism depends on EDC concentration within the body, the observed reduction of the metabolic rate should be less than expected from the effect on V_{max}. Thus, it is impossible to distinguish kinetically between the two metabolic pathways for EDC using the present experimental protocol. However, the two pathways can be more conclusively verified kinetically using in vitro methods and this study is under way.

The reliance on initial rate measurements in in vivo gas uptake studies is misleading because the early rate represents a composite of tissue equilibration and metabolism. V_{max} for EDC is thus a composite of one or more metabolic pathways plus other factors such as tissue deposition and urinary excretion.

Acknowledgement. The authors are grateful to the National Institute for Occupational Safety and Health (NIOSH), Cincinnati, Ohio, U.S.A. for the unlimited use of their facility.

References

Anders MW, Livesey JC (1980) Metabolism of 1,2-dihaloethanes. In: Ames B, Infante P, Reitz R (eds) Banbury Report. 5. Ethylene dichloride: A potential health risk? Cold Spring Harbor Laboratory Publication, New York, pp 331–341

Andersen ME (1981) A physiologically based toxicokinetic description of the metabolism of inhaled gases and vapors: Analysis at steady state. Toxicol Appl Pharmacol 60: 509–526

Andersen, ME, Gargas ML, Jones RA, Jenkins LJ (1980) Determination of the kinetic constants for metabolism of inhaled toxicants in vivo using gas uptake measurements. Toxicol. Appl Pharmacol 5: 100–116

Baars AJ, Jansen M, Breimer DD (1978) The influence of phenobarbital, 3-methyl cholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on glutathione S-transferase activity of rat liver cytosol. Biochem Pharmacol 24: 2487–2494

Benson AM, Cha YN, Bueding E, Heine JS, Talalay P (1979) Elevation of extrahepatic glutathione S-transferase and epoxide hydratase activities by 2(3)-tert-butyl-4-hydroxyanisole. Cancer Res 39: 2971–2977

Bolt HM, Kappus H, Buchter A, Bolt W (1976) Disposition of [1,2-^{14}C] vinyl chloride in rat. Arch Toxicol 34: 153–162

Bolt HM, Filser JG, Buchter A (1981) Inhalation pharmacokinetics based on gas uptake studies. III. A pharmacokinetic assessment in man of "peak concentrations" of vinyl chloride. Arch Toxicol 48: 213–228

Cha Y-N, Bueding E (1979) Effect of 2(S)-tert-butyl-4-hydroxyanisole administration on the activities of several hepatic microsomal and cytoplasmic enzymes in mice. Biochem Pharmacol 28: 1917–1921

Cha Y-N, Heine HS, Moldeus P (1982) Differential effects of dietary and intraperitoneal administration of antioxidants on the activities of several hepatic enzymes of mice. Drug Metab Dispos 10: 434–435

Filser JG, Bolt HM (1979) Pharmacokinetics of halogenated ethylenes in rats. Arch Toxicol 42: 123–136

Filser JG, Bolt HM (1981) Inhalation pharmacokinetics based on gas uptake studies. I. Improvement of kinetic models. Arch Toxicol 47: 279–292

Filser JG, Bolt HM (1983) Inhalation pharmacokinetics based on gas uptake studies. IV. The endogenous production of volatile compound. Arch Toxicol 521: 123–133

Fiserova-Bergerova V, Vlach J, Cassady JC (1980) Predictable "individual differences" in uptake and excretion of gases and lipid soluble vapors simulation study. Br J Ind Med 37: 42–49

Fossa AA, Carlson GP (1983) Antiarrhythmic effects of disulfiram in various cardiotoxic models. Pharmacology 26: 164–171

Fossa A, White JF, Carlson GP (1983) Antiarrhythmic effects of disulfiram on epinephrine-induced cardiac arrhythmias in rabbits exposed to trichloroethylene. Toxicol Appl Pharmacol 66: 109–117

Gargas ML, Andersen ME (1979) Closed atmosphere gas uptake studies and their validation by direct metabolite determination. In: Proceedings of the Tenth Annual Conference on Environmental Toxicology, AFAMRL-TR-79-121, Air Force Aerospace Medical Research Laboratory, Wright Patterson AFB, Ohio (ADA 086 341 pp 74–92), USA

Gehring PJ, Watanabe PG, Park CN (1978) Resolution of dose-response toxicity data for chemicals requiring metabolic activation: Example – vinyl chloride. Toxicol Appl Pharmacol 44: 581–591

Gillette JR (1979) Effects of induction of cytochrome P_{450} enzymes on the concentration of foreign compounds and their metabolites and on the toxicological effects of these compounds. Drug Metab Rev 10 (1): 59–87

Gillette JR (1980) Kinetics of decomposition of chemically unstable metabolites in the presence of nucleophiles: Derivation of equations used in graphical analyses. Pharmacology 20: 64–86

Gillette JR, Pang KS (1977) Theoretical aspects of pharmacokinetic drug interactions. Clin Pharmacol Ther 22: 623–638

