The Effects of Subacute and Subchronic Oral Exposure to cis-1,2-Dichloroethylene in Rats

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Running Head: Toxicity of Ingested cis-1,2-Dichloroethylene

ABSTRACT

Cis-1,2-dichloroethylene was administered by daily gavage to male and female Sprague-Dawley-derived Charles River rats. Male and female rats received 1.0, 3.0, 10.0 and 20.0 mM/kg/day during the 14-day subacute study. Doses gavaged during the 90-day subchronic study were 0.33, 1.00, 3.00 and 9.00 mM/kg/day. There were no compound-related deaths, nor were any compound related histopathology lesions demonstrated. There were significant decreases in both hemoglobin and hematocrit levels in both sexes after the 90-day exposure period, and decreases in female red blood cell counts. Significant changes in serum phosphorous were seen in both male and female exposure groups after both subacute and subchronic exposures. There were significant increases in serum calcium concentrations in male rats in both subacute and subchronic exposure groups. Significant increases in liver-to-body weight ratios were seen after 14- and 90-day treatment of male and female rats. This study demonstrates consistent indications of toxicity at subacute and subchronic exposure levels as low as 3 mM/kg/day. Implications of liver abnormalities were demonstrated at the lowest subacute exposure levels (1 mM/kg/day) and kidney abnormalities were demonstrated at the lowest subchronic exposure level (0.33 mM/kg/day).

INTRODUCTION

Cis-1,2-Dichloroethylene (cis-DCE, CAS No. 156-59-2) is commercially produced as an isomeric mixture by chlorination of acetylene, separated from the trans-1,2-dichloroethylene isomer (trans-DCE, CAS No. 156-60-5) by fractional distillation (1,2). Isomers of dichloroethylene (DCE) are colorless, light liquids and have a sweet, slightly irritating chloroform-like odor (1,2). They are commonly used as solvents for fats, phenol, camphor and other compounds. They are also used to retard fermentation (1). Cis-DCE is only slightly water soluble, and has been detected in drinking water at concentrations not exceeding 1.0 7μg/L (1,3). Low-level cis-DCE contamination of chlorinated drinking water may be expected, since acetylene is not the only chlorination substrate to produce isomeric mixtures of DCE (2).

Human toxicology data on cis-DCE are sparse; however, symptoms of drowsiness, dizziness and narcosis have been reported (2). A human threshold limit value (TLV) of 200 ppm has been established, but chronic toxicity data are toxicity data are not available (1).

Jenkins et al. (4) reported significant inhibition of liver glucose-6-phosphatase, liver tyrosine transaminase and plasma alanine transaminase activities in male rats 20 hours after a single oral administration of 15.5 mM cis-DCE/kg in corn oil. Liver alkaline phosphatase activity was significantly increased in a dose-dependent fashion after both 4.1 and 15.5 mM cis-DCE/kg exposure. Using isolated rat liver, Bonse et al. (5) demonstrated liver enzyme leakage (alanine aminotransaminase and aspartate aminotransaminase) into the perfusate after 60 minutes perfusions of a cis-DCE saturated perfusion solution. Bonse et al. (5) also demonstrated that 1 to 3% of the absorbed cis-DCE was converted to dichloroacetic acid and 8 to 10% to dichloroacetaldehyde in the isolated perfused liver.

Filser and Bolt (6) demonstrated that *in vivo* metabolism of both trans- and cis-DCE can be described as zero-order kinetics at higher concentrations (saturated metabolic conditions), and first-order kinetics at lower concentrations (unsaturated metabolic conditions). Freundt and Macholz (7) studied the effects of inhaled trans- and cis-DCE on hexobarbital sleep time and zoxazolamine paralysis time in rat. They observed a dose-dependent increase in both sleep time and paralysis time following

a single 8-hour inhalation exposure to either compound at 200-, 600- and 1000-ppm dose levels. Trans-DCE did not demonstrate significantly increased paralysis time at the 200-ppm dose level. Barnes et al. (8) were unable to demonstrate an increase in hexobarbital sleep time after a 90-day drinking water exposure to 100, 1000, or 2000 mg/L trans-DCE in male or female CD-1 mice. Studies of the interaction between trans- and cis-DCE and rat hepatic cytochrome P-450 conducted by Costa and Ivanetich (9) show the P-450 system to be rate-limiting in DCE metabolism. These studies indicated that DCE inactivates P-450 binding site for DCE, with concurrent lose of P-450. Other studies indicate that there appears to be no evidence that cis-DCE is either mutagenic (10) or a direct-acting hepatocarcinogen (11).

