Acute, Subacute, and Subchronic Oral Toxicity Studies of 1,1-Dichloroethane in Rats: Application to Risk Evaluation

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1,1-Dichloroethane (DCE) is a solvent that is often found as a contaminant of drinking water and a pollutant at hazardous waste sites. Information on its short- and long-term toxicity is so limited that the U.S. EPA and ATSDR have not established oral reference doses or minimal risk levels for the volatile organic chemical (VOC). The acute oral LD₅₀ in male Sprague-Dawley (S-D) rats was estimated in the present study to be 8.2 g/kg of body weight (bw). Deaths appeared to be due to CNS depression and respiratory failure. In an acute/subacute experiment, male S-D rats were given 0, 1, 2, 4, or 8 g DCE/kg in corn oil by gavage for 1, 5, or 10 consecutive days. The animals were housed in metabolism cages for collection of urine and sacrificed for blood and tissue sampling 24 h after their last dose. There were decreases in body weight gain and relative liver weight at all dosage levels, as well as increased renal nonprotein sulfhydryl levels at 2 and 4 g/kg after 5 and 10 days. Elevated serum enzyme levels, histopathological changes, and abnormal urinalyses were not manifest. For the subchronic study, adult male S-D rats were gavaged with 0.5, 1, 2, or 4 g DCE/kg 5 times weekly for up to 13 weeks. Animals receiving 4 g/kg exhibited pronounced CNS depression. One 2-g/kg rat died during week 6. There were very few manifestations of organ damage in animals that succumbed or in survivors at any dosage level. Decreases in bw gain and transient increases in enzyme levels and blood urea nitrogen were not elevated, nor were glycosuria or proteinuria present. Chemically induced histological changes were not seen in the liver, kidney, lung, brain, adrenal, spleen, stomach, epididymis, or testis. Hepatic microsomal cytochrome P450 experiments revealed that single, high oral doses of DCE did not alter total P450 levels, but did induce CYP2E1 levels and activity and inhibit CYP1A1 activity. These effects were reversible and regressed with repeated DCE exposure. There was no apparent progression of organ damage during the 13-week subchronic study, nor appearance of adverse effects not seen in the short-term exposures. One g/kg orally (po) was found to be the acute, subacute, and subchronic LOAEL for DCE, under the conditions of this investigation. In each instance, 0.5 g/kg was the NOAEL.

Key Words: 1,1-dichloroethane; volatile organic chemical (VOC); halocarbon; CYP2E1; (noncancer) risk evaluation

1,1-Dichloroethane (DCE) is a short-chain, chlorinated aliphatic hydrocarbon (i.e., halocarbon). It is used primarily as an intermediate in the synthesis of other halocarbons and high-vacuum rubber. DCE is utilized to a limited extent as a degreaser, cleaning agent, and finish remover (ATSDR, 1990). Environmental releases occur primarily by volatilization, but DCE can also be discharged into surface waters and soils. Vogel and McCarty (1987) reported that 1,1,1-trichloroethane (TRI) can be rapidly biotransformed via reductive dehalogenation to DCE under methanogenic conditions present in landfills. Pellizzari (1982) found DCE in ambient air in the proximity of industrial and chemical waste disposal sites. The volatile organic chemical (VOC) was identified in groundwater, in combination with TRI and trichloroethylene (TCE), at 10% of hazardous waste sites on the National Priorities List (Fay and Mumtaz, 1996). DCE was one of the 5 most frequently detected VOCs, other than trihalomethanes, in a nationwide survey of 945 municipal water supplies (Westrick et al., 1984). DCE was recently included with TCE, TRI, and tetrachloroethylene in an Interaction Profile (ATSDR, 2001).

DCE is currently assigned the classification of C (Possible Human Carcinogen) by the U.S. Environmental Protection Agency (U.S. EPA, 1990). A 78-week bioassay was conducted by the National Cancer Institute (NCI), in which B6C3F1 mice and Osborne-Mendel rats of both sexes received high doses of DCE by gavage (NCI, 1978). There were dose-related, marginal increases in certain tumors in female rats and male and female mice. These findings were considered to be indicative of possible carcinogenicity, but not to be conclusive. Klaunig et al. (1986) supplied male B6C3F1 mice with drinking water containing 835 or 2500 mg/l (ppm) DCE for up to 52 weeks. DCE did not affect the incidence or number of liver or lung tumors in diethylnitrosamine-initiated mice or in mice receiv-
ing DCE only. At present, the EPA (1990) does not list a quantitative estimate of carcinogenic risk for ingestion or inhalation of DCE in its IRIS database.

Very few studies have been conducted to assess the toxicity potential of inhaled or ingested DCE. An LC50 is apparently unavailable. Widely different acute oral LD50s of 1120 and 14,100 mg/kg in male rats were cited in secondary literature sources (RTECS, 2001; Spector, 1956). Hofmann (1971) published the only account of a comprehensive investigation of the toxic potential of DCE, a 26-week inhalation study in 4 species. The NCI (1978) gavaged mice and rats with DCE for 6 weeks in order to establish maximally tolerated doses, but only body weight and lethality were monitored. Body weight gain, survival, and histopathology were assessed in chronic cancer bioassays (Klaunig et al., 1986; NCI, 1978). No acute, subacute, or subchronic oral study results are apparently available. No reports of injury or death of humans due to DCE exposure were found in the literature. The U.S. Agency for Toxic Substances and Disease Registry (ATSDR) (1990) refrained from establishing acute, intermediate, or chronic minimal risk levels (MRLs), due to insufficient data. The EPA (1990) determined that there were no suitable data for calculating an oral reference dose (RfD). This still appears to be the case.

