Influence of Exposure Route and Oral Dosage Regimen on 1, 1-Dichloroethylene Toxicokinetics and Target Organ Toxicity

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ABSTRACT

The objective of this investigation was to elucidate the effects of route of exposure and oral dosage regimen on the toxicokinetics (TK) of 1,1-dichloroethylene (DCE). Fasted male Sprague-Dawley rats that inhaled 100 or 300 ppm for 2 h absorbed total systemic doses of (10 or 30 mg/kg DCE, respectively. Other groups of rats received 10 or 30 mg/kg DCE by intravenous injection, bolus gavage (by mouth), or gastric infusion (g.i.) over a 2-h period. Serial microblood samples were taken from the cannulated, unanesthetized animals and analyzed for DCE content by gas chromatography to obtain concentration versus time profiles. Inhalation resulted in substantially higher peak blood concentrations and area under blood-concentration time curves (AUC0 inf) than did gastric infusion of the same dose over the same time frame at each dosage level, although inhalation (AUC0 inf) values were only modestly higher. Urinary N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyltranspeptidase (GGT) activities were monitored as indices of kidney injury in the high-dose groups. NAG and GGT excretion were much more pronounced after inhalation than gastric infusion. Administration of DCE by gavage also produced much higher Cmax and AUC0 inf than did 2-h g.i., although AUC0 inf values were not very different. The 30 mg/kg bolus dose produced marked elevation in serum sorbitol dehydrogenase, an index of hepatocellular injury. Administration of this dose by inhalation and gastric infusion was only marginally hepatotoxic. These findings demonstrate the TK and target organ toxicity of DCE vary substantially between different exposure routes, as well as dosage regimens, making direct extrapolations untenable in health risk assessments.

Home use of volatile organic chemical (VOC)-contaminated tap water commonly results in exposure by multiple routes. Previously, toxicity/carcinogenicity risk assessments focused primarily on the amount of chemical in the water ingested. It is now recognized that inhalation during showering and other water-use activities also contribute significantly to one’s systemically absorbed dose (Weisel and Jo, 1996; Gordon et al., 2006). There is little information, however, on the relative quantities and toxicities of VOCs absorbed from different portals. Health risk assessments of ingested VOCs must often be conducted on the basis of inhalation toxicity data, with direct extrapolation from one route of exposure to another. In addition, oral cancer and noncancer studies of VOCs in rodents have usually used daily gavage dosing. The relevance of these single bolus data to human risks is questionable, as people typically consume contaminated water in small, divided doses over the course of the day.

1,1-Dichloroethylene (DCE) was selected for the current study. It is used primarily as a chemical intermediate and in the production of polyvinylidene copolymers used to produce flexible films (e.g., Saranwrap, Velon) for food packaging. Environmental releases occur primarily by evaporation, although some DCE is released to soil, groundwater, and surface waters. DCE is also formed by biotic and abiotic degradation of common VOCs, including 1,1,1-trichloroethane, trichloroethylene (TCE), and perchloroethylene (ATSDR, 1994). These are the most frequently found organic chemicals in groundwater in the proximity of hazardous waste sites (Pohl et al., 2008) and in drinking water in the United States (Moran et al., 2007).

Exposure to low levels of DCE in environmental media is of concern, largely because of the cytotoxic and carcinogenic potential of VOCs. The primary target organs of DCE are the liver, lungs, and kidneys (ATSDR, 1994; IRIS, 2002, http://www.epa.gov/iris). Maltoni et al. (1985) describe significant

ABBREVIATIONS: VOC, volatile organic chemical; DCE, 1,1-dichloroethylene; TCE, trichloroethylene; S-D, Sprague-Dawley; TK, toxicokinetic; g.i., gastric infusion; Vg, minute volume; f, respiratory rate; VT, tidal volume; ECD, electron capture detector; SDH, sorbitol dehydrogenase; NAG, N-acetyl-β-D-glucosaminidase; GGT, γ-glutamyltranspeptidase; AUC, area under the blood-concentration time curve; F, oral bioavailability; GSH, glutathione.
increases in kidney adenocarcinomas (males) and pulmonary adenomas (both sexes combined) in Swiss mice inhaling 25 ppm DCE for 1 year. Lee et al. (1978) also report an increased incidence of pulmonary adenomas in male CD-1 mice inhaling 55 ppm DCE for a year, but not in rats. No tumors due to DCE were seen in Sprague-Dawley (S-D) rats that inhaled up to 75 ppm for 18 months (Quast et al., 1986). The species and gender specificity of the renal tumors and cytotoxicity were attributed by Speerschneider and Dekant (1995) to higher expression of cytochrome P450 IIE1 (CYP2E1), resulting in greater bioactivation of DCE in kidneys of male mice. Quast et al. (1983) supplied male and female S-D rats with drinking water containing up to 200 ppm DCE (daily dose, 20–30 mg/kg) for 2 years. There were no exposure-related neoplasic changes. Ponomarkov and Tomatis (1980), however, observed a modest increase in liver adenomas and carcinomas in rats gavaged once weekly with of the chemical (50 mg/kg). Thus, it appears that the toxicity and carcinogenicity potential of DCE are species- and gender-dependent and influenced significantly by route of exposure and oral dosage regimen as well.