The available literature does not provide threshold oral exposure, toxicity data that are needed to promulgate safe drinking water standards. The following studies were designed and conducted to provide such toxicologic data.

METHODS

Cis-DCE (Lot Nos. 08715 PL and 8409 PK)(97% pure) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Male and

female rats were exposed to cis-DCE for 14 or 90 consecutive days in separate experiments. The exposure to cis-DCE was by daily oral gavage in a corn oil vehicle at a volume of 3 mL/kg animal body weight.

Male and female Sprague-Dawley rats approximately 70 days old were obtained from from Charles River Breeding Laboratories (N. Wilmington, MA). Rats were quarantined for one week, then rats of each sex were randomly divided into five groups of 10 rats each. The rats were held at 21 to 24°C, 40 to 60% relative humidity and a 12-hour day/night cycle. Each 14-day exposure group received by gavage either 1, 3, 10 or 20 mM/kg/day (32, 98, 293, or 878 mg/kg) of cis-DCE.

The 90-day exposure groups were obtained from the same commercial source and maintained under the same conditions as the 14-day groups. Each 90-day exposure group received by gavage either 0.33, 1, 3 or 9 mM/kg/day of cis-DCE.

Food and water were available *ad libitum*, and consumption data were recorded twice weekly during the exposure period. Body weight data were taken weekly. At the end of the specified exposure period, all animals were sacrificed and specimens were

collected for clinical chemistry, hematology and histopathology studies.

Necropsy

Rats were starved 18 hours prior to pentobarbital anesthesia. At sacrifice, bodies and major organs (adrenal gland, gonads, heart, kidneys, liver, lungs, spleen and thymus) were weighed and recorded. Two 5-mL blood samples were taken for hematological analysis and for clinical chemistries. Tissues were fixed in 10% buffered formalin. Tissue was sectioned and then stained with hematoxylin-eosin. One half of the controls and all of the highest exposure group were analyzed for histopathology by a board-certified pathologist. These tissues include: all gross lesions, duodenum, skin, jejunum, mandibular lymph nodes, messenteric lymph nodes, tongue, mammary glands, salivary gland, thigh muscle, ileum, sciatic nerve, colon, sternum, femur or vertebrae, marrow, cecum, thymus, lungs and bronchi, liver, heart, aorta, pancreas, thyroid, spleen, parathyroids, kidneys, esophagus, adrenal, stomach, urinary bladder, seminal vesicles, prostate, testes, including epididymis, ovaries, uterus, nasal cavity, nasal turbinates, brain, pituitary, preputial or clitoral glands, and Zymbal's gland.

Clinical chemistries

Serum clinical chemistry levels were determined using diagnostic kits and a Baker Encore Chemistry Analyzer (Allentown,PA). The following serum clinical chemistry determinations were performed: alanine aminotransaminase, aspartate aminotransaminase, lactate dehydrogenase, creatinine, cholesterol, phosphorous, calcium, glucose and blood urea nitrogen.

Hematology

Samples were evaluated using a Coulter Counter, Model ZBI (Hialeah, Fl) for hemoglobin, hematocrit, mean corpuscular volume, red blood cell count, white blood cell count and reticulocytes count.

Statistics

Analysis of variance techniques were used to analyze each of the response measures when data was normally distributed (12). However, because of the nature of some of the clinical chemistry measures (extreme values, high variability), a nonparametric analysis of variance procedure, the Kruskal-Wallis test, was used (on these measures) to look for differences among the dose groups

(13). Males and females were considered separately in all statistical analyses.

RESULTS

14-Day Subacute Oral Toxicity

Male and female rats in the high-dose groups demonstrated central nervous system depression, secretions about the nose and mouth and signs of discomfort. These effects were most apparent immediately after dosing. Five of the 20 animals (2 males and 3 females) in this dose group died. Two animals (1 male and 1 female) in the next highest dose group died as well. All animal mortality took place within the initial week of dosing.

Body and organ weights. Both male and female rats demonstrated significant changes in body weight gains (Tables 1 and 2). The tendency in females was toward maximum weight gain among the middle dose groups with a decreased weight gain at the higher doses. Males showed a similar and significant pattern in final body weight.