Judging from the very limited information available, it appears that the central nervous system (CNS), kidneys, and liver are the most likely target organs for DCE. DCE and most other VOCs are CNS depressants (Evans and Balster, 1991). The current threshold limit value of 100 ppm (ACGIH, 2000) is intended to prevent decrements in psychophysiological functions of workers. Swelling of renal proximal convoluted tubules, proteinuria, and glucosuria are reported in mice given 4800 mg/kg by ip injection (Plaa and Larson, 1965). Pronounced nephrotoxicity was seen in cats, but not in other species subjected repeatedly over a period of months to 500 and 1000 ppm DCE vapor (Hofmann et al., 1971). A small, delayed increase in relative liver weight was observed in female rats following 10 days of inhalation of 6000 ppm (Schwetz et al., 1974). Since DCE is metabolized by hepatic microsomal P450s (McCall et al., 1983; Van Dyke and Wine et al., 1971), the VOC may be able to induce certain P450s. Cytochrome P450 2E1 (CYP2E1) would be a logical candidate for induction, as the isozyme plays a major role in the metabolism of structurally related halocarbons (Guengerich et al., 1991).

The overall objective of this investigation was to characterize the acute, subacute, and subchronic oral toxicity of DCE in adult, male Sprague-Dawley rats. Emphasis was placed upon assessment of possible renal and hepatic effects. Experiments were also conducted to test the hypothesis that DCE induces hepatic P450s likely responsible for its metabolism. The subchronic study was designed to determine whether effects progressed with repeated DCE exposure, and whether injury not apparent on short-term exposures was manifest upon longer-term administration of the chemical. An additional aim was to identify lowest-observed-adverse-effect levels (LOAELs) and no-observed-adverse-effect levels (NOAELs) for acute, subacute, and subchronic DCE ingestion.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley (S-D) rats were obtained from Harlan (Indianapolis, IN) for toxicity studies and from Charles River (Raleigh, NC) for follow-up P450 induction experiments. The animals were acclimated for at least one week to a 12-h light/dark cycle, with light from 0600–1800 h. The rats were then randomly assigned to dosage groups. Groups of 4–6 rats were housed together in stainless steel cages in negative flow glove racks in a temperature- and humidity-controlled biosafety suite. Some rats were housed individually for 24-h urine collections in Nalgene® metabolism cages. All animals were supplied withRalston Purina Chow 5001 and tap water ad libitum throughout the acclimation and testing periods.

Chemicals and dosing. 1,1-Dichloroethane (DCE), of 99.99% purity, was supplied by Dow Chemical Co. (Freeport, TX). All other chemicals and biologicals were obtained from Sigma Chemical Co. (St. Louis, MO), Mazola® corn oil, from Best Foods/CPC International Inc. (Englewoods Cliffs, NJ), was used as a diluent. Appropriate quantities of DCE were incorporated into sufficient corn oil to yield a 2:1 (DCE: corn oil) dilution on the day of dosing. The approximate volume (ml) of corn oil received by a 250-g rat at each DCE dosage level was as follows: 0.5 mg/kg, 0.15 ml; 1 g/kg, 0.3 ml; 2 g/kg, 0.6 ml; 4 g/kg, 1.3 ml; 8 g/kg, 2.6 ml; 12 g/kg, 3.8 ml; and 16 g/kg, 5.1 ml). Controls received 1 ml of corn oil. The solutions were administered as a bolus by gavage, using a curved, ball-tipped intubation needle affixed to a glass syringe. Dosings were conducted in a biohazard hood between 0900 and 1000 h, or 3 h to 4 h into the rats’ light/inactive cycle. The dosed animals were returned to their cages, or maintained for 24 h in the metabolism cages.

Acute and subacute toxicity study protocols. In the first experiment, a single dose of DCE was administered orally in corn oil to groups of 8 male S-D rats of 250–300 g. The dosage levels were 0, 1, 2, 4, 8, 12, and 16 g/kg bw. The number of deaths that occurred within 2 weeks was recorded.

In the second experiment, five dosage levels of DCE (0, 1, 2, 4, and 8 g/kg) were given daily by gavage to groups of 8 (8 g/kg group only) or 24 rats of 250–300 g for up to 10 days. All of the 8-g/kg group members and 1/3 of the members of the other groups received one dose of DCE and were placed into metabolism cages for a 24-h urine collection, before weighing, etherization, and sampling of blood and tissues. After administration of the fifth and tenth daily doses, a 24-h urine was collected from 8 of the remaining animals in the 0–1-, 2–2-, and 4–4-g/kg groups prior to sacrifice. Blood and tissue samples were again taken for serum enzyme analyses and histopathological examination. Protein and glucose levels, as well as the activities of selected enzymes were measured in the urine.

Subchronic study protocol. Groups of 15 male S-D rats of 180–200 g were gavaged with 0.5, 1.0, 2.0, or 4.0 g DCE/kg bw in corn oil 5 times weekly for up to 13 weeks. Controls received corn oil only. Body weight was recorded after administration of the fifth dose each week, and a mortality record was kept; 24-h urine specimens were collected every 2 weeks from one-half of the rats in each group. The other halves served as blood donors on weeks 0, 4, 8, and 12. These animals were lightly etherized and ~2 ml of blood taken by needle puncture of the caudal artery. Moribund rats were removed/ killed and examined for gross morphological changes. Excessive mortality in the highest dosage (4 g/kg) group necessitated termination of the survivors after the 11th week. These animals were etherized, and blood and tissues were taken for analyses. Members of the other dosage groups were similarly processed after 13 weeks of the regimen, so that serum enzyme and BUN measurements and histopathological examinations could be performed.