Toxicokinetic (TK) studies can provide insight into the influence of exposure route and dosage regimen on target organ dosimetry and resulting toxic/carcinogenic potential of DCE. VOCs absorbed from the gastrointestinal tract are subject to first-pass elimination by the liver and lungs. In contrast, inhaled VOCs directly enter the arterial circulation and should be rapidly delivered to organs in relatively large amounts. Lee et al. (1996) demonstrated in rats that the liver and lungs, acting in concert, removed up to 60% of low doses of TCE. Liu et al. (2009) subsequently found that oral bioavailability was just ~12 to 16% in rats given 100 μg/kg TCE, an environmentally relevant dose. DCE should also be efficiently removed, as it is also extensively metabolized and is more volatile than TCE. Although gavage dosing has commonly been used for sake of convenience in chronic toxicity and cancer bioassays of VOCs, this can result in such a rapid rate of delivery to the liver that cytotoxicity may occur and much of the absorbed dose avoids first-pass metabolism (Lee et al., 2000). Thus, it might be anticipated that administration of DCE by gavage will result in more pronounced damage of the liver and extrahepatic organs than will administration of the same total amount in divided doses.

The overall objective of the current investigation was to characterize the effects of exposure route and oral dosage regimen (bolus versus prolonged gastric infusion) on the bioavailability and hepatorenal toxicity of DCE in rats. Experiments were designed to test the following hypotheses: 1) administration of equivalent oral and inhaled doses of DCE will result in significantly different internal doses, with more pronounced differences at lower dosage levels; 2) inhalation will result in higher arterial DCE levels and greater extrahepatic (i.e., renal injury) than ingestion; and 3) consumption of DCE as an oral bolus will result in higher arterial concentrations, as well as more severe liver and kidney damage, than will repetitive ingestion of the same total dose over an extended period.

Materials and Methods

Chemicals. DCE of 99% purity was purchased from Aldrich Chemical Co. (Milwaukee, WI). The purity was verified by gas chromatography (GC). All other chemicals and biochemicals were obtained from Sigma-Aldrich (St. Louis, MO). Acepromazine hydrochloride and ketamine hydrochloride were purchased from Fort Dodge Laboratories (Fort Dodge, IA). Bayer Corp. (Shawnee Mission, KS) supplied xylazine hydrochloride.

Animals. Male S-D rats were obtained from Charles River Breeding Laboratories (Raleigh, NC) and maintained on a 12-h light/dark cycle, with light from 7:00 AM to 7:00 PM. They were housed in groups of three in stainless steel wire mesh cages in a negative airflow animal rack. The rack was kept in a biohazards facility in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility. The rats were maintained in a temperature (22 ± 1 °C) and humidity (55 ± 10%) controlled room for the 2-h 100 ppm inhalation regimen. Tap water and food (Purina Rat Chow 5001; Purina, St. Louis, MO) were available ad libitum during this acclimation period. Body weights at the time of the TK and toxicity experiments were 325 to 375 g and 140 to 150 g, respectively. The larger rats were necessary for inhalation experiments and for serial microblood sampling protocols. The experimental protocols were reviewed and approved by the University of Georgia Animal Care Committee.

Animal Preparation. All rats were surgically cannulated for the TK experiments. Each animal was anesthetized by intramuscular injection of 0.1 ml/100 g b.wt. of a “cocktail” consisting of ketamine hydrochloride (100 mg/ml), acepromazine maleate (10 mg/ml), and xylazine hydrochloride (20 mg/ml) (3:2:1, v/v/v). An indwelling cannula (PE-50 polyethylene tubing) was implanted into the right carotid artery of each rat. The cannula was inserted into the artery toward the heart until the tip rested just above the aortic arch. The tubing was then securely ligated to the vessel. A second cannula was placed in the right jugular vein of some rats for intravenous dosing. For gastric infusion (g.i.) experiments, a ventral incision was made in the abdominal wall, and a flare-tipped cannula was implanted into the fundus of the stomach. All cannulas were passed under the skin and exteriorized at the nape of the neck, so the animals could move about freely without disturbing the cannulas upon recovery. Water was provided, but food was withheld during the 24-h postsurgical recovery period before dosing. Although fasting alone resulted in increased liver CYP2E1 activity, fasted S-D rats on which cannula surgery was performed exhibited CYP2E1 activity similar to that of nonfasted controls (data not shown).