14 DAY
Dose levels
kidney
0, 1, 3, 10, 20
F XX XX
M X
XX = sig relative & absolu
X = sig absolute

There was a consistent, dose-related increase in both liver weight and liver-to-body weight ratios in both sexes. Kidney weight and kidney-to-body weight ratios were elevated in females in a dose-dependent manner. Both kidney weight and kidney-to-body weight ratios were significantly increased in females, at 10 and 20-mM/kg/day exposure levels. Only at the 10-mM/kg/day dosage level did male rats demonstrate a significant increase in kidney weight. Male brain and spleen weight measurements revealed significant, but not dose-dependent decreases in weight. Testes demonstrated a significant increase in wet weight to body weight ratios (Table 2).

14 DAY
LIVER
0, 1, 3, 10, 20
F XX
M X

Only liver weights and liver-to-body weight ratios were significantly different from controls at the lowest dose level. This difference was evident in both sexes. This was also the only finding of all the 14-day exposure measurements that was significantly different from the control group at the lowest exposure level.

Clinical chemistries. Both male and female rats demonstrated increases in serum cholesterol and phosphorous. The increase in cholesterol appears to be dose-related (Tables 1 and 2). Female rats also showed significant changes in serum creatine

and blood urea nitrogen levels (Table 1). Male rats' serum calcium increased in a dose-dependent fashion (Table 2).

Hematology. Females exhibited a significant decrease in hematocrit at the 3-, 10-and 20-mM/kg/day dosing levels. A decrease in the red blood cell count of female rats was demonstrated when 3 mM/kg/day-exposed groups were compared to control groups (Table 1). There were no significant hematology findings among male rats.

Food and water consumption. There was a significant increase in water consumption and water consumption per body weight among both male and female rats, particularly at the highest dose (Tables 1 and 2). Food consumption increased significantly only as a percent female body weight and then only in the 10-mM/kg/day dose group.

Histopathological findings. There were no significant compound-related lesions.

90-Day Subchronic Oral Toxicity

During the 90-day exposure period, one female rat died in each of the 0.33- and 1.00-mM/kg/day exposure groups. One male

32 mg/kg/day

rat died in the control group, 3 males died in the 3 mM/kg/day group and 4 male rats died in the 9-mM/kg/day exposure groups. All animal mortality occurred within the first week of dosing. In most cases, animals found dead also demonstrated mild pulmonary congestion. No other consistent finding could be established, nor could a relationship to compound dosing be established.

Body and organ weights. Male rats demonstrated a significant decrease in body weight gain only at the highest exposure level. Female, but not male rats, again demonstrated a significant increase in liver weight when exposed to 1, 3 and 9 mM/kg/day (Tables 3 and 4). Both sexes showed an increase in liver-to-body weight ratios at the 1-, 3- and 9-mM/kg/day exposure level.

Female rats also demonstrated variable and significant increases in thymus weight and thymus-to-body weight ratio at the 9-mM/kg/day dose level (Table 3). Adrenal-to-body weight ratios were only significant when compared to ratios from female rats exposed to 0.33 and 9 mM/kg/day (the 9 mM/kg/day value was larger).

When kidney weight was expressed as a percent of body weight, ratios from all compound exposed male rats were significantly increased over control ratios. These kidney weight-to-body weight data are the only data generated in the 90-day study in which the lowest exposure group (0.33 mM/kg/day) demonstrate statistical difference from the control group (Table 4).

Clinical chemistries. Female serum phosphorous levels were increased at the 1- and 3-mM/kg/day exposure levels only (Table 3). Serum phosphorus was, however, decreased in male rats exposed to 0.33 mM cis-DCE/kg/day for 90-days (Table 4). There was a concurrent increase in serum calcium concentrations in male rats at the 0.33- and 1-mM/kg/day exposure levels (Table 4). Other significant clinical chemistry findings in male rats were decreases in creatinine and blood urea nitrogen at the highest dosing level.

Hematology. Hemoglobin, hematocrit, and red blood cell counts were decreased in female rats exposed to 3 mM/kg/day of the test compound. Hematocrit was also decreased in female rats at 9 mM/kg/day. Male rats demonstrated decreases in hemoglobin between the 3- and 9-mM/kg/day exposure groups. Decreases in hematocrit

were demonstrated at the 1-, 3- and 9-mM/kg/day exposure levels (Tables 3 and 4).

Food and water consumption. There were no significant changes in food consumption. Female rats exposed at the 1-mM/kg/day level increased their water consumption as a percent of body weight (Table 3). Males rats increased total daily water consumption when exposed to 9 mM/kg/day (Table 4).