Urinalyses. Routine procedures were used for urinalyses. Urinary protein was measured by the Ponceau-S + protein complex color reaction (Pesce and Strande, 1973). Glucose was estimated by a technique involving the reaction of
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glucose with o-toluidine under hot acidic conditions (Dubowski, 1962). The urine samples were dialyzed against distilled water (volume ratio of 1: 800) at 4°C using a Spectrator® membrane (molecular weight cutoff—12,000) for at least 4 h. The dialyzed urine was used for measuring levels of: (a) the lysosomal enzymes acid phosphatase (ACP) (Stroo and Hook, 1977) and N-acetylgalactosaminidase (NAG) (Niebes, 1972); and (b) the brush-border enzymes alkaline phosphatase (ALP; Wright et al., 1972) and maltase (MAL) (Dahlqvist, 1964). Each of these was assayed spectrophotometrically by measuring p-nitrophenol formation at 400 nm. Protein concentrations were measured in both dialyzed and undialyzed urine samples, in order to derive a factor to correct for dilution during dialysis. Activities of the enzymes in urine, as well as protein and glucose levels, were calculated as units/ml and mg/ml, respectively, and expressed as total quantities voided over 24 h.

Clinical chemistry. At sacrifice, each rat was anesthetized with ether and blood taken by closed chest cardiac puncture. Serum was prepared and stored at −20°C prior to analyses. Standard spectrophotometric procedures, involving the conversion of NADH to NAD over time, were used to quantify s-orbitol dehydrogenase (SDH) (Gerlach and Hibi, 1974) and alanine aminotransferase (ALT) (Mattenheimer, 1971) activities in serum. Ornithine-carbamyl transferase (OCT) activity in serum was determined by the technique of Drotman (1971). Activities of the enzymes are functions of dose that was assessed by one-way analysis of variance. The significance of apparent differences over time between values for adjacent time points was evaluated by Student-Newman-Keuls’ test. The significance of differences between 0 time and relatively late time points was assessed by Duncan’s Multiple Range test. The minimum level of significance selected for these tests was p ≤ 0.05. The method of Litchfield and Wilcoxon (1949), as performed using an AVTEK Toxicological Statistics, Ver. 2.1, program, was utilized to estimate the LD10.

Histopathology. After sacrifice, a 1-cm wide strip of the left median lobe of the liver was removed and placed into buffered formalin for fixation. Other tissues taken for histological examination included the kidney, lung, brain, adrenal, spleen, testis, and epididymis. The stomach was also examined in the subchronic study. The tissue specimens were routinely processed into paraffin; 2-μm thick sections were stained with hematoxylin and eosin (H&E). The slides were coded and examined in a single-blind fashion by a veterinary pathologist.

Hepatic P450 assays and experiments. Male S-D rats from Charles River were used in follow-up P450 experiments. These animals’ livers were perfused in situ with cold normal saline in order to remove as much blood as possible. A 5-g portion of the central lobe was then taken for preparation of microsomes by differential centrifugation. The standard methods of Lowry et al. (1951) and Omura and Sat0 (1964) were used to quantify hepatic microsomal protein and total P450 levels, respectively. CYP2E1 activity was estimated by measuring p-nitrophenol hydroxylation (PNP-OH). The hydroxylation of PNP to 4-nitrophenol (4-NC) was determined according to Koop (1990). A substrate concentration of 100 mM was used for determining P450s. All 4-NC assays were performed at 37°C and pH 7.4.

RESULTS

Acute and subacute study. CNS depression and death were the major adverse effects of high, single or repeated oral doses of DCE. Rats receiving a single dose of ≥ 2 g DCE/kg initially exhibited excitement, followed by progressive motor impairment and sedation. The magnitude and duration of sedation were dose-dependent. In the initial experiment, the acute oral LD10 was determined to be 8.2 g/kg, with 95% confidence limits of 4.8–14.1 g/kg. The number of fatalities in groups of 8 rats at each dosage level (in g/kg) were as follows: 0/8 – 0; 0/8 – 1; 0/8 – 2; 0/8 – 4; 4/8 – 8; 5/8 – 12; and 8/8 – 16. All deaths occurred within 24 h of dosing.

The acute/subacute DCE treatment regimen caused reductions in body weight gain and in liver weight. Mean body weights in the 1-, 2-, 4-, and 8-g/kg groups were lower than in controls after the initial dose, but they were not significantly different from one another (Fig. 1). Three subjects in the 8-g/kg group died by 24 h. Decreases in body weight gain in the 1-, 2-, and 4-g/kg groups were dose-dependent over much of the remainder of the 10-day regimen. Rats in 2- and 4-g/kg groups did not gain weight during this period. Relative kidney weights did not affect DCE (Table 1). Absolute liver weights were reduced substantially at 5 and 10 days. There were comparable decreases in relative liver weight at all 3 dosage levels, despite the significant reductions in body weight gain in these groups. Moderation of the effect on liver weight was apparent at 10 days, with only the 2- and 4-g/kg group means slightly lower than controls.