Inhalation Exposures. Exposures to DCE were conducted as described by Dallas et al. (1989). Each cannulated rat was placed in a restraining tube (Battelle, Geneva, Switzerland) of the type used for nose-only exposures. A face mask with a miniaturized one-way breathing valve (Hans Rudolph Inc., St. Louis, MO) was secured to the animal’s head with elastic straps. The mask was designed to fit 300- to 400-g rats, so that the valve entry port was immediately adjacent animal’s nares and valve dead space was minimized. The valve included sampling ports, so the concentration of DCE in the separate inhaled and exhaled breath streams could be monitored. Desired concentrations of DCE were generated in a 70-l gas sampling bag (Calibrated Instruments, Airdsley, NY). The bag was connected in series by Teflon tubing to a pneumotachograph, the breathing valve, and an empty 70-l gas collection bag. Each rat was acclimated to the mask and restraining tube for 1 h until stable breathing patterns were established. Two-hour exposures to 100 or 300 ppm DCE were initiated between 10:00 AM and 12:00 PM. Serial breath and micro blood samples were taken during and up to 3 h after exposure for DCE analysis.

Respiratory Measurements. The airflow created by an animal’s inspiration was recorded, so the minute volume (V\textsubscript{E}), respiratory rate (f), and tidal volume (V\textsubscript{T}) could be determined during and after the inhalation exposures. A mean value for these parameters was obtained for each rat by averaging measurements made at 10-min intervals. Mean values (±S.D.) (n = 6) for the 2-h 100 ppm inhalation sessions were as follows: V\textsubscript{E} = 230 ± 18 ml/min, f = 136 ± 6 breaths/min, and V\textsubscript{T} = 1.7 ± 0.2 ml. Mean values (±S.D.) (n = 6) for...
2-h 300 ppm sessions were as follows: $V_r = 216 \pm 37 \text{ ml/min}$, $f = 135 \pm 23$ breaths/min, and $V_E = 1.6 \pm 0.2 \text{ ml}$.

Alveolar levels and cumulative uptake of DCE could be estimated from the exhaled breath data, $f$, $V_E$, and dead space volume. A certain amount of the inhaled air resides in the valvular and physiological (i.e., upper respiratory tract) dead space and is exhaled without participating in alveolar gas exchange. The alveolar concentration was calculated by correcting for the contribution of DCE in this dead space to the exhaled breath concentration. Subtraction of the concentration of DCE in the alveolar air from that in the inhaled air yielded an estimation of the amount of compound that was taken up into the body each sampling period (Dallas et al., 1989). Thereby, it was possible to monitor percentage of systemic uptake and cumulative uptake during the 2-h exposures.

**Oral and Intravenous Dosage Regimens.** Ten and 30 mg/kg DCE, the approximate cumulative doses absorbed during the 2-h 100 and 300 ppm inhalation sessions, were administrated to unanesthetized, freely moving rats intravenously, by gavage (by mouth), and by gastric infusion. Aqueous emulsions were prepared just before dosing using 5% Alkamula (Rhone Poulenc, Cranbury, NJ). The concentration of DCE in each emulsion was verified by GC headspace analysis. A total volume of 5 ml/kg DCE emulsion was administered orally. A glass syringe and curved blunt-tipped intubation needle served to give the oral bolus. A microprocessor-controlled P22 syringe infusion pump (Harvard Apparatus Inc., Holliston, MA) was used to infuse DCE via an indwelling gastric cannula for 2 h. Ten and 30 mg/kg DCE were injected over 10 s into the right jugular vein of other groups of rats. Serial blood samples were taken from the arterial cannula during and after exposure from all groups and analyzed for their DCE content.

**Analytical Procedure.** DCE concentrations in inhaled and exhaled air were measured with an MT560 GC (Tracer Instruments, Austin, TX) equipped with an electron capture detector (ECD). Air samples were taken with a gastight syringe and injected directly onto an 8-×-1/8-in. stainless steel column packed with 0.1% AT 1000 on Graphpak (Alltech Associates, Deerfield, IL). Operating temperatures were 150°C, injection port; 360°C, ECD; and 70°C, column oven. The nitrogen (carrier gas) flow rate was 40 ml/min, with an additional make-up gas flow rate of 30 ml/min to the detector.

