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INFLUENCE OF EXPOSURE ROUTE AND ORAL DOSAGE REGIMEN ON 1,1-DICHLOROETHYLENE TOXICOKINETICS AND TARGET ORGAN TOXICITY

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Running Title: 1,1-DCE Kinetics & Toxicity: Exposure Route & Dosage Regimen

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Nonstandard Abbreviations: 1,1-dichloroethylene (DCE), volatile organic chemical (VOC), toxicokinetic (TK), physiologically-based toxicokinetic (PBTK) model, sorbitol dehydrogenase (SDH), N-acetyl-β-D-glucosaminidase (NAG), gamma-glutamyltranspeptidase (GGT)

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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 DCE/kg, respectively. Other groups of rats received 10 or 30 mg DCE/kg by i.v. injection, bolus gavage (p.o.), or gastric infusion (g.i.) over a 2-h period. Serial micro blood 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 concentration time curves (AUC^2_0) than did g.i. of the same dose over the same time-frame at each dosage level, though inhalation (AUC^∞_o) values were only modestly higher. Urinary N-acetyl-β-D-glucosaminidase (NAG) and gamma-glutamyltranspeptidase (GGT) activities were monitored as indices of kidney injury in the high-dose groups. NAG and GGT excretion were much more pronounced following inhalation than g.i. Administration of DCE by gavage also produced much higher C_{max} and AUC^2_0 values than did 2-h g.i., though AUC^∞_0 values were little 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 g.i. 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.

Introduction

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 contributes significantly to one's systemically absorbed dose (Gordon et al., 2006; Weisel and Jo, 1996). 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. Also, oral cancer and non-cancer studies of VOCs in rodents have usually employed 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 utilized to produce flexible films (e.g., Saranwrap®, Velon®) for food packaging. Environmental releases occur primarily by evaporation, though 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 (TRI), trichloroethylene (TCE) and perchloroethylene (PERC) (ATSDR, 1994). These are the most frequently found organic chemicals in groundwater in the proximity of hazardous waste sites (Fay, 2006) and in drinking water in the U.S. (Moran et al., 2007).

Exposure to low levels of DCE in environmental media is of concern, largely because of the VOC's cytotoxic and carcinogenic potential. The primary target organs of DCE are the liver, lungs and kidneys (ATSDR, 1994; IRIS, 2002). Maltoni et al. (1985) describe significant

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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 P450IIE1 (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

neoplastic changes. Ponomarkov and Tomatis (1980), however, observed a modest increase in

liver adenomas and carcinomas in rats gavaged once weekly with 50 mg/kg of the chemical.

Thus, it appears that DCE's toxicity and carcinogenicity potential 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 DCE's target organ dosimetry and resulting toxic/carcinogenic potential.

VOCs absorbed from the gastrointestinal (GI) 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 – 16% in rats given 100 µg

TCE/kg, 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 utilized 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: a) Administration of equivalent oral and inhaled doses of DCE will result

in significantly different internal doses, with more pronounced differences at lower dosage-

levels; b) Inhalation will result in higher arterial DCE levels and greater extrahepatic (i.e., renal

injury) than ingestion; c) 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.

Methods

Chemicals. 1,1-Dichloroethylene (DCE) of 99% purity was purchased from Aldrich Chemical

Co. (Milwaukee, WI). The purity was verified by gas chromatography. All other chemicals and

biochemicals were obtained from Sigma Chemical Co (St. Louis, MO). Acepromazine

hydrochloride and ketamine hydrochloride were purchased from Fort Dodge Laboratories (Fort

Dodge, IA). Bayer Corporation (Shawnee Mission, KS) supplied xylazine hydrochloride.

Animals. Male Sprague-Dawley (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 AM to 7

PM. They were housed in groups of 3 in stainless steel wire mesh cages in a negative air-flow animal rack. The rack was kept in a biohazards facility in an AAALAC-approved facility. The rats were maintained in a temperature ($25^{°}$ C)- and humidity (40%)-controlled room for at least 1 week prior to use. 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 325-375 g and 140-150 g, respectively. The larger rats were necessary for inhalation experiments and for serial micro blood 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 i.m. injection of 0.1 ml/100 g   b.w. 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 i.v. dosing. For gastric (g.i.) infusion experiments, a ventral incision was made in the abdominal wall, and a flare-tipped cannula 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 cannulation surgery was performed exhibited CYP2E1 activity similar to that of non-fasted controls (data not shown).

Inhalation Exposures. Exposures to DCE were conducted as described by Dallas et al. (1989). Each cannulated rat was placed into a restraining tube (Battelle-Geneve, 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, Ardsley, 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-h 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 post exposure for DCE analysis.

Respiratory Measurements. The airflow created by an animal's inspiration was recorded, so the minute volume (V_E), respiratory rate (f), and tidal volume (V_T) could be determined during and following the inhalation exposures. A mean value for these parameters was obtained for each rat by averaging measurements made at 10-min intervals. Mean values ($\pm$ S.D.) (n = 6) for the 2-h, 100-ppm inhalation sessions were: $V_E = 230 \pm 18$ ml/min; f = 136 $\pm$ 6 breaths/min; and $V_T = 1.7 \pm 0.2$ ml. Mean values ($\pm$ S.D.) (n = 6) for 2-h, 300-ppm sessions were: $V_E = 216 \pm 37$ ml/min; f = 135 $\pm$ 23 breaths/min; and $V_T = 1.6 \pm 0.2$ 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

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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 systemic uptake and cumulative uptake

during the 2-h exposures.