There was very little evidence of injury of the liver, kidneys, or other organs examined. Serum SDH and ALT activities...
were not significantly altered from controls at 1, 5, or 10 days at any dosage level of DCE (data not shown). Hepatic microsomal cytochrome P450 levels exhibited slight, sporadic increases that were neither dose- nor time-dependent (Table 2). No significant alterations in hepatic NPSH levels were measured during the 10-day experiment. Renal NPSH levels, in contrast, were slightly elevated on days 5 and 10 in both the 2- and 4-g/kg animals. BUN levels were not significantly affected. DCE did not produce proteinuria, glycosuria, or enzumuria. Protein and glucose levels and NAG, ACP, ALP and MAL activities did not exhibit toxicologically significant alterations at any dosage level during the 10-day study (data not shown). Gross morphological changes were not evident in organs of animals that succumbed to DCE. No chemically associated lesions were found in H&E-stained sections of the liver, kidney, lung, brain, adrenal, spleen, testis, or epididymis of rats sacrificed at 1, 5, or 10 days. Mild, focal pneumonitis was occasionally seen in the lungs of animals, particularly in controls.

Subchronic study.Repeated oral administration of DCE, even in lethal amounts, produced very few manifestations of tissue damage. Rats receiving 4 g/kg, the highest dose, experienced protracted narcosis after each day’s dosing. Accordingly, their body weight gain was consistently lower than that of controls and other DCE-treated groups (Fig. 2). There was a progressive increase in the number of deaths, from the initial week of exposure until week 11, when the 7 surviving 4-g/kg rats were terminated (Fig. 3). Pulmonary congestion was the only anomaly evident upon gross examination of tissues of moribund and dead rats. One death occurred in the 2-g/kg group during the sixth week of DCE ingestion. The 2-g/kg animals exhibited moderate CNS depression following each day’s dosing. Their body weight gain was significantly lower than that of controls from the fourth week until the end of the 13-week study (Fig. 2). There were no fatalities in the 0.5 or 1.0 g/kg groups, nor reductions in body weight gain.

Subchronic ingestion of high doses of DCE did not appear to injure the liver. No elevations of serum SDH or OCT were manifest at any dosage level after 4, 8, or 12 weeks of the repetitive exposure regimen (data not shown). A slight differ-

![FIG. 1. Body weight gain/loss of rats gavaged with 0, 1, 2, or 4 g DCE/kg bw for up to 10 consecutive days. Each point represents the mean ± SD for a group of 8–16 rats at each monitoring period. Values for all DCE-treated groups were significantly (p < 0.05) lower than controls from days 1–10. Group means that are significantly different from one another on day 10 are designated by a different letter.](https://academic.oup.com/toxsci/article-abstract/64/1/135/1637719)

### TABLE 1

<table>
<thead>
<tr>
<th>Evaluation day</th>
<th>Daily DCE dose (g/kg)</th>
<th>Liver weight</th>
<th>Kidney weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute (g)</td>
<td>Relative (g/100 g bw)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>12.4 ± 0.8</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.9 ± 0.7</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.6 ± 1.0</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11.6 ± 0.7</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10.1 ± 1.2</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>12.6 ± 1.0</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10.0 ± 0.5***</td>
<td>3.5 ± 0.1***</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.1 ± 0.5***</td>
<td>3.5 ± 0.2***</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.6 ± 0.6***</td>
<td>3.4 ± 0.2***</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>12.8 ± 1.0</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11.4 ± 0.7**</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.7 ± 1.1***</td>
<td>3.3 ± 0.3**</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.1 ± 0.7**</td>
<td>3.4 ± 0.3**</td>
</tr>
</tbody>
</table>

**Note.** Rats of 250–300 g were dosed orally with 1,1-dichloroethane (DCE) daily for up to 10 days. Liver and kidney weights were measured after 1, 5 and 10 consecutive days of DCE administration. Values are expressed as mean ± SD for groups of 8 male rats. 

**Significantly different from control at p ≤ 0.01. ***p ≤ 0.001.**
ence in hepatocyte histology was observed in the 4-g/kg survivors sacrificed at 11 weeks. There was a mild condensation and change in cytoplasmic staining, consistent with glycogen mobilization. No other histological changes were seen in the liver at this or at lower DCE treatment levels. Relative liver weights were not significantly different from controls after 11 and 13 weeks for the 4-g/kg group and other dosage groups, respectively. The mean values ± SD (n = 15 at start of study) were: 0 g/kg, 3.3 ± 0.5 g; 0.5 g/kg, 3.1 ± 1.1 g; 1.0 g/kg, 3.2 ± 0.2 g; 2.0 g/kg, 3.1 ± 0.2 g; and 4.0 g/kg, 3.2 ± 0.4 g.

DCE had very limited nephrotoxic potential under the conditions of this subchronic study. BUN levels were not significantly elevated at any exposure level in rats gavaged repetitively with DCE for 4, 8, or 12 weeks (data not shown). Neither urinary protein nor glucose excretion was increased over controls after 2, 4, 6, 8, 10, or 12 weeks of the dosage regimen (data not shown). Urinary elimination of ACP was elevated in

![FIG. 2.](https://example.com/figure2.png)  
**FIG. 2.** Body weight gain of rats gavaged with 0, 1, 2, or 4 g DCE/kg 5 times weekly for up to 13 weeks. Brackets encase the mean ± SD for groups of 15 rats at the beginning of the experiment. SD bars are omitted from some data points for clarity. An asterisk indicates a statistically significant difference (p ≤ 0.05) from control, from the designated time point until the end of the study. A different letter designates group means that are significantly different from one another at 11 weeks.

![FIG. 3.](https://example.com/figure3.png)  
**FIG. 3.** Survival of rats receiving 0, 0.5, 1.0, 2.0, or 4.0 g DCE/kg by gavage 5 times weekly for up to 13 weeks. Surviving members of the 4.0 g/kg group were sacrificed after 11 weeks. Each point represents the mean percentage survival in groups originally numbering 15 animals.