Blood DCE levels were quantified by GC headspace analysis. The volume of blood taken at a given sampling time depended upon the anticipated DCE concentration. Ten- to 100-μl samples were transferred to chilled headspace vials, which were immediately capped with a polytetrafluoroethylene-lined septum and tightly crimped. The vials were placed into the HS-6 headspace autosampler of a Sigma 300 GC (PerkinElmer Life and Analytical Sciences, Boston, MA), equipped with an electron capture detector and a 8-×-1/8-in. stainless steel column packed with FFAP Chromasorb W-AW (80–100 mesh; Alltech Associates). Operating temperatures were as follows: 200°C, injection port, 360°C, ECD; and 70°C, column oven. The carrier gas was 5% argon-methane at a flow rate of 40 ml/min, with a make-up gas flow rate of 20 ml/min to the ECD.

**Toxicity Experiments.** The objectives of these experiments were to elucidate the hepatorenal toxicity of inhaled and ingested DCE and to determine the effect of oral dosage regimen on liver and kidney injury. The higher exposure levels (i.e., 300 ppm and 30 mg/kg) used in the TK studies were used. Fasted male S-D rats of 140 to 150 g were selected for this particular experiment, because Andersen and Jenkins (1977) found older rats were much less susceptible to DCE-induced hepatotoxicity and that fasting markedly increased the severity of liver injury. Use of rats as the animal model necessitated these measures to enhance DCE toxicity, as rats metabolically active less of the chemical than mice and are less susceptible to it (Dowseley et al., 1995).

Groups of 140- to 150-g male S-D rats were subjected to 1) 300 ppm DCE vapors for 2 h, 2) 30 mg/kg DCE p.o., or 3) 30 mg/kg DCE g.i. over 2 h. For inhalation exposures, the animals were maintained individually in a 26.5-l glass chamber equipped with a small fan. The chamber had a port for introduction of DCE and for withdrawal of air samples. These samples were taken every 10 min during the 2-h exposures, and their DCE content was measured by a GC with a flame ionization detector. The chamber concentration ($\pm$ S.E.; $n = 8$) decreased gradually from 306 ± 11 ppm at the beginning of the session to 235 ± 13 ppm at the end of 2 h. As in the TK experiments, orally administrated DCE was incorporated into a 5% aqueous Alkamula emulsion and given in a total volume of 5 ml/kg p.o. or g.i. Controls received 5 ml/kg saline p.o. Blood was taken from the tail artery at 2 h and by cardiac puncture 24 h after dosing for measurement of sorbitol dehydrogenase (SDH) activity by a standard spectrophotometric procedure.

A second subset of rats was kept for evaluation of urinary enzyme excretion over two successive 12-h periods after conclusion of the DCE exposure regimens. These animals were maintained in Nalgene plastic metabolism cages for collection of 12- and 24-h urine over ice. Total N-acetyl-β-D-glucosaminidase (NAG) and γ-glutamyltranspeptidase (GGT) activities were measured colorimetrically in urine voided over ice during each 12-h collection period. NAG and GGT are localized predominantly in the brush border of renal proximal tubular cells.

**TK Analyses.** The intravenous DCE time course data were fitted to a two-compartment model. Data from animals receiving DCE orally were analyzed by noncompartmental analysis. A two-compartment model with a constant input function was fitted to the concentration versus time data for inhalation. Arenal under the blood-concentration time curve (AUC) for the first 2 h ($\text{AUC}_2$), $\text{AUC}_{\infty}$, apparent clearance, and distribution and terminal half-lives $t_1/2$ and $t_1/2p$ were obtained using WinNonlin from Pharsight (Mountain View, CA). The maximal blood concentration ($C_{\text{max}}$) and the time to reach $C_{\text{max}}$ ($T_{\text{max}}$) were observed values. Bioavailability ($F$) was calculated as follows: $F = \frac{\text{AUC}_{\infty}}{\text{dose}_i/v} / \frac{\text{AUC}_{\infty}}{v} x \frac{\text{dose}_{i,v}}{\text{dose}_{i,v}}$.

The model that was the “best fit” for the DCE blood concentration data was determined by model selection criteria including weighted sum of residuals, Akaike’s Information Criteria, $F$ test, and Schwarz criteria.