Oral and I.V. Dosage Regimens. Ten and 30 mg DCE/kg, the approximate cumulative doses

absorbed during the 2-h, 100- and 300-ppm inhalation sessions, were administered to

unanesthetized, freely-moving rats i.v., by gavage (p.o.), and by gastric infusion (g.i.). Aqueous

emulsions were prepared just prior to dosing using 5% Alkamuls® (Rhone Poulenc, Cranbury,

NJ). The concentration of DCE in each emulsion was verified by gas chromatography (GC)

headspace analysis. A total volume of 5 ml/kg of 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, Southnatick, MA)

was used to infuse DCE via an indwelling gastric cannula for 2 h. Ten and 30 my DCE/kg were

injected over 10 sec into the right jugular vein of other groups of rats. Serial blood samples were

taken from the arterial cannula during and post exposure from all groups and analyzed for their

DCE content.

Analytical Procedure. DCE concentrations in inhaled and exhaled air were measured with a

Tracor MT560 gas chromatograph (GC) (Tracor Instruments, Austin, TX) equipped with an

electron capture detector (ECD). Air samples were taken with a gas-tight syringe and injected

directly onto an 8-ft x ⅛-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 (Perkin-Elmer, Norwalk, CT), equipped with an electron capture detector and a 8-ft x ⅛-in stainless steel column packed with FFAP Chromasorb W-AW (80-100 mesh) (Alltech Associates, Deerfield, IL). Operating temperatures were: 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) employed in the TK studies were utilized. Fasted male S-D rats of 140 to 150 g were selected for this particular experiment, since 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 activate less of the chemical than mice and are less susceptible to it (Dowsley et al., 1995).

Groups of 140- to 150-g male S-D rats were subjected to: (a) 300 ppm DCE vapors for 2 h; (b) 30 mg DCE/kg p.o.; or (c) 30 mg DCE/kg 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 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 238 ± 13 ppm at the end of the 2 h. As in the TK experiments, orally-administered DCE was incorporated into a 5% aqueous Alkamuls® emulsion and given in a total volume of 5 ml/kg p.o. or g.i. Controls received 5 ml saline/kg p.o. Blood was taken from the tail artery at 2 h and by cardiac puncture 24 h post 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 urines over ice. Total N-acetyl-β-D-glucosaminidase (NAG) and gamma-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.

Toxicokinetic (TK) Analyses. The i.v. 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. Area under the blood concentration time curve (AUC) for the first 2 h (AUC^2_0), AUC^∞_0, apparent clearance (CL), and distribution and terminal half-lives alpha ($t\frac{1}{2}\alpha$) and beta ($t\frac{1}{2}\beta$) were obtained using WinNonlin from Pharsight

(Cary, NC). The maximum blood concentration (C_{max}) and the time to reach C_{max} (T_{max}) were observed values. Bioavailability (F) was calculated as follows (Equation 1):

$$F = \frac{AUC_{oral} \, Dose_{iv}}{AUC_{iv} \, Dose_{oral}}$$

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 (AIC), F test and Schwarz criteria (SC).

Statistical analyses. Most results are expressed as mean ± S.D. Comparisons between rate of chemical administration (oral gavage vs. gastric infusion) and route of administration (gastric infusion vs. 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 $t\frac{1}{2}$, were made with one-way ANOVA 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 6 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 (Figs. 1A and B). The C_{max}, AUC^2_0 and AUC^∞_0 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 post 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 post 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

systemic uptake diminished somewhat during the first 20 min, due to increase in DCE content in

the body and returning pulmonary blood. Thereafter, uptake remained relatively constant at 65 –

72% at near steady-state (Fig. 2). Uptake appeared to be largely independent of exposure level

during the 2-h sessions, as 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

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± 1.7 mg/kg, respectively. Thus, total systemic uptake was directly proportional to the inhaled concentration.

Exposure Route. Ten and 30 mg DCE/kg were administered over 2 h by g.i. and the arterial blood time-courses contrasted with their corresponding inhalation profiles, in order 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 g.i. 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 post exposure relative to the inhalation groups' levels. As a result, AUC^{∞}_0 values for the respective 300-ppm inhalation and 30 mg/kg g.i. groups did not differ significantly (Table 1). The AUC^2_0 and C_{max} values for the inhalation group, however, were substantially higher. Similar dosimetry relationships were manifest at the lower exposure level, though 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 p.o. and g.i. 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}, AUC^2_0 and AUC^{∞}_0, respectively (Table 1). The 3-fold increase in g.i. dose resulted in 9.5-, 8.9- and 8.5-fold increases in C_{max}, AUC^2_0 and AUC^{∞}_0 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 g.i. groups, however, resulted in AUC^{∞}_0 and F values that were not substantially different from those for the corresponding p.o. groups. The p.o. groups' C_{max} and AUC^2_0 values, however, were markedly higher.

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The TK of DCE was not dose-dependent in the i.v. dosage range studied here. Ten and 30 mg DCE/kg were injected i.v. 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 (α) or the terminal elimination phase (β) (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, though it did tend to be lower in the 10 mg/kg groups for each of the three exposure scenarios (data not shown). Bioavailability (F) was 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, though 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). Interestingly, 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 g.i. rats 2 and 24 h post 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

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for 120 min (Fig. 8A). The amounts of GGT eliminated during each 12-h period post exposure

were comparable. Urinary GGT amounts were not elevated over controls 12- or 24-h post

exposure in animals dosed by gavage or g.i. 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 g.i. 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 g.i. 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 g.i. This seemed a logical approach to a

rather complex problem, namely comparing the kinetics and toxicity of a chemical entering the

systemic 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_{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 GI tract of fasted rats, as reflected by T_{maxs} 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_{max}, AUC^2_0, AUC^∞_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 (Fig. 4), due to the elevated arterial concentrations (2 – 3 µg/ml) and the high rate of renal blood flow. Jackson and Conolly (1985) observed increases in kidney/b.w. ratio and in serum urea nitrogen and creatinine, as well as morphological changes in the renal cortex of fasted male S-D rats that inhaled ≥ 250 ppm DCE for 4 h. It is worthy of note in the current study that the exposure route had an even greater effect on TK at the lower dosage, where nephrotoxicity was not evaluated.