### TABLE 2

<table>
<thead>
<tr>
<th>Evaluation day</th>
<th>Daily DCE dose (g/kg)</th>
<th>Liver microsomal CYP450 NPSH</th>
<th>Liver</th>
<th>Kidney</th>
<th>Blood urea nitrogen</th>
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</thead>
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<tr>
<td>1</td>
<td>0</td>
<td>0.86 ± 0.06</td>
<td>8.3 ± 0.4</td>
<td>3.8 ± 0.3</td>
<td>ND</td>
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<td>1</td>
<td>0.86 ± 0.07</td>
<td>8.2 ± 0.5</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
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<td>0.87 ± 0.07</td>
<td>9.5 ± 0.5</td>
<td>ND</td>
<td>ND</td>
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</tr>
<tr>
<td>4</td>
<td>0.83 ± 0.09</td>
<td>10.1 ± 0.7</td>
<td>2.7 ± 0.4</td>
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</tr>
<tr>
<td>8*</td>
<td>1.06 ± 0.05*</td>
<td>8.3 ± 0.6</td>
<td>2.6 ± 0.2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.89 ± 0.08</td>
<td>8.2 ± 0.5</td>
<td>2.8 ± 0.3</td>
<td>21.2 ± 4.2</td>
</tr>
<tr>
<td>1</td>
<td>1.03 ± 0.11*</td>
<td>7.3 ± 1.6</td>
<td>2.9 ± 0.4</td>
<td>21.1 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.84 ± 0.12</td>
<td>7.7 ± 0.5</td>
<td>4.2 ± 0.4***</td>
<td>18.7 ± 2.8</td>
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</tr>
<tr>
<td>4</td>
<td>0.95 ± 0.06</td>
<td>8.5 ± 0.4</td>
<td>3.7 ± 0.6*</td>
<td>21.8 ± 1.6</td>
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</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0.85 ± 0.05</td>
<td>8.4 ± 0.5</td>
<td>3.2 ± 0.3</td>
<td>19.3 ± 1.6</td>
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<td>0.96 ± 0.05***</td>
<td>8.8 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>19.9 ± 1.4</td>
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</tr>
<tr>
<td>2</td>
<td>0.98 ± 0.12*</td>
<td>9.0 ± 1.4</td>
<td>3.8 ± 0.5**</td>
<td>21.0 ± 2.9</td>
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<tr>
<td>4</td>
<td>0.78 ± 0.09</td>
<td>9.1 ± 0.5*</td>
<td>4.4 ± 0.4***</td>
<td>23.9 ± 3.7</td>
<td></td>
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</table>

**Note.** Groups of 8 male S-D rats were gavaged with 0, 1, 2, or 4 g DCE/kg for up to 10 consecutive days. Parameter values are expressed as mean ± SD. ND, not determined. Liver microsomal CYP450 is given in nmol/mg protein, NPSH in mmol/g tissue, blood urea nitrogen in mg/dl.

*One group of 8 rats received a single 8 g/kg dose and was sacrificed 24 h later.

*Significantly different at p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.
the 2- and 4-g/kg rats at 6 weeks, while both ACP and NAG excretion were increased over controls in the 1, 2, and 4 g/kg animals at 8 weeks (Fig. 4). No other anomalies were evident, other than an apparent decrease in the 2-g/kg NAG value at 6 weeks, a relatively high ACP control value at 12 weeks, and unusually low ACP excretion in each DCE-treated group at 12 weeks.

Histopathological examination of the kidney and other extrahepatic tissues did not reveal chemically induced changes. Mild nephropathy and pulmonary inflammation, however, were present in some control and DCE-treated rats (Table 3). Slight degeneration of the tubular epithelium, with reactive hyperplasia as well as tubular dilation and the presence of occasional casts characterized the nephropathy. The pulmonary inflammation consisted of interstitial infiltration by mixed inflammatory cells and thickening of alveolar septa. These modest changes, a frequent finding in male rats of this age, tended to be distributed at the end of the bronchioles. Interestingly, the incidence of nephropathy appeared to be highest in controls and to be inversely related to DCE dose. NCI (1978) also observed a lower incidence of nephropathy in DCE-dosed rats than in controls. Sections of brain, adrenal, spleen, testis, epididymis, and stomach were normal.

**P450 induction experiments.** A single, high oral dose of DCE (8 g/kg) did not alter hepatic microsomal P450 levels, but did influence the activity of some P450s. Neither total P450 levels nor CYP2B1/2 activity varied significantly over a 48-h period following dosing (data not shown). There was, however, an increase of \( \approx 75\% \) in CYP2E1 activity within 1 h (Fig. 5). CYP2E1 activity then appeared to rise gradually to an apparent maximum 24-h post dosing and to diminish thereafter. In contrast, CYP1A1 activity dropped by \( \approx 60\% \) during the first h.

**TABLE 3**

Nephropathy and Pulmonary Inflammation in Subchronic Study

<table>
<thead>
<tr>
<th>DCE dose (g/kg)</th>
<th>0</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Rats examined(^b)</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>No. Rats with lesions (% with lesions)</td>
<td>(70)</td>
<td>(67)</td>
<td>(53)</td>
<td>(43)</td>
<td>(29)</td>
</tr>
<tr>
<td>Pulmonary inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Rats examined(^b)</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>No. Rats with lesions</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>(% with lesions)</td>
<td>(20)</td>
<td>(27)</td>
<td>(67)</td>
<td>(36)</td>
<td>(43)</td>
</tr>
</tbody>
</table>

*Note. Groups of male S-D rats were gavaged with 0, 0.5, 1.0, 2.0, or 4.0 g DCE/kg bw 5 times weekly for up to 13 weeks.