**Statistical Analyses.** Most results are expressed as mean ± S.D. Comparisons between rate of chemical administration (oral gavage versus gastric infusion) and route of administration (gastric infusion versus inhalation data) were done with Student’s $t$ test with a significance level of $p \leq 0.05$. Comparisons of TK parameters that are not usually dependent on rate/route of administration, such as clearance and $t_1/2$, were made with one-way analysis of variance coupled with the Holm-Sidak multiple comparisons test with a significance level of $p \leq 0.05$.

**Results.**

**Inhalation.** The target chamber concentrations for the DCE inhalation exposures in the kinetics experiments were 100 and 300 ppm. The actual concentrations inhaled by the animals were determined by analyses of air samples taken from sampling ports immediately adjacent to the animal’s miniaturized breathing valve. The inhaled DCE concentrations for the six rats in each group at the initiation of exposures were 101.6 ± 0.8 and 310.0 ± 3.5 ppm (mean ± S.E.) for the 100 and 300 ppm groups, respectively.

Blood concentration versus time profiles revealed that inhaled DCE was rapidly absorbed into and eliminated from the body (Fig. 1). Inhaled DCE was readily available for distribution to body tissues, in that arterial blood concentrations were >50% of near steady state at the first sampling time (i.e., 2 min). Blood concentrations in the 100 and 300 ppm groups reached near equilibrium within 10 to 15 min and increased asymptotically for the duration of the 2-h
exposures (Fig. 1, A and B). The $C_{\text{max}}$, $AUC_0^{2}$, and $AUC_0^{\infty}$ values increased 4.7-, 3.9-, and 3.9-fold, respectively, as the inhaled concentration increased from 100 to 300 ppm (Table 1). DCE concentrations in the exhaled breath exceeded alveolar concentrations during the inhalation exposures, due to the contribution of unabsorbed DCE in the respiratory dead space (Fig. 1). The converse was true after exposure. Exhaled breath levels were relatively low, due to dilution with fresh air residing in the dead space. Alveolar and exhaled breath levels of DCE exhibited a biexponential decline after exposure, with initial precipitous drops. The terminal elimination half-lives for the 100 and 300 ppm groups were both 50 min (Table 1).

Systemically absorbed doses of DCE were determined from the inhalation data. Subtraction of the quantity of DCE exhaled from that inhaled, with allowance for dead space, yielded the amount and percentage of chemical taken up each sampling time. Percentage of systemic uptake diminished somewhat during the first 20 min, due to increase in DCE

### TABLE 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>$C_{\text{max}}$ (mg/l)</th>
<th>$T_{\text{max}}$ (min)</th>
<th>$t_{1/2}$</th>
<th>$AUC_0^{2}$ (µg·min/ml)</th>
<th>$AUC_0^{\infty}$ (µg·min/ml)</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/kg p.o.</td>
<td>2.2 ± 0.8a</td>
<td>7 ± 5a</td>
<td>47 ± 19a</td>
<td>44 ± 15a</td>
<td>51 ± 16a</td>
<td>24.4</td>
</tr>
<tr>
<td>10 mg/kg g.i.</td>
<td>0.2 ± 0.1b,A</td>
<td>146 ± 34b,A</td>
<td>78 ± 16a,A</td>
<td>10 ± 3b,A</td>
<td>33 ± 10a,A</td>
<td>17.1</td>
</tr>
<tr>
<td>100 ppm inhal</td>
<td>0.6 ± 0.1b</td>
<td>55 ± 18b</td>
<td>50 ± 24a,A</td>
<td>62 ± 9B</td>
<td>72 ± 10B</td>
<td>39.4</td>
</tr>
<tr>
<td>30 mg/kg p.o.</td>
<td>8.9 ± 4.1c</td>
<td>5 ± 3a</td>
<td>88 ± 29a</td>
<td>222 ± 64c</td>
<td>233 ± 8c</td>
<td>46.5</td>
</tr>
<tr>
<td>30 mg/kg g.i.</td>
<td>1.9 ± 0.7d,C</td>
<td>128 ± 32b,C</td>
<td>92 ± 38a,B</td>
<td>89 ± 37d,C</td>
<td>279 ± 107c,C</td>
<td>40.1</td>
</tr>
<tr>
<td>300 ppm inhal</td>
<td>2.8 ± 0.7d</td>
<td>90 ± 35c</td>
<td>50 ± 20a,B</td>
<td>241 ± 57d</td>
<td>279 ± 73s,C</td>
<td>55.7</td>
</tr>
</tbody>
</table>