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 centrilobular hepatocytes,

bronchiolar Clara cells and renal proximal tubular cells (Forkert, 2001; Speerschneider and

Dekant, 1995). 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 PERC, though

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_{max}, but

remain elevated longer. The corresponding g.i. and p.o. AUC^{∞}_{0} values are therefore not

substantially different (Table 1). C_{max} and AUC^{2}_{0} are obviously much better dosimeters for liver

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damage by orally-administered DCE. Rao and Recknagel (1969) report carbon tetrachloride

($CC1_4$) is absorbed, taken up by the liver, and produces lipoperoxidation and covalent binding

within 5 min of oral administration to rats. Sufficiently large bolus doses of DCE and $CC1_4$

apparently produce hepatocellular concentrations that exceed the capacity of the cells'

detoxification (e.g., GSH conjugation) and repair mechanisms.

There have been a number of instances in which adverse effects of VOCs ingested in

divided doses were very different from those seen in experiments employing oral bolus dosing.

Chloroform ($CHC1_3$), 1, 1- and 1, 2-dichloroethane have produced a high incidence of

hepatocellular carcinoma when administered to B6C3F1 mice by gavage. Jorgenson et al.

(1985) and Klaunig et al. (1986) found no evidence of hepatic tumors when comparable doses

were supplied in the animals' drinking water. Larson et al. (1994) and Coffin et al. (2000) found

$CHC1_3$ to enhance hepatocellular proliferation and/or to decrease methylation of the promoter

region of the *c-myc* gene in mouse liver when given by gavage. Little if any change occurred in

mice ingesting equivalent daily doses of the chemical in water *ad libitum*. La et al. (1996)

measured greater DNA binding and cellular proliferation in the liver, kidney and forestomach of

male B6C3F1 mice gavaged with 1, 2, 3-trichloropropane than in mice receiving the halocarbon

in drinking water. The latter research groups did not conduct TK experiments, but it is quite

likely their and the other dissimilar responses to the disparate dosage regimens were attributable

to substantial differences in target organ dosimetry. Use of results from gavage studies are thus

likely to often substantially overestimate toxic and carcinogenic risks to the liver of VOCs in

food or drinking water.

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, CC1$_4$) 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_{max} and AUC^2_0 are obviously appropriate dosimeters, or determinants of hepatocytotoxicity in the present scenario rather than AUC^∞_0. Interestingly, the very high arterial DCE levels (and assumed higher renal exposure) in bolus animals did not result in more pronounced kidney injury than in g.i. 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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Acknowledgements

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FOOTNOTES

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FIGURE LEGENDS

1.   Time-courses of DCE concentrations in exhaled breath (•), alveolar air (o) and arterial

blood (♦) of rats inhaling 100 (A) and 300 (B) ppm DCE for 120 min, followed by a 180- to

210-min post exposure period. Each point and brackets represent the mean ± S.D. for 6 rats.

2.   Percentage systemic uptake of DCE during 120 min of inhalation of 100 or 300 ppm

DCE. Each point and brackets represent mean ± S.D. for 6 rats.

3.   Cumulative systemic uptake of DCE during inhalation of 100 or 300 ppm DCE for 120

min. Each point represents the mean ± S.D. for 6 rats.   The connecting lines are drawn point to

point.

4.   Effect of exposure route on arterial blood DCE concentration time-courses. One group of

rats inhaled 100 ppm DCE for 120 min, while another group received an equivalent dose of 10

mg DCE/kg by constant gastric infusion (g.i.) over a 120-min period (A). Other groups of rats

were similarly exposed to 300 ppm and 30 mg/kg (B). Brackets encase mean ± S.D. for groups

of 6 rats.

5.   Influence of oral dosage regimen on arterial blood DCE concentration time-courses. In

A, rats were given 10 mg DCE/kg as an aqueous emulsion by gavage (p.o.) or by constant gastric

infusion (g.i.) over 120 min. In B, rats were similarly administered 30 mg/kg. Brackets encase

mean ± S.D. for groups of 6 rats.

6.   Concentrations of DCE in the arterial blood of rats following i.v. injection of 10 or 30 mg DCE/kg. Brackets encase mean ± S.D. for groups of 6 animals.

7.   Serum sorbitol dehydrogenase (SDH) levels 2 and 24 h after rats were untreated (controls) or given 30 mg DCE/kg 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 6 rats. Values that are significantly different from one another are designated by different superscripts.

8.   Urinary excretion of (A) gamma-glutamyltranspeptidase (GGT) and (B) *N*-acetyl-*β-D* glucosaminidase (NAG) activities during successive 12-h periods in controls and in rats following administration of 30 mg DCE/kg 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 6 rats. Different superscripts designate statistically significant differences.

TABLE 1

Influence of dose, route and pattern of exposure on DCE toxicokinetic parameter estimates

Dose (mg/kg) p.o. & g.i. (ppm) inhal	C_{max} (mg/L)	T_{max} (min)	$t\frac{1}{2}$ (min)	AUC^2_0 (µg-min/ml)	AUC^∞_0 (µg-min/ml)	F (%)
10 p.o.	2.2 ± 0.8^a	7 ± 5^a	47 ± 19^a	44 ± 15^a	51 ± 16^a	24.4
10 g.i.	$0.2 \pm 0.1^{b,A}$	$146 \pm 34^{b,A}$	$78 \pm 16^{a,A}$	$10 \pm 3^{b,A}$	$33 \pm 10^{a,A}$	17.1
100 inhal	0.6 ± 0.1^B	55 ± 18^B	$50 \pm 24^{a,A}$	62 ± 9^B	72 ± 10^B	39.4
30 p.o.	8.9 ± 4.1^c	5 ± 3^a	88 ± 29^a	222 ± 64^c	233 ± 8^c	46.5
30 g.i.	$1.9 \pm 0.7^{d,C}$	$128 \pm 32^{b,C}$	$92 \pm 38^{a,B}$	$89 \pm 37^{d,C}$	$279 \pm 107^{c,C}$	40.1
300 inhal	2.8 ± 0.7^D	90 ± 35^C	$50 \pm 20^{a,B}$	241 ± 57^D	$279 \pm 73^{c,C}$	55.7

Rats were administered 10 or 30 mg DCE/kg as an oral bolus (p.o.) or over a 2-h period by constant gastric infusion (g.i.). Other groups of rats inhaled (inhal) 100 or 300 ppm DCE for 2 h. Serial arterial samples were taken for DCE analysis during and after exposures to characterize blood profiles. Values are means ± S.D. for groups of 6 rats. Values that are significantly different ($p < 0.05$) from one another are designated by different superscripts. Lower case letters are used for comparison of p.o. and g.i. values, while upper case letters are used for comparing g.i. and inhal data.