\(^a\)Due to multiple fatalities at 4.0 g/kg, survivors were sacrificed after 11 weeks.

\(^b\)Number of animals examined at termination of study.

**FIG. 4.** Urinary excretion of acid phosphatase (ACP) and N-acetylglucosaminidase (NAG) in rats given 0, 0.5, 1.0, 2.0, or 4.0 g DCE/kg by gavage 5 times weekly for up to 13 weeks. Bars represent mean values for groups of 7 or 8 rats. SD is not shown for the sake of clarity. Asterisks indicate a statistically significant difference from control values at the same sampling time.

**FIG. 5.** Relative time courses of alteration of CYP2E1 and CYP1A1 activities in liver microsomes of rats given a single oral dose of 8 g DCE/kg bw. CYP2E1 activity was estimated by measuring hydroxylation of \( p \)-nitrophenol to 4-nitrocatechol (4-NC). CYP1A1 activity was assessed by measurement of \( \theta \)-deethylation of ethoxyresorufin to resorufin. Values are expressed as mean ± SD for groups of 6 rats. SD bars are omitted from some data points for clarity. Values for each isozyme, that are significantly different at different sampling times, are designated by a different upper or lower case letter.
after dosing, and decreased again between 6 and 12 h. The enzyme seemed to recover somewhat between 24 and 48 h.

To determine if the DCE-induced increases in CYP2E1 activity were associated with increases in CYP2E1 protein, Western-blot analyses of CYP2E1 expression in liver microsomes were made in the rats killed 1, 6, 12, 24, and 48 h after the single 8-g/kg po dose (Fig. 6). A representative blot of samples taken at each time point (Fig. 6A) and a bar graph showing the mean ± SD of pixel density for multiple assays at each time point (Fig. 6B) demonstrate modest induction due to DCE exposure. The largest increase in the CYP2E1 protein level was observed at 24 h, when it was 2.6-fold higher than that at 1 h.

DCE produced a dose-dependent increase of CYP2E1 activity and inhibition of CYP1A1 activity, as measured 24 h after oral dosing (Fig. 7). Total P450 levels and CYP2B1/2 activity, however, were not affected at any treatment level (data not shown). The lowest doses of DCE to significantly alter CYP1A1 and CYP2E1 activities were 1 and 4 g/kg, respectively. Inhibition of CYP1A1 activity was maximal at doses of 2 g/kg and higher. Maximal induction of CYP2E1 activity occurred in response to 8 g/kg, the highest DCE dose administered.

Repetitive oral exposures to DCE did not result in progressive changes in hepatic microsomal CYP2E1 activity. A single oral bolus of 4 g/kg produced a marked increase in CYP2E1 activity and in inhibition of CYP1A1 activity (Fig. 8). Despite daily administration of 4 g DCE/kg, CYP2E1 activity diminished ~50% between days 1 and 5 and remained constant thereafter. In contrast, CYP1A1 activity was lower on day 5 than day 1, but had increased somewhat by day 10.

DISCUSSION

DCE exhibited a very low order of toxicity in orally dosed male S-D rats. The acute oral LD₅₀ was estimated to be 8.2 g/kg bw. Disparate acute oral LD₅₀ values of 1,120, and 14,100 mg/kg for male rats have previously been cited by RTECS (2001) and Spector (1956), respectively. Animals in the current studies exhibited the continuum of CNS effects described by Evans and Balster (1991) for VOCs, including motor excitation, sedation, motor impairment, anesthesia, coma, and death by respiratory depression. Poor metabolism of DCE (Mitoma et al., 1985) likely contributes to maintenance of very high systemic levels of the parent compound for prolonged periods.

FIG. 6. Western-blot analyses of CYP2E1 expression in liver microsomes from rats given 8 g DCE/kg, po. CYP2E1 protein content in microsomes isolated from livers of male S-D rats was analyzed using a polyclonal goat antirat antibody to CYP2E1, alkaline phosphatase-conjugated secondary antibody, substrate, and color development. Protein band density was calculated using NIH Image software, ver. 1.6.2. (A) Representative Western blot showing time course of CYP2E1 protein expression after DCE treatment. (B) Time course of CYP2E1 protein expression after DCE treatment. Results are pixel numbers calculated for each band and represent means ± SD of measurements from microsomes obtained from 4 to 6 rats. Bars with different superscript letters above them are significantly different (p < 0.05) from one another.

FIG. 7. Dose dependency of effects of DCE on hepatic microsomal P450 isozenzymes. Rats were gavaged with 0, 0.5, 1, 2, 4, or 8 g DCE/kg bw and killed 24 h later for measurement of CYP2E1 and CYP1A1 activities. CYP2E1 activity was estimated by measuring hydroxylation of p-nitrophenol to 4-nitrocatechol (4-NC). 0-Deethylation of ethoxyresorufin to resorufin were used as the index of CYP1A1 activity. Bracket encases mean ± SD for groups of 10 rats. Mean values for each isozyme that are significantly different at different doses are designated by a different lower- or upper-case letter.
Such lipophilic compounds partition into and accumulate in neuronal membranes. Engelke et al. (1996) proposed that the presence of VOC molecules in cholesterol-filled interstices between sphingolipids and phospholipids changes membrane fluidity, thereby altering intercellular communication and normal ion movements. Alternatively, it has been hypothesized that VOCs partition into and interact with hydrophobic regions of proteins, influencing membrane-bound enzyme activities and/or receptor specificities (Balster, 1998). The extremely high doses of DCE, like many other VOCs, may also induce cardiac arrhythmias and/or autonomic effects, including reductions in heart rate and peripheral vascular resistance.