Values that are significantly different ($p < 0.05$) from one another are designated by different superscripts. Lowercase letters are used for comparison of p.o. and g.i. values, whereas uppercase letters are used for comparing g.i. and inhal data.
content in the body and returning pulmonary blood. Thereafter, uptake remained relatively constant at 65 to 72% at near steady state (Fig. 2). Uptake appeared to be largely independent of exposure level during the 2-h sessions, because the 100- and 300-ppm values did not differ significantly from one another at any sampling time. Cumulative uptake over the 2-h exposures could be determined by summing of uptake for each time-period and by monitoring respiratory minute volume. Cumulative systemic uptake is plotted against duration of exposure in Fig. 3. The mean (±S.D.) total systemically absorbed doses at 2 h in the 100- and 300-ppm groups were 9.7 ± 0.9 and 29.7 ± 1.7 mg/kg, respectively. Thus, total systemic uptake was directly proportional to the inhaled concentration.

**Exposure Route.** Ten and 30 mg/kg DCE were administered over 2 h by gastric infusion and the arterial blood time courses contrasted with their corresponding inhalation profiles, to elucidate the influence of route of exposure on internal dosimetry. It is obvious in Fig. 4 that the pattern of systemic uptake and elimination for each gastric infusion group was quite different from that for its corresponding inhalation group. Blood DCE levels progressively increased during the 2-h infusions and then declined at a slow rate after exposure relative to the levels of the inhalation groups. As a result, AUC₀ values for the respective 300 ppm inhalation and 30 mg/kg g.i. groups did not differ significantly (Table 1). The AUC₀ and C_max values for the inhalation group, however, were substantially higher. Similar dosimetry relationships were manifest at the lower exposure level, although differences in the route-dependent kinetic parameters were more pronounced (Table 1).

**Oral Dosage Regimen.** The influence of oral dosage regimen on the TK of DCE was evaluated by comparing arterial blood profiles of the oral bolus and gastric infusion groups (Fig. 5). DCE was rapidly absorbed after it was given as an oral bolus. Blood levels were maximal within 5 to 7 min. With the 3-fold increase in bolus dose, there were 4-, 5-, and 4.6-fold increases in C_max, AUC₀, and AUC₀ values, respectively (Table 1). The 3-fold increase in gastric infusion dose resulted in 9.5-, 8.9-, and 8.5-fold increases in C_max, AUC₀, and AUC₀ values, respectively. Peak blood levels in these animals were markedly lower than those observed in the corresponding bolus groups. Prolonged elevation of blood levels in the gastric infusion groups, however, resulted in AUC₀ and F values that were not substantially different from those for the cor-
responding by mouth groups. The $C_{\text{max}}$ and AUC$_{0\rightarrow\infty}$ values of the oral bolus groups, however, were markedly higher.

The TK of DCE was not dose-dependent in the intravenous dosage range studied here. Ten and 30 mg/kg DCE were injected intravenously as a reference to determine bioavailability. The blood-concentration profiles are shown in Fig. 6.

An initial rapid drop in blood levels is followed by a relatively slow decline. There were no changes with dose in clearance or in half-life during the distribution phase ($\alpha$) or the terminal elimination phase ($\beta$) (Table 2). There was a 2.6-fold increase in AUC$_0$ with the 3-fold increase in dosage. Half-life did not vary significantly with dose, irregardless of oral dosage regimen or route of exposure (Table 1). Clearance did not vary significantly with dose, although it did tend to be lower in the 10 mg/kg groups for each of the three exposure scenarios (data not shown). The $F$ values were also consistently lower in each instance at the lower dose (Table 1). First-pass hepatic and pulmonary elimination was clearly most efficient in the 10 mg/kg g.i. group ($F = 17\%$), in which the liver was presented with the lowest DCE levels at the slowest rate. The elimination rate constants ($K_{12}$ and $K_{21}$) indicated a rapid transfer from blood to tissues, followed by a slower release from tissues back to blood (Table 2).

**Toxicity.** The extent of liver damage by DCE was influenced substantially by the oral dosage regimen, although only slightly by the exposure route. All three modes of administration produced statistically significant increases in serum SDH activity over controls 2 and 24 h after dosing (Fig. 7). It is interesting that the pronounced elevation at 2 h in the gavaged rats was followed by only a modest rise at 24 h. This pattern suggests rapid damage of the more susceptible hepatocytes. The phenomenon was not exhibited by the other exposure groups. SDH activity in rats inhaling DCE was modestly higher than in the gastric infusion rats 2 and 24 h after exposure.