Histopathological finding. There were no significant compound-related lesions.

DISCUSSION

1,2-DCE exists in both cis and trans isomeric forms. Since the great bulk of the toxicology literature addresses the trans isomer, these studies were designed to address some of the literature gaps concerning the cis isomer of 1,2-DCE.

A large variety of compound-related effects were demonstrated in this study, even at relatively low exposure levels. One effect, kidney weight-to-body weight ratio (in male

rats) was significantly increased at the lowest 90-day exposure level of 0.33 mM/kg/day.

Cis-DCE effects on organ weight and body weight ratios, such as the one noted above (concerning kidney weight-to-body weight ratio) are interesting, but in light of the negative histopathological findings, these effects are difficult to interpret. A trend toward a decrease in body weight gain, significant only at the highest dose level, was seen in 90-day exposed male rats. The significant increase in kidney-to-body weight ratio may be due at least in part to decreased body weight gain. However, the increase in kidney weight was a relatively consistent finding and may require further experimentation to better understand this observation.

The group of male rats exposed for 90 days is the only group that does not show a significant increase in gross liver weight. However, this group does demonstrate a significant increase in liver-to-body weight ratios. All exposure groups (14- and 90-day, male and female) demonstrated an increase in liver weight-to-body weight ratios. The threshold for significance in all groups was 1 mM/kg/day. In light of the negative histology data this effect

may be a hypertrophy and hyperplasia similar to that induced by phenobarbital.

Other less consistent changes in organ weights and organ weight-to-body weight ratios may require more data for interpretation. Though difficult to interpret, effects such as decreases in brain weight after only 14 days of exposure ($p < 0.001$) may have serious implications. However, further study is indicated to determine the cause and consequence of this temporary loss of brain weight.

Some of the clinical chemistry effects are very small decreases in measures that are usually associated with toxicity when they increase (blood urea nitrogen, serum creatinine, and serum aspartate aminotransaminase). However, serum cholesterol did increase slightly and transiently. There was an apparent alteration of calcium and phosphorous metabolism. This was expressed by an increase in serum calcium levels in male rats at both 14 and 90 days and by a increase in serum phosphorus levels in all groups except 90-day males, where it decreased.

Hematology data indicated that cis-DCE may cause or facilitate anemia. Both sexes at 90 days in several dose groups

demonstrated significantly decreased hemoglobin, hematocrit and, in females, decreased red blood cell count. There were consistent increases in drinking water consumption in both sexes. Since the test compound was administered by gavage this effect must be considered to be a compound-related effect, and cannot be associated with water palatability. Determination of whether this effect is due to influence on renal, central nervous system or other organ or organ system(s) will require more data. Changes in final weight and body weight gain were not consistent with food consumption data, and require other causes or contributing effects for an explanation.

Hayes et al. (14) investigated the effects of drinking water trans-DCE exposure for 90 days on male and female Sprague-Dawley rats, using essentially the same indicators of toxicity as were used in this study. The major differences between the Hayes et al. (14) 90-day study and this 90-day study is the initial age of the rats, 26 versus 70 days, respectively, and the method of exposure, in 1% emulphor drinking water suspension versus by corn oil gavage. Hayes et al. (14) used drinking water trans-DCE doses of up to 43.2 mM/kg/day, and only three significant findings were reported. They were: increased female kidney weight-to-body weight (or kidney weight-to-brain weight) ratios at 19.3 and 43.2

mM/kg/day and increased ovarian weight to body weight (but not to brain weight) ratios at 19.3 mM/kg/ day (only). No effect was reported in male rats. No effects were observed at 5.4 mM/kg/day in either sex. Chieco et al. (15) observed large differences in the liver toxicity of 1,1-DCE due to the exposure vehicle. 1,1-DCE in corn oil gavage was demonstrated to be 2 to 4 times more liver toxic than equal doses of 1,1-DCE administered by 0.5% Tween 80 gavage. Though histopathology studies were conducted on lung, spleen, kidney, adrenals, stomach, duodenum, and suprahepatic vena cava, only liver demonstrated pathology. Cis- and trans-DCE exposure did not cause any measurable liver histopathology. Liver blood flow is unique in its relationship with the gastro-intestinal (GI) tract due to the entero-hepatic portal vein. Since the relevance of neither the vehicle nor the duration of oral exposure has been addressed in organs other than the liver, further experimentation will be required to draw firm conclusions about the comparibility of the toxicity of cis- and trans-DCE. Primafacia, the data reported in this paper suggest that considerable differences do exist between the cis- and trans-DCE observable effect thresholds.