Although short-term administration of high oral doses of DCE caused dose-dependent CNS depression and fatalities in male S-D rats in the present study, there was little if any evidence of liver injury. Decreases in body weight gain were accompanied by reductions in absolute and relative liver weight after 5 and 10 days at the 1, 2, and 4 g/kg dosage levels. These effects may have also been manifest had a lower dose of DCE been administered. Schwetz et al. (1974) reported dose-dependent decreases in food intake and body weight gain in pregnant S-D rats that inhaled 3800 or 6000 ppm DCE for 7 h on days 6 through 15 of gestation. Absolute and relative liver weights of similarly exposed, nonpregnant females were unaffected. Serum ALT was not elevated in the pregnant or the nonpregnant rats. DiVincenzo and Krasavage (1974) saw no histopathological changes in the liver nor elevated serum OCT levels in male guinea pigs given ≤ 750 mg DCE/kg, ip. No increases in serum enzymes or chemically induced histological changes in the liver or in any other organ examined were seen in rats in the current study.

Our subacute oral dosage regimen in rats did not appear to adversely affect the kidney, another potential target organ of DCE. Hofmann et al. (1971) saw pronounced kidney injury in a small number of cats that inhaled 500 ppm DCE 6 h/day, 5 days/week for 1 month and 1000 ppm similarly for a second month. Nephrotoxicity was not evident in rats, guinea pigs, or rabbits subjected to the same exposure regimen. Plaa and Larson (1965) assessed DCE nephrotoxicity in male Swiss mice given 1.2, 2.4, or 4.8 g/kg as a single ip injection. Phenolsulfonphthalein excretion was not altered, but the 2 higher doses did produce proteinuria (measured qualitatively) and swelling of proximal convoluted tubules. These findings in mice are in contrast to the lack of proteinuria, sustained enzyrnmia, and histological changes in kidneys of rats given comparable single or multiple oral bolus doses of DCE in the current study.

DCE’s apparent lack of cytotoxicity in rats may be due to the nature of its metabolism. Male Osborne-Mendel rats were shown to metabolize only ~7.4% of a 700 mg/kg oral bolus dose of the chemical (Mitoma et al., 1985). The remainder was largely exhaled. Male B6C3F1 mice metabolized considerably more of an even higher dose (i.e., 29.2% of 1800 mg/kg, po). The mouse’s relatively high metabolism, coupled with its greater capacity to exhale VOCs, explains why mice could tolerate a much higher dose (i.e., 2885 versus 764 mg/kg, po, for male mice and rats, respectively) in the 78-week cancer bioassay by NCI (1978). Mitoma et al. (1985) report that covalent binding of radiolabel to liver proteins is significantly higher in mice than in rats dosed with 14C-DCE by corn oil gavage. It is not clear whether there are qualitative species differences in DCE metabolism, as no applicable mouse data could be located. Hepatic microsomal metabolism of DCE by male Long-Evans rats yields large quantities of acetic acid, a relatively small amount of 2,2-dichloroethanol, and traces of mono- and dichloroacetic acids (McCall et al., 1983). Phenobarbital pretreatment of rats enhances DCE metabolism (McCall et al., 1983) and covalent binding (Colacci et al., 1985) in the liver. SKF-525A decreases metabolism of DCE to mono- and dichloroacetic acids (McCall et al., 1983) and reduces covalent binding (Colacci et al., 1985). Mitoma et al. (1985) observe that the modest degree of hepatic covalent binding and the overall metabolism of DCE are saturated in male Osborne-Mendel rats given 175 mg/kg, po. The apparent lack of DCE cytotoxicity in rats in the current study would thus appear to be due primarily to the limited extent of DCE metabolism and to the innocuous nature of acetic acid, the major metabolite.

The results of the current cytochrome P450 experiments partially support the hypothesis that DCE induces liver P450s likely responsible for its metabolism. Although the identity of the P450s that catalyze DCE oxidation has (have) not been established, there is considerable evidence that implicates CYP2E1. Pretreatment of rats with ethanol, a classic CYP2E1 inducer, stimulates hepatic DCE metabolism in vitro (Guengerich et al., 1991). Sato et al. (1980) demonstrated that CYP2E1 was the major catalyst in human liver for oxidation of
11 halocarbon congeners. Nakajima and Sato (1979) found a 1-day fast of male and female Wistar rats to markedly enhance the hepatic metabolism of DCE and a series of other VOCs. Ethanol and acetone, CYP2E1 substrates, are believed to induce the enzyme primarily by binding to it and thereby protecting it from degradation by cAMP-dependent phosphorylation (Roberts et al., 1995; Ronis et al., 1991). DCE may act similarly at the posttranslational level, as CYP2E1 activity and protein levels increase significantly within 1 h of DCE administration. TCE (Lee et al., 2000) and TRI (Bruckner et al., 2001) also rapidly enhance hepatic CYP2E1 activity in male S-D rats. Results of the present study show that the increase in liver microsomal CYP2E1 activity (Fig. 5), as a consequence of acute treatment with DCE, is due to an increase in CYP2E1 protein content (Fig. 6). Comparison of the time-courses for DCE-induced changes in CYP2E1 activity and protein levels showed the 2 to be very similar, with maximal increases (between 2- and 3-fold) observed 24 h after exposure, with slightly lower values at 48 h. Whether the increased CYP2E1 protein levels are due to increased synthesis or decreased degradation, however, is unknown.