The route dependence of kidney injury by DCE was quite different from that of the liver. Urinary GGT excretion was markedly elevated over controls in rats that inhaled 300 ppm DCE for 120 min (Fig. 8A). The amounts of GGT eliminated during each 12-h period after exposure were comparable. Urinary GGT amounts were not elevated over controls 12- or 24-h after exposure in animals dosed by gavage or gastric infusion. Exposure route- and time-dependent changes in urinary NAG excretion largely paralleled the changes in GGT, the other brush-border enzyme (Fig. 8B). There appeared to be modest increases over controls in urinary excretion of NAG in the gastric infusion group at 12 and 24 h, but the apparent increases were not sufficient to be statistically significant. Administration of DCE as an oral bolus did not enhance GGT or NAG excretion over that in controls or the gastric infusion group.

**Discussion**

There is a paucity of TK data relevant to route-to-route and dosage regimen-to-regimen extrapolations. Few study protocols have included comparable exposures of rodents to a VOC orally and by inhalation. In such cases, the test chemical has typically been administered over different time frames (e.g., inhalation for several h versus ingestion of a single bolus). Systemically absorbed doses have rarely been determined for inhalation exposures. Our experimental approach was unique in that it involved administration of equivalent doses of chemical over the same time frame by inhalation and gastric infusion. This seemed a logical approach to a rather complex problem, namely, comparing the kinetics and toxicity of a chemical entering the sys-
temic circulation at different locations from different portals at different rates.

Findings in the present study demonstrate that route of exposure has a significant effect on the TK of DCE. The lungs are an optimal site for absorption of DCE and other VOCs, due to their relatively large surface area, high blood perfusion rate, and intimate alveolar-capillary interfaces (Bruckner et al., 2008). Rapid, extensive absorption of DCE into the arterial circulation occurs with the small, uncharged, lipophilic molecule. Blood concentrations are more than 50% of their $C_{\text{max}}$ 2 min after initiation of exposures. Relatively high concentrations of DCE are thus quickly reaching the lungs, kidneys, and other tissues of animals inhaling the chemical. DCE is also rapidly absorbed from an aqueous emulsion in the gastrointestinal tract of fasted rats, as reflected by $T_{\text{max}}$ values of 5 and 7 min. Nevertheless, the VOC is subject to first-pass hepatic and pulmonary elimination. Their influence on gastrically infused DCE is evidenced by relatively low $C_{\text{max}}$, $AUC_0^\infty$, $AUC_\infty^0$, and bioavailability values, notably at the lower dose (Table 2), which is apparently more efficiently removed by the liver. Lee et al. (1996) report that presystemic elimination of TCE in rats is inversely proportional to dose in this dosage range.

Route of administration had a pronounced influence on the extent of kidney injury by the higher DCE dosage used in the current study. The kidneys were subjected for 2 h to relatively large quantities of the chemical during inhalation of 300 ppm DCE for 120 min. The height of each bar represents the mean ± S.D. for six rats. Values that are significantly different from one another are designated by different superscripts.

TABLE 2
Pharmacokinetic parameter estimates for intravenous DCE
Serial blood samples were taken from rats for DCE analysis after intravenous injection of 10 or 30 mg DCE/kg. Values are means ± S.D. for groups of six rats.

<table>
<thead>
<tr>
<th>Dose</th>
<th>$t_{1/2}$</th>
<th>$t_{1/2}$</th>
<th>CL</th>
<th>$AUC_0^\infty$</th>
<th>$K_{12}$</th>
<th>$K_{21}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/kg</td>
<td>4.5 ± 2.2</td>
<td>62.6 ± 16</td>
<td>65 ± 17</td>
<td>165 ± 50</td>
<td>0.086 ± 0.04</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>4.9 ± 0.8</td>
<td>64.3 ± 21</td>
<td>72 ± 24</td>
<td>402 ± 114*</td>
<td>0.086 ± 0.02</td>
<td>0.021 ± 0.005</td>
</tr>
</tbody>
</table>
* Significantly different from corresponding 10-mg/kg value at $p \leq 0.05$. No other parameters vary significantly with dose.

Fig. 7. SDH levels 2 and 24 h after rats were untreated (controls) or given 30 mg/kg DCE by gavage (p.o.), by constant gastric infusion (g.i.), or by inhalation of 300 ppm DCE for 120 min. The height of each bar represents the mean ± S.D. for six rats. Values that are significantly different from one another are designated by different superscripts.

Fig. 8. Urinary excretion of GGT (A) and NAG (B) activities during successive 12-h periods in controls and in rats after administration of 30 mg/kg DCE by gavage (p.o.) or by constant gastric infusion (g.i.) over 2 h, as well as inhalation (inh) of 300 ppm DCE for 2 h. Bar heights represent mean ± S.D. for groups of six rats. Different superscripts designate statistically significant differences.