Four consistently occurring effects were demonstrated in this study. Whether these four effects are related to one another or

arise independently cannot be addressed by the data generated here. These are consistent findings among most or all of the cis-1,2-DCE exposure groups; 1) increased liver size without histopathologic findings; 2) increases in kidney-to-body weight ratios; 3) alterations in serum phosphorous concentrations and 4) a tendency toward anemia as evidenced by decreases in serum hemoglobin and hematocrit. Of these effects, only the tendency toward anemia demonstrates accumulative toxicity, as it appears to be more severe after 90-days then after 14-days of exposure.

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TABLE 1

FINDINGS OF THE 14-DAY CIS-1,2-DICHLOROETHYLENE TOXICITY STUDY IN FEMALE RATS

(4 pp.)

Table 1. Findings of the 14-Day cis-1,2-Dichloroethylene Toxicity Study in Female Rats

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Body Weight						
Final (g)	NS	232 (8.24)	243 (15.8)	243 (11.5)	240 (17.0)	238 (13.1)
Gain (g)	0.05	2.93[a] (7.83)	11.7[a,b] (8.62)	11.9[b] (9.40)	6.54[ab] (7.25)	6.06[a] (12.4)
Average Daily Consumption (ADC)						
Water ADC (g/day)	<0.001	32.1[a] (3.28)	36.1[a] (5.73)	35.1[a] (4.84)	40.8[a] (3.08)	55.0[b] (6.38)
Water ADC (% Body Weight)	<0.001	13.9[a] (1.27)	14.9[a,b] (2.25)	14.5[a,b] (1.96)	16.8[b] (0.68)	23.4[c] (3.41)
Food ADC (g/day)	NS	15.1 (1.24)	17.8 (2.20)	17.2 (1.45)	18.5 (3.37)	15.0 (1.56)
Food ADC (% Body Weight)	0.008 Control <10 mM	6.54 (0.45)	7.35 (0.77)	7.10 (0.57)	7.47 (0.94)	6.51 (0.43)
Organ Weight						
Adrenal Weight (g)	NS	0.09 (0.02)	0.10 (0.03)	0.12 (0.05)	0.11 (0.01)	0.12 (0.04)
Adrenal (% Body Weight x 10)	NS	0.39 (0.06)	0.42 (0.10)	0.49 (0.20)	0.45 (0.06)	0.49 (0.15)

Table 1 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Brain Weight (g)	NS	1.91 (0.10)	1.86 (0.21)	1.94 (0.12)	1.85 (0.13)	1.83 (0.07)
Brain (% Body Weight)	NS	0.82 (0.03)	0.77 (0.10)	0.80 (0.05)	0.77 (0.06)	0.77 (0.06)
Kidney Weight (g)	0.004	1.77[a] (0.12)	1.96[ab] (0.27)	1.97[ab] (0.10)	2.12[b] (0.17)	2.05[b] (0.23)
Kidney (% Body Weight)	0.004	0.77[a] (0.05)	0.81[ab] (0.08)	0.81[ab] (0.04)	0.88[b] (0.07)	0.86[b] (0.07)
Liver Weight (g)	<0.001	7.09[a] (1.09)	8.60[b] (1.39)	8.81[b] (0.64)	9.57[bc] (1.08)	10.2[c] (1.21)
Liver (% Body Weight)	<0.001	3.07[a] (0.49)	3.53[b] (0.40)	3.63[b] (0.23)	3.97[bc] (0.29)	4.26[c] (0.31)
Ovaries Weight (g)	NS	0.19 (0.05)	0.22 (0.06)	0.22 (0.04)	0.20 (0.04)	0.20 (0.08)
Ovaries (% Body Weight)	NS	0.08 (0.02)	0.09 (0.02)	0.09 (0.02)	0.09 (0.01)	0.08 (0.03)
Spleen Weight (g)	NS	0.53 (0.08)	0.59 (0.13)	0.58 (0.07)	0.56 (0.04)	0.47 (0.06)
Spleen (% Body Weight)	NS	0.23 (0.03)	0.24 0.05)	0.24 (0.03)	0.23 (0.01)	0.20 (0.02)