Cessation of food intake (due to CNS depression) may contribute to CYP2E1 induction at later time points. Brown et al. (1995) observed a slight increase in hepatic CYP2E1 activity in male F-344 rats fasted for 8 h, and pronounced increases after 16 and 24 h. CYP1A1 activity was unaffected by the fasting. CYP2E1 is known to be a high-affinity, low-capacity enzyme, while CYP2B1/2 is a low-affinity, high-capacity enzyme active in metabolism of high doses of TCE (Nakajima et al., 1992). Phenobarbital pretreatment of male rats enhances DCE metabolism and covalent binding in the liver (Colacci et al., 1985; McCall et al., 1983). Thus, the lack of effect of DCE on CYP2B1/2 in the current study was unexpected. We also failed to anticipate the inhibition of CYP1A1, a P450 isoform that is unaffected by TRI (Bruckner et al., 2001) and would not be expected to play a major role in the metabolism of DCE or other halocarbons.

Findings in this investigation may be useful in assessing noncarcinogenic risks of acute and subacute DCE ingestion. There was no evidence of toxic injury of the liver, kidneys or any other organ examined microscopically. The increases in renal NPSH levels in the 2- and 4-g/kg groups after 5 and 10 days of DCE dosing are of uncertain toxicological significance. Glutathione is reported to play an important role in the metabolism of 1,2-dichloroethane, but not DCE (McCall et al., 1983). The dose-dependent reductions in body weight gain can be attributed to prolonged narcosis, likely with attendant decreases in food and water intake. Unfortunately, food and water consumption was not monitored. The lower relative liver weights in all 3 dosage groups were unexpected, since VOCs typically enhance liver weight in rodents. It is worthy of note that relative liver and kidney weights at the termination of the subchronic study were not different from controls. The lowest acute dose to significantly inhibit hepatic CYP1A1 activity was 1 g/kg. There was no progression in magnitude of any biological effect over the 10 consecutive days of DCE administration. Thus the LOAEL for decreases in body and liver weights and CYP1A1 activity was 1 g/kg for acute and subacute gavage dosing of adult male S-D rats. These effects were relatively mild and reversible. The NOAEL for CYP1A1 inhibition was 0.5 DCE g/kg.

DCE, in most respects, proved to be no more toxic to rats on subchronic than on subacute oral administration. There was a progressive increase in the number of fatalities over time at 4 g/kg in the long-term experiment. There was little experimental evidence of organ damage despite the mortality. Tissue injury was not progressive during the subchronic study, nor did signs of toxicity appear that were not observed with the short-term exposures. The same was true in the NCI (1978) bioassay. Despite decreased survival of DCE-treated rats in that chronic study, the only lesions seen were manifestations of chronic murine pneumonia. Male and female B6C3F1 mice gavaged with as much as 2885 and 3331 mg DCE/kg/day were not injured. Klaunig et al. (1986) also failed to find lesions in the same strain of mice ingesting up to 543 mg/kg/day in drinking water for 52 weeks. As described previously, Hofmann et al. (1971) observed kidney injury in cats, but not in rabbits, guinea pigs or S-D rats subjected 6 h/day, 5 days/week to 500 ppm DCE vapor for 13 weeks, followed by the same regimen of 1000 ppm for 13 more weeks. There were occasional, minor increases in urinary ACP and NAG excretion in the 1-, 2-, and 4-g/kg rats in the present 13-week study, but the changes were not consistent or dose-dependent. CNS depression and one death were observed at the 2-g/kg dosage level. It is likely that CNS effects would have been detected at lower doses, had sensitive, objective measures of animal performance or behavior been employed. Nevertheless, based on enzymuria, 0.5 and 1.0 g/kg were the subchronic NOAEL and LOAEL, respectively, under the conditions of this investigation.

Most industrial and environmental DCE exposures appear to present little risk of toxicity, judging from the results of the present investigation. DCE concentrations in contaminated groundwater are typically in the μg/l (ppb) range. The daily DCE dose received by a 70-kg individual who consumes 2 liters of water per day containing 200 μg/l can be estimated as follows: 200 μl × 2 liters × 1 (assuming 100% absorption) ÷ 70 kg = 5.7 μg/kg. The current time-weighted average (TWA) for occupational inhalation exposure to DCE is 100 ppm (400 mg/m³) (ACGIH, 2000). The systemic dose of DCE received by a 70-kg employee during an 8-h exposure to 100 ppm can be calculated as follows: 400 mg/m³ × 0.6 (assuming 60% absorption) × 0.01 m³ (alveolar ventilation rate) × 480 min ÷ 70 kg = 16.5 mg/kg. These representative occupational and environmental dosages are orders of magnitude lower than the acute LD₅₀ and the subacute and subchronic LOAELs in the present study. The biological responses (i.e., decreased liver CYP1A1 activity and lower liver and body weights) seen at the LOAEL could be considered adaptive, rather than adverse
responses. Each of the effects diminished in magnitude upon repeated DCE administration. Manifestations of liver and kidney injury that are elicited by many halocarbons were absent, even at lethal dosages. CNS depression may be the most sensitive and appropriate toxic end point to use in risk assessments of DCE. As alluded to, CNS depression was likely present in the rats given \( \leq 1000 \text{ mg/kg, po.} \)

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