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TABLE 2

Pharmacokinetic parameter estimates for i.v. DCE

Dose (mg/kg)	$t\frac{1}{2}_\alpha$ (min)	$t\frac{1}{2}_\beta$ (min)	CL (L/min-kg)	AUC^∞_0 (μg-min/ml)	K12 (min)$^{-1}$	K21 (min)$^{-1}$
10	4.5 ± 2.2	62.6 ± 16	65 ± 17	165 ± 50	0.086 ± 0.04	0.02 ± 0.02
30	4.9 ± 0.8	64.3 ± 21	72 ± 24	402 ± 114[*]	0.086 ± 0.02	0.021 ± 0.005

Serial blood samples were taken from rats for DCE analysis following i.v. injection of 10 or 30 mg DCE/kg. Values are means ± S.D. for groups of 6 rats. [*]Significantly different from corresponding 10 mg/kg value at $p \leq 0.05$. No other parameters vary significantly with dose.
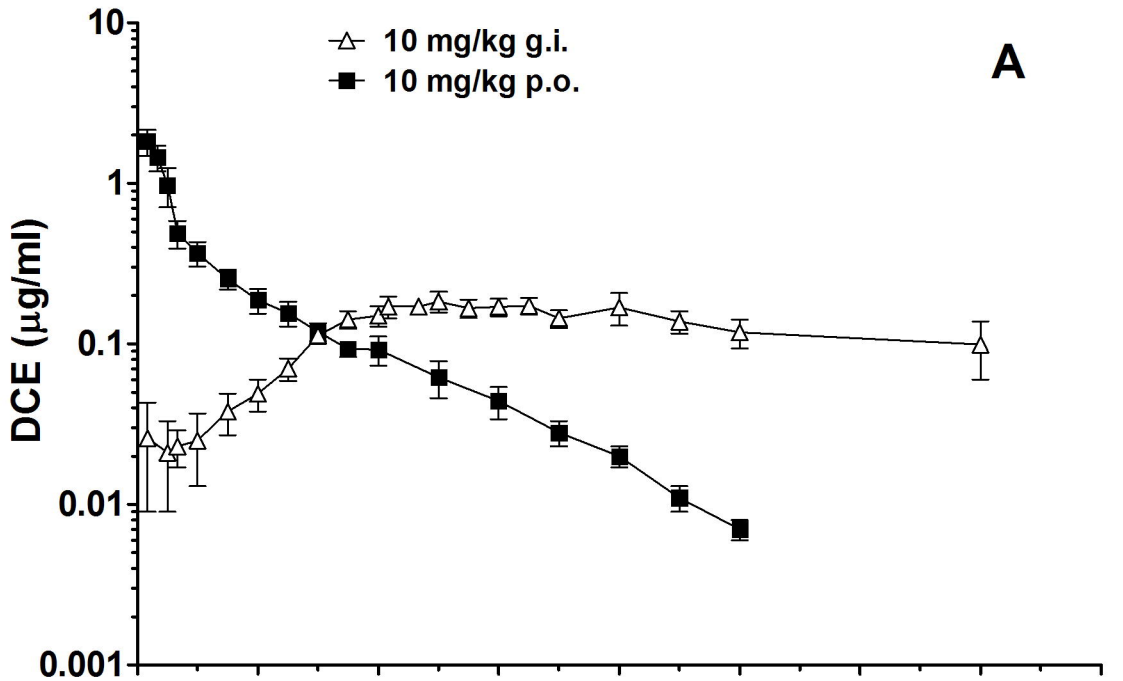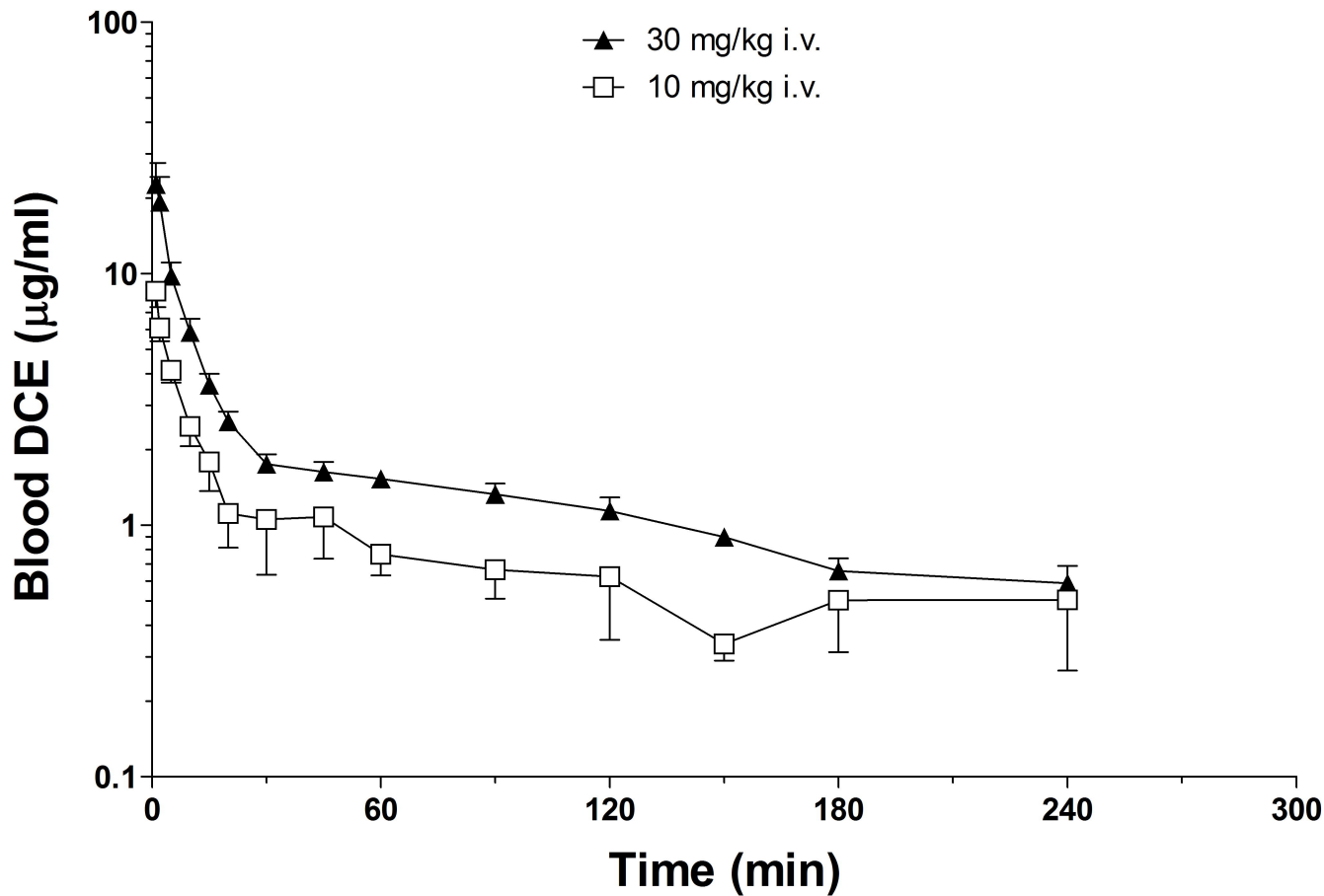
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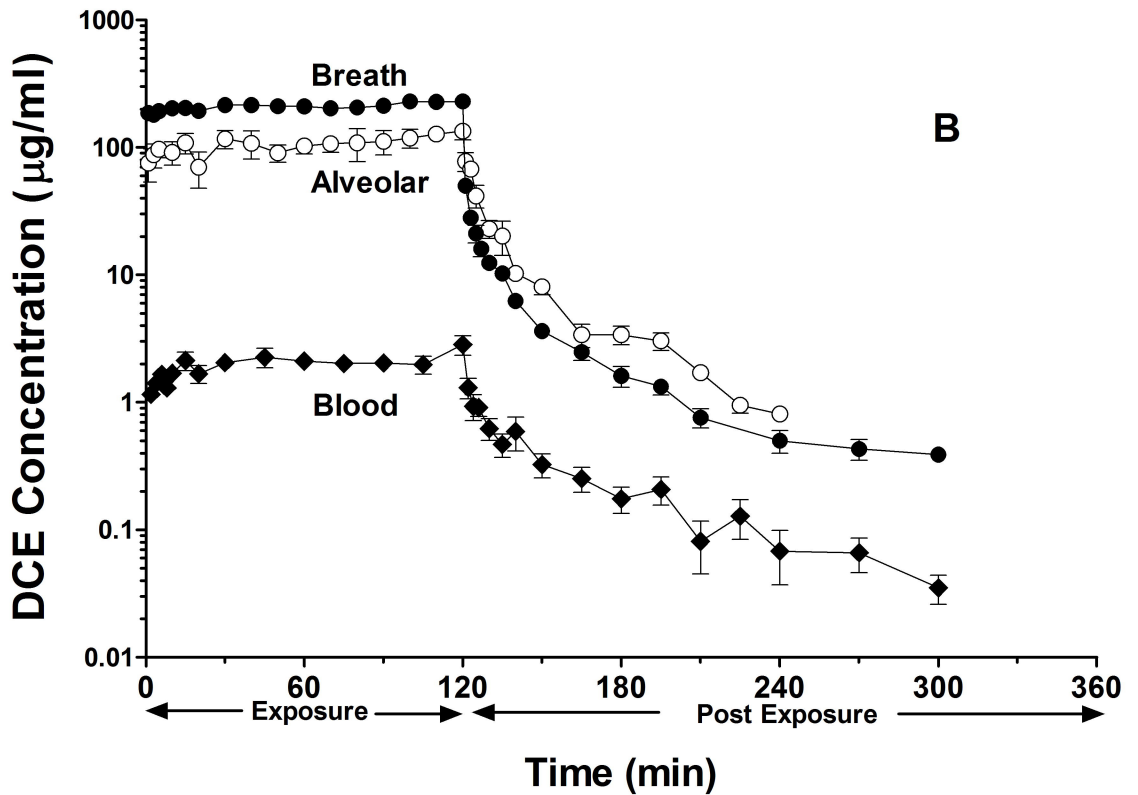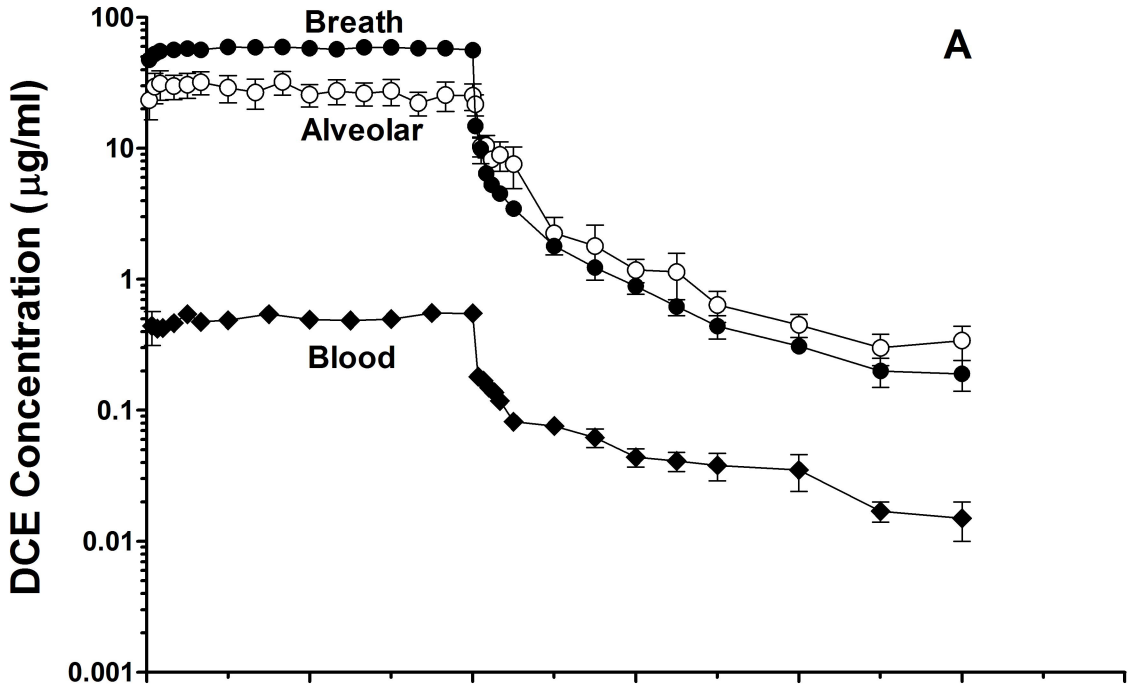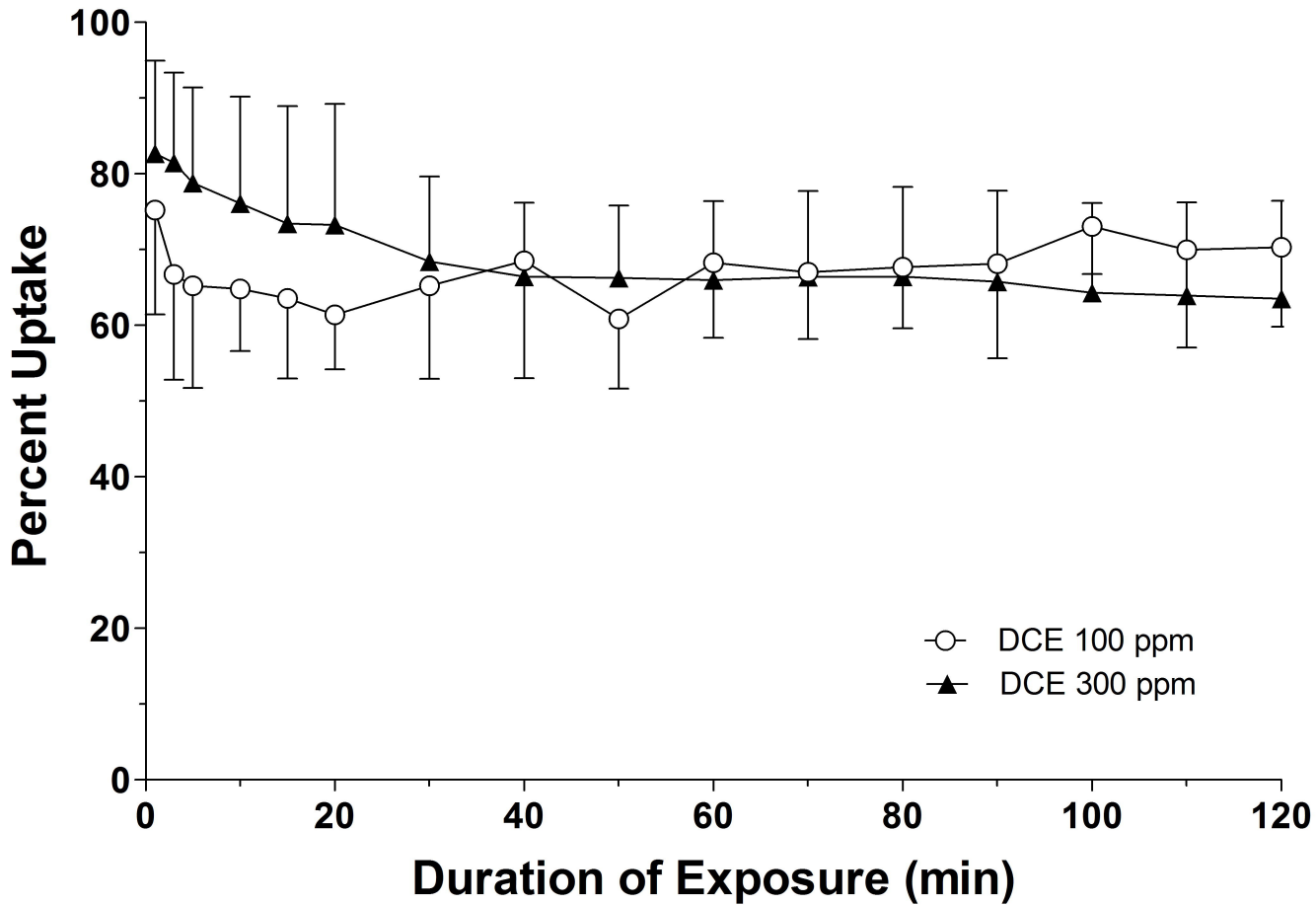
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Figure 2