Table 1 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Thymus Weight (g)	NS	0.38 (0.09)	0.48 (0.11)	0.44 (0.12)	0.43 (0.12)	0.38 (0.11)
Thymus (% Body Weight)	NS	0.16 (0.04)	0.20 (0.04)	0.18 (0.05)	0.18 (0.04)	0.16 (0.04)
Clinical Chemistry						
AST (IU/L)	NS	37.6 (9.62)	38.4 (10.0)	41.5 (7.84)	41.3 (8.56)	48.4 (8.73)
Blood Urea Nitrogen (mg/dL)	0.004	20.7[a] (3.15)	18.6[a,b] (2.84)	17.8[b] (2.49)	15.0[b] (2.47)	17.2[b] (3.54)
Calcium (mg/dL)	NS	11.5 (0.37)	11.3 (1.03)	11.3 (0.94)	11.4 (0.66)	11.3 (0.59)
Creatinine (mg/dL)	0.05	0.59[a,b] (0.11)	0.57[a,b] (0.07)	0.59[a,b] (0.09)	0.52[a] (0.07)	0.67[b] (0.10)
Cholesterol (mg/dL)	0.02	73.6[a] (12.3)	84.9[a,b] (9.60)	86.1[a,b] (19.6)	92.3[a,b] (20.7)	103.6[b] (13.3)
Phosphorous (mg/dL)	0.02	7.18[a] (1.02)	8.50[b] (1.44)	8.21[a,b] (0.78)	8.00[a,b] (0.61)	8.79[b] (0.78)

Table 1 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Hematology						
Hematocrit (%)	0.005	37.7[a] (1.82)	34.7[a,b] (2.55)	33.4[b] (1.68)	33.6[b] (2.05)	33.5[b] (0.77)
Hemoglobin (g/dL)	NS	14.3 (0.51)	13.6 (0.77)	13.3 (0.84)	13.5 (0.79)	13.5 (0.37)
Red Blood Cell (10^6/mL)	0.04 Control > 3mM	6.30 (0.32)	5.94 (0.52)	5.77 (0.32)	5.80 (0.28)	5.72 (0.17)

[a,b]Dose groups with the same letter are not significantly different ($\alpha = 0.05$).

TABLE 2

FINDINGS OF THE 14-DAY CIS-1,2-DICHLOROETHYLENE TOXICITY STUDY IN MALE RATS

(4 pp.)

Table 2. Findings of the 14-Day cis-1,2-Dichloroethylene Toxicity Study in Male Rats

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	.1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Body Weight						
Final (g)	0.02	403[a] (34.3)	390[a,b] (17.5)	408[a] (16.1)	391[a,b] (27.9)	369[b] (25.0)
Gain (g)	0.02	56.6[a,b] (7.83)	57.2[a,b] (8.62)	69.0[b] (9.40)	54.3[a,b] (7.25)	26.3[a] (12.4)
Average Daily Consumption (ADC)						
Water ADC (g/day)	0.03	52.2[a,b] (7.16)	50.6[a] (2.27)	52.9[a,b] (7.05)	56.0[a,b] (5.83)	64.5[b] (9.22)
Water ADC (% Body Weight)	<0.001	13.0[a] (1.65)	13.0[a] (0.74)	13.0[a] (1.49)	14.2[a] (0.85)	18.1[b] (1.60)
Food ADC (g/day)	NS	27.9 (2.02)	28.3 (2.67)	29.6 (2.05)	29.2 (2.88)	25.9 (3.62)
Food ADC (% Body Weight)	NS	6.93 (0.44)	7.25 (0.56)	7.26 (0.45)	7.37 (0.50)	7.31 (0.53)
Organ Weight						
Adrenal Weight (g)	NS	0.10 (0.02)	0.11 (0.04)	0.11 (0.05)	0.12 (0.04)	0.12 0.03).
Adrenal (% Body Weight x 10)	NS	0.26 (0.06)	0.29 (0.10)	0.27 (0.12)	0.31 (0.10)	0.32 (0.10)