DCE is biotransformed by CYP2E1 in rodents to at least three reactive metabolites: DCE epoxide; 2-chloroacetyl chloride; and 2,2-dichloroacetaldehyde. Forkert (2001) and Simmonds et al. (2004) conclude from studies of mouse liver and lung that DCE epoxide is the most important cytotoxic metabolite. Dowsley et al. (1999) also find CYP2E1-mediated
DCE epoxide formation in human liver and lung microsomes. Martin et al. (2003) report that mitochondrial dysfunction is an early event in hepatotoxicity. DCE electrophilic metabolites can damage hepatocytes (Jones and Liebler, 2000) and renal tubular cells (Brittebo et al., 1993) by binding covalently to their proteins and nucleic acids. DCE cytotoxicity and covalent binding are greatest in murine cells with the highest CYP2E1 content, namely, centrifibular hepatocytes, bronchiolar Clara cells, and renal proximal tubular cells (Speerschneider and Dekant, 1995; Forkert, 2001). Cummings et al. (2001) report higher CYP2E1 content in proximal than in distal tubular cells in kidneys of male F344 rats. Nephrotoxicity in the present study was evidenced by urinary excretion of large amounts of brush-border enzymes.

The hepatic glutathione (GSH)/renal β-lyase biotransformation pathway plays a major role in the nephrotoxicity and carcinogenicity of halocarbons such as TCE and perchloroethylene, although this is not the case for DCE (Dekant, 1996). DCE epoxide and 2-chloroacetyl chloride are detoxified by GSH conjugation and hydrolysis, respectively. GSH affords cells protection until DCE metabolites are formed in quantities sufficient to deplete the tripeptide (Brittebo et al., 1993; Forkert, 2001). 2,2-Dichloroacetaldehyde, a minor metabolite of DCE in mouse liver microsomes that undergoes GSH conjugation and subsequent metabolism by renal β-lyase, has not been detected in vivo (Forkert, 2001).

There are very few experimental data available with which to contrast the TK of a VOC ingested in divided doses with that when the total dose is given as a single oral bolus. Internal or systemically absorbed doses are usually unknown when VOCs are consumed in water. The intersubject rate and pattern of ingestion vary widely in different circumstances. Under the exposure condition (i.e., 2-h constant gastric infusion) selected for the current investigation, arterial blood levels progressively rise for the 2 h. The levels never approach the bolus $C_{\text{max}}$, but remain elevated longer. The corresponding gastric infusion and oral $AUC_{\text{inf}}$ values are therefore not substantially different (Table 1). $C_{\text{max}}$ and $AUC_{\text{inf}}$ are obviously much better dosimeters for liver damage by orally administered DCE. Rao and Recknagel (1969) report carbon tetrachloride (CC14) is absorbed, taken up by orally administered DCE. Rao and Recknagel (1969) re-

In conclusion, findings in the current investigation illustrate that basic features of experimental designs of toxicology studies of VOCs can substantially alter the chemicals’ TK, and in turn the magnitude of adverse effects they produce. Oral administration of toxic members (e.g., DCE and CC14) of this class of chemicals as an oral bolus results in their rapid absorption and delivery to the liver in such large quantities, that once metabolically activated can exceed the capacity of hepatocellular protective and repair mechanisms. Such thresholds may not be reached when the same total dose is ingested in divided doses over a longer period. The blood $C_{\text{max}}$ and $AUC_{\text{inf}}$ are obviously appropriate dosimeters or determinants of hepatocytotoxicity in the present scenario rather than $AUC_{\text{inf}}$. It is interesting to note that the very high arterial DCE levels (and assumed higher renal exposure) in bolus animals did not result in more pronounced kidney injury than in gastric infusion animals. It is quite possible that DCE was eliminated so rapidly from the systemic circulation by metabolism and exhalation that it did not remain long enough in the kidney to deplete GSH sufficiently to initiate cytotoxicity. Chieco et al. (1981) demonstrated that DCE was more injurious to rats when cytotoxic levels were present for a period of sufficient duration to significantly depress GSH. Inhaled DCE may have produced marked GSH depletion and renal injury as a result of maintenance of relatively high arterial and renal DCE levels throughout the 2-h exposure. It is clear from the current study that direct extrapolation of results from ingestion experiments to predict hazards of an inhaled VOC may very well underpredict risks to extrahepatic organs such as the kidney. The foregoing data illustrate the importance of designing experimental protocols that are as close to actual human exposure scenarios as possible.

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