Gold LS (1980) Human exposure to ethylene dichloride. In: Ames B, Infante P, Reitz R (eds) Banbury Report ≠5. Ethylene dichloride: A potential health risk? Cold Spring Harbor Laboratory Publication, New York, pp 209–225

Guengerich FP, Watanabe PG (1979) Metabolism of ^{14}C - and [^{36}(Cl]-labeled vinyl chloride in vivo and in vitro. Biochem Pharmacol 28: 589–596

Guengerich FP, Crawford VM, Domoradzi JY, MacDonald TL, Watanabe PG (1980) In vitro activation of 1,2-dichloroethane by microsomal and cytosolic enzymes. Toxicol Appl Pharmacol 55: 303–317

Hallier E, Filser JG, Bolt HM (1981) Inhalation pharmacokinetics based on gas uptake studies. II. Pharmacokinetics of acetone in rats. Arch Toxicol 47: 293–304

Hunter AL, Neal RA (1975) Inhibition of hepatic mixed function oxidase activity in vitro and in vivo by various thiono-sulfur-containing compounds. Biochem Pharmacol 4: 2199–2205

Igwe OJ, Que Hee SS, Wagner WD (1984) Proposed mechanism for the toxic interaction between disulfiram (DSF) and 1,2-dichloroethane. Presented at the International Conference on Organic Solvent Toxicity, Oct. 15–17 Stockholm, Sweden. Abstract Nr. 166, p 35

Kaplowitz N, Kuhlenkamp J, Clifton G (1975) Drug induction of hepatic glutathione S-transferases in male and female rats. Biochem J 146: 351–356

McKenna MJ, Zempel JA, Braun WH (1979) The pharmacokinetics of inhaled methylene chloride in rats. In: Proceedings of the Ninth Annual Conference on Environmental Toxicology, AFAMRL-TR-79-68. Air Force Aerospace Medical Research Laboratory, Wright Patterson Air Force Base, Ohio (ADA 074-837 pp 183–199), USA

Pohl LR, Martin JL, Taburet AM, George JW (1980) Oxidative bioactivation of haloforms into hepatotoxins. In: Coon MF, Conney AH, Eastbrook RW, Gelboin HV, Gillette JR, O'Brien PJ (eds) Microsomes, drug-oxidations and chemical carcinogenesis. Academic Press, New York, Vol II, p 881

Reichert D, Henschler D (1978) Uptake and hepatotoxicity of 1,1-dichloroethylene by isolated blood-perfused rat liver. Int. Arch. Occup Environ Health 41: 169–178

Reitz RH, Fox TR, Ramsey J, Quast JF, Lang-Vasrdt PW, Watanabe PG (1982) Pharmacokinetics and macromolecular interactions of ethylene dichloride in rats after inhalation or gavage. Toxicol Appl Pharmacol 62: 190–204

Roth RA, Wiersman DA (1979) Role of the lungs in total body clearance of circulating drugs. Clin Pharmacokinet 4: 355–367

SAS User's Guide: Statistics, 1982 Edition, SAS Institute, Inc., Cary, NC: SAS Institute, Inc., p 584

Sato A, Nakajima T, Fujimara Y, Hirosawa K (1974) Pharmacokinetics of benzene and toluene. Int Arch Arbeitsmedizin 33: 169–182

Sato A, Nakajima T (1979a) A structure-activity relationship of some chlorinated hydrocarbons. Arch Environ Health 34: 69–75

Sato A, Nakajima T (1979b) Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Br J Ind Med 36: 231–234

Sparnins VL, Venegas PL, Wattenberg LW (1982) Glutathione S-transferase activity: enhancement by compounds inhibiting chemical carcinogenesis and by dietary constituents. J. Natl. Cancer Inst 68 (3): 493–496

Spreafico F, Zuccato E, Marcucci F, Sironi M, Puglialunga S, Madonna M, Mussini E (1980) Pharmacokinetics of ethylene dichloride in rats treated by different routes and its long-term inhalatory toxicity. In: Ames B, Infante P, Reitz R (eds) Banbury Report #5. Ethylene dichloride: A potential health risk? Cold Spring Harbor, Laboratory Publication, New York, pp 107–133

Van Bladeren PJ, Breimer DD, Rotteveel-Smij GMT, de Knijff P, Mohn GR, Van Meeteren-Walchli B, Bujs W, Van Der Gen A (1981) The relation between the structure of vincinal dihalogen compounds and their mutagenic activation via conjugation to glutathione. Carcinogenesis 2: 499–505

Watanabe PG, Zempel JA, Pegg DG, Gehring PJ (1978) Hepatic macromolecular binding following exposures to vinyl chloride. Toxicol Appl Pharmacol 44: 571–579

Young JD, Braun WH, Gehring PJ (1978) Dose-dependent fate of 1,4-dioxane in rats. J Toxicol Environ Health 4: 709–726

Received January 7, 1986/Accepted June 23, 1986