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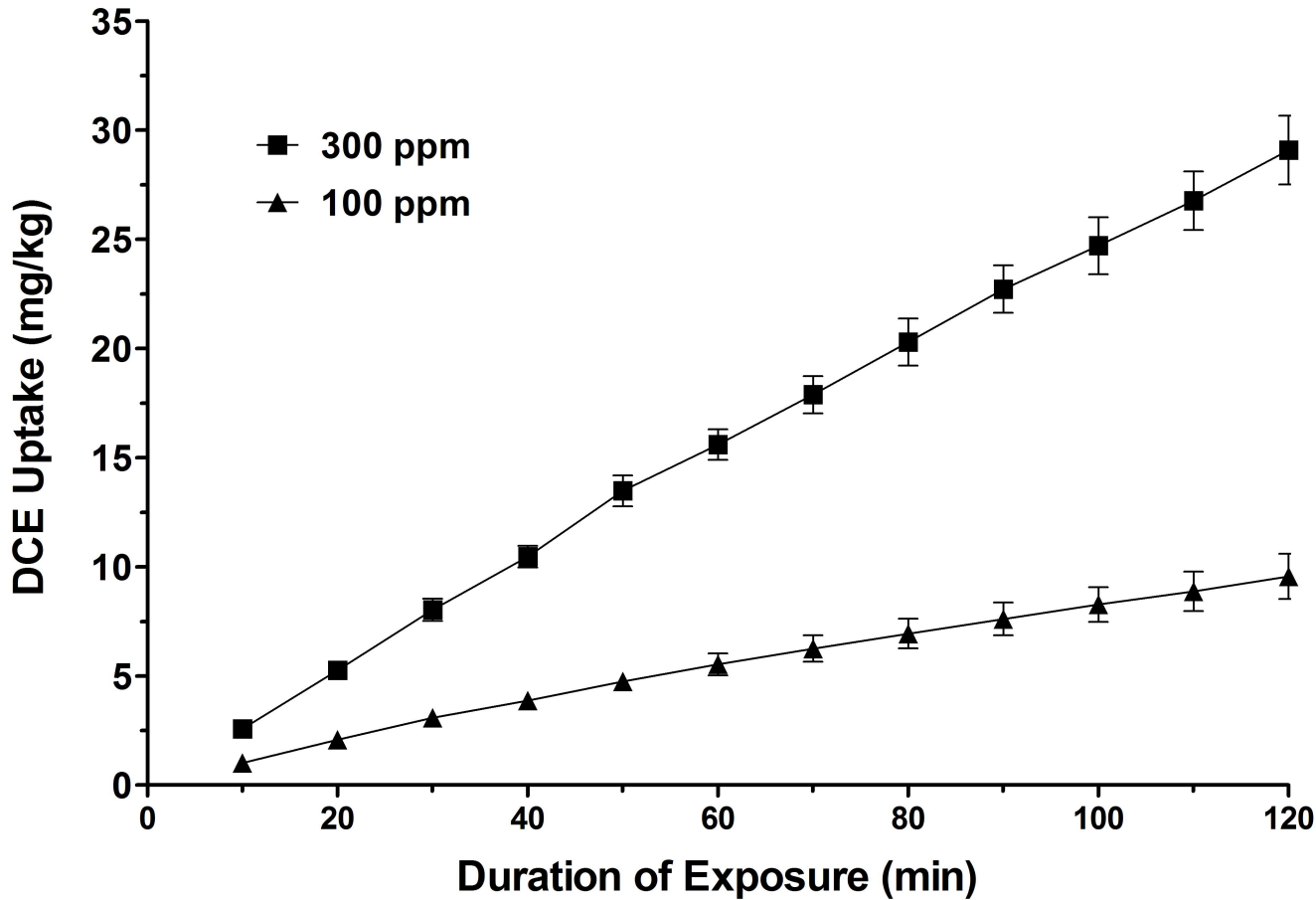
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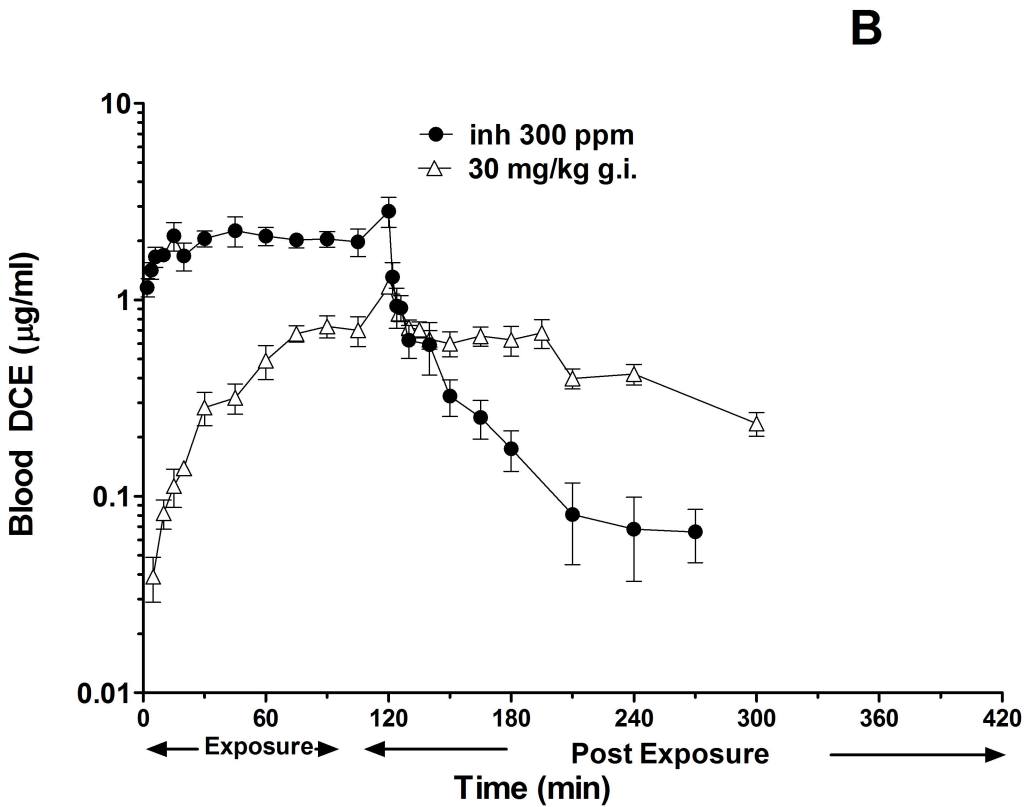