Table 2. Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Brain Weight (g)	<0.001	2.15[a] (0.12)	1.87[c] (0.27)	2.10[a,b] (0.10)	1.99[b,c] (0.17)	1.98[b,c] (0.23)
Brain (% Body Weight)	NS	0.54 (0.07)	0.48 (0.05)	0.52 (0.03)	0.51 (0.03)	0.54 (0.05)
Kidney Weight (g)	NS	3.32 (0.50)	3.45 (0.48)	3.84 (0.49)	3.88 (0.38)	3.49 (0.55)
Kidney (% Body Weight)	0.003 (0<3, 0<10 only)	0.82 (0.08)	0.88 (0.09)	0.94 (0.11)	0.99 (0.07)	0.94 (0.12)
Liver Weight (g)	0.002	13.4[a] (2.18)	15.0[a,b] (1.90)	16.0[a,b] (1.76)	17.2[b] (2.62)	16.9[b] (1.74)
Liver (% Body Weight)	<0.001	3.31[a] (0.30)	3.83[b] (0.37)	3.90[b] (0.30)	4.40[c] (0.51)	4.57[c] (0.36)
Spleen Weight (g)	0.005	0.86[a] (0.11)	0.83[a] (0.11)	0.88[a] (0.17)	0.82[a,b] (0.05)	0.80[b] (0.11)
Spleen (% Body Weight)	NS	0.21 (0.02)	0.21 (0.03)	0.22 (0.04)	0.21 (0.02)	0.18 (0.03)
Testes Weight (g)	NS	3.15 (0.90)	3.18 (0.24)	3.47 (0.25)	3.12 (1.04)	3.52 (0.32)
Testes (% Body Weight)	<0.001	0.78[a,b] (0.22)	0.82[a] (0.04)	0.85[a,b] (0.04)	0.80[b,c] (0.27)	0.96[c] (0.05)

Table 2. Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Thymus Weight (g)	NS	0.64 (0.14)	0.54 (0.19)	0.66 (0.19)	0.61 (0.15)	0.58 (0.12)
Thymus (% Body Weight)	NS	0.16 (0.03)	0.14 (0.05)	0.16 (0.04)	0.15 (0.03)	0.16 (0.04)
Clinical Chemistry						
AST (IU/L)	NS	43.3 (8.53)	44.4 (6.59)	44.9 (10.1)	38.7 (8.83)	53.3 (12.3)
Blood Urea Nitrogen (mg/dL)	NS	15.5 (4.34)	16.6 (2.93)	16.2 (3.48)	15.4 (2.29)	16.1 (3.38)
Calcium (mg/dL)	0.002	9.95[a] (1.33)	10.6[a,b] (0.92)	10.7[a,b] (0.75)	10.9[b] (0.26)	11.1[b] (0.26)
Creatinine (mg/dL)	NS	0.05 (0.01)	0.04 (0.01)	0.05 (0.01)	0.04 (0.01)	0.05 (0.01)
Cholesterol (mg/dL)	0.03	60.0[a,b] (11.9)	55.8[a] (14.3)	59.5[a,b] (11.7)	69.6[a,b] (18.0)	75.1[b] (12.5)
Phosphorous (mg/dL)	0.008	8.51[a] (0.68)	8.59[a] (0.75)	9.79[b] (0.64)	9.46[a,b] (0.84)	9.16[a,b] (1.36)

Table 2. Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Hematology						
Hematocrit (%)	NS	38.3 (2.54)	36.5 (3.04)	39.1 (1.28)	37.9 (2034)	36.5 (1.99)
Hemoglobin (g/dL)	NS	14.2 (0.89)	13.9 (0.97)	14.5 (0.37)	14.2 (0.69)	14.2 (0.59)
Red Blood Cell (10^6/mL)	NS	6.39 (0.37)	6.22 (0.50)	6.49 (0.35)	6.38 (0.40)	6.22 (0.41)

[a,b,c]Dose groups with the same letter are not significantly different ($\alpha = 0.05$).

TABLE 3

FINDINGS OF THE 90-DAY CIS-1,2-DICHLOROETHYLENE TOXICITY STUDY IN FEMALE RATS

(4 pp.)

Table 3. Findings of the 90-Day cis-1,2-Dichloroethylene Toxicity Study in Female Rats