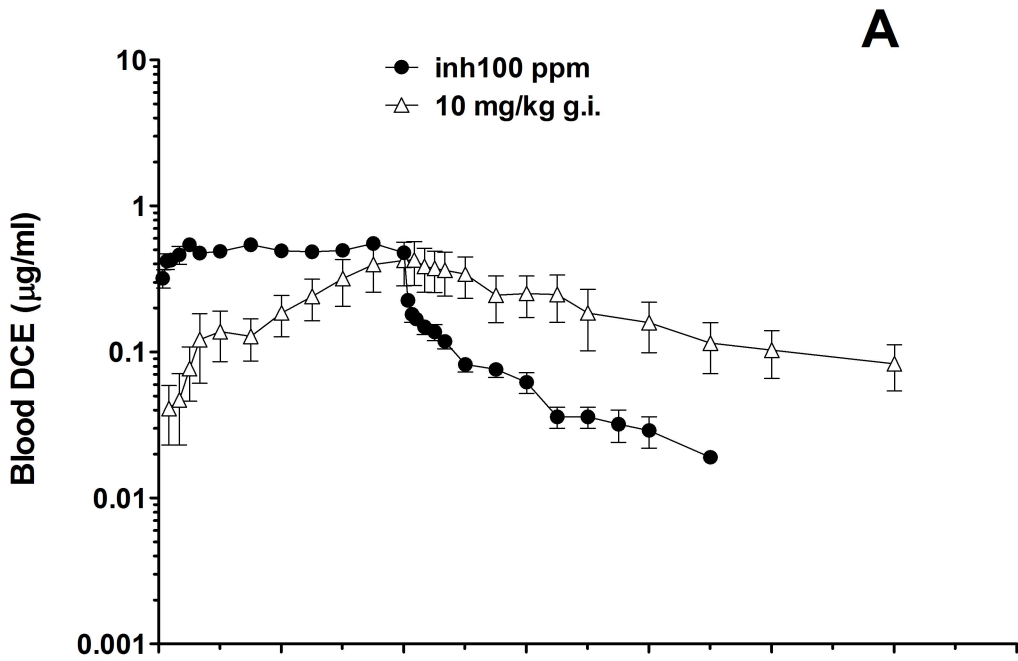
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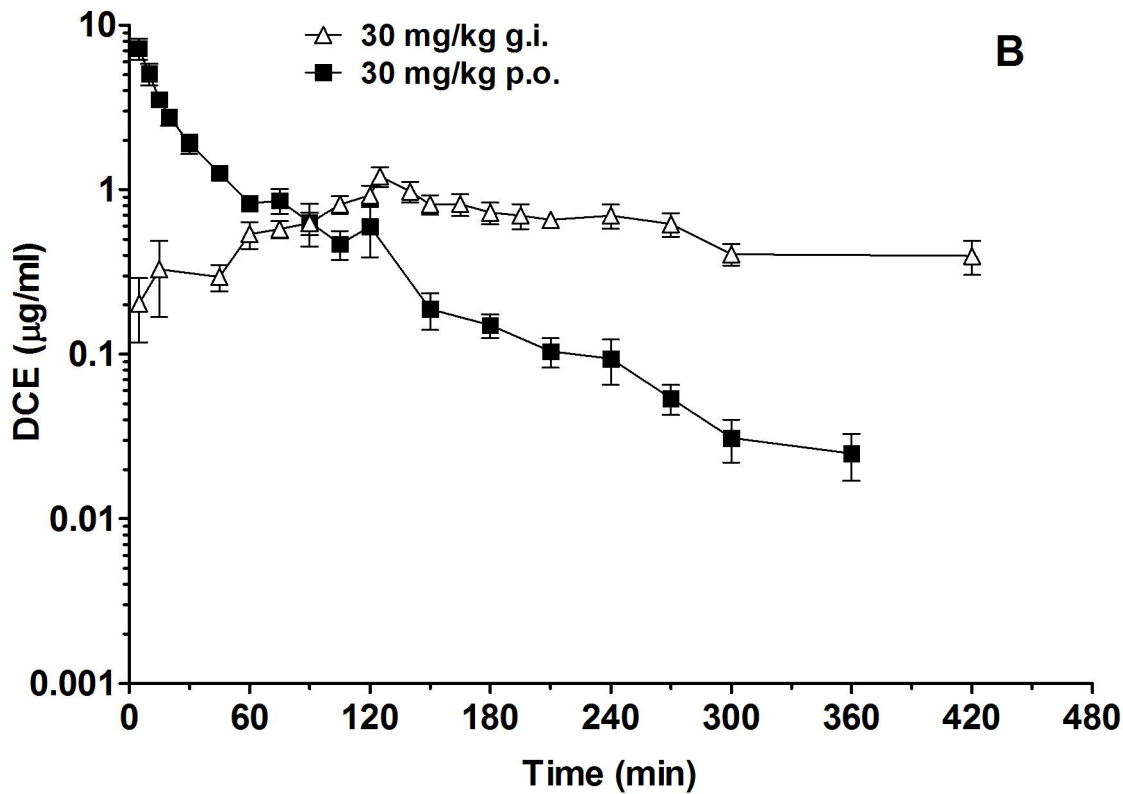
Figure 6

Figure 7

Figure 8