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	10 mM Mean (SD)	20 mM Mean (SD)
Body Weight						
Final (g)	NS	53.7 (17.7)	68.4 (22.5)	55.8 (21.5)	48.0 (17.2)	51.5 (28.7)
Gain (g)	NS	315 (23.4)	316 (26.7)	305 (38.2)	303 (24.8)	301 (40.7)
Average Daily Consumption (ADC)						
Water ADC (g/day)	NS	39.7 (3.63)	39.8 (3.12)	49.5 (12.5)	46.2 (8.16)	44.2 (1.54)
Water ADC (% Body Weight)	<0.01	12.6[a] (1.12)	12.7[a] (1.13)	16.1[b] (3.35)	15.5[ab] (3.66)	14.9[ab] (2.04)
Food ADC (g/day)	NS	18.0 (1.14)	18.1 (0.83)	18.7 (1.73)	18.7 (1.95)	18.1 (1.32)
Food ADC (% Body Weight)	NS	5.74 (0.44)	5.78 (0.68)	6.15 (0.65)	6.18 (0.53)	6.08 (0.64)
Organ Weight						
Adrenal Weight (g)	NS	0.10 (0.02)	0.09 (0.02)	0.10 (1.02)	0.11 (0.02)	0.12 (0.02)
Adrenal (% Body Weight)	0.025	0.31[ab] (0.04)	0.29[a] (0.06)	0.34[a,b] (0.06)	0.36[ab] (0.08)	0.40[b] (0.10)

Table 3 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 mM Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Brain Weight (g)	NS	2.08 (0.24)	1.98 (0.13)	2.01 (0.09)	1.96 (0.12)	1.97 (0.12)
Brain (% Body Weight)	NS	0.66 (0.08)	0.63 (0.04)	0.67 (0.08)	0.65 (0.05)	0.66 (0.09)
Kidney Weight (g)	NS	2.18 (0.22)	2.24 (0.29)	2.53 (1.01)	2.55 (0.49)	2.55 (0.37)
Kidney (% Body Weight)	NS	0.69 (0.06)	0.71 (0.05)	0.82 (0.23)	0.85 (0.21)	0.85 (0.06)
Liver Weight (g)	0.001	8.89[a] (0.81)	9.16[a] (0.56)	9.80[a,b] (1.55)	10.2[a,b] (0.89)	11.0[b] (1.34)
Liver (% Body Weight)	<0.001	2.82[a] (0.19)	2.91[a] (0.18)	3.21[b] (0.22)	3.36[b] (0.18)	3.67[c] (0.27)
Ovaries Weight (g)	NS	0.17 (0.02)	0.20 (0.04)	0.21 (0.06)	0.21 (0.04)	0.22 (0.06)
Ovaries (% Body Weight)	NS	0.06 (0.01)	0.06 (0.02)	0.07 (0.02)	0.07 (0.01)	0.07 (0.02)
Spleen Weight (g)	NS	0.68 (0.10)	0.57 (0.08)	0.57 (0.20)	0.60 (0.11)	0.56 (0.10)
Spleen (% Body Weight)	NS	0.22 (0.03)	0.18 (0.03)	0.19 (0.05)	0.20 (0.04)	0.19 (0.03)

dose response

Table 3 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 mM Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Thymus Weight (g)	0.02	0.31[a] (1.09)	0.45[a] (1.39)	0.30[a,b] (0.64)	0.34[a,b] (1.08)	0.35[b] (1.21)
Thymus (% Body Weight x 10)	0.02	0.99[a] (0.18)	1.40[a] (0.27)	1.00[a,b] (0.29)	1.11[a,b] (0.33)	1.16[b] (0.31)
Clinical Chemistry						
AST (IU/L)	0.02	39.3[a] (9.09)	32.0[a,b] (6.86)	28.0[a,b] (5.62)	26.0[a,b] (12.7)	27.5[b] (6.74)
Blood Urea Nitrogen (mg/dL)	NS	20.1 (2.44)	16.7 (2.60)	20.0 (8.55)	19.0 (9.02)	19.0 (3.68)
Calcium (mg/dL)	NS	10.9 (0.33)	11.1 (0.58)	10.7 (0.23)	9.86 (3.36)	10.8 (0.20)
Creatinine (mg/dL)	NS	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.05 (0.01)	0.05 (0.01)
Cholesterol (mg/dL)	NS	88.0 (15.8)	91.3 (27.6)	87.7 (22.2)	81.7 (16.0)	108 (29.4)
Phosphorous (mg/dL)	<0.001	6.15[a] (1.02)	6.89[a,c] (1.44)	8.27[b] (0.78)	7.73[b,c] (0.61)	7.28[a,b,c] (0.78)

Table 3 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 mM Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Hematology						
Hemoglobin (g/dL)	0.02	15.2[b] (0.27)	14.7[ab] (0.66)	14.5[ab] (0.72)	14.3[a] (0.64)	14.6[ab] (0.51)
Hematocrit (%)	<0.001	41.3[b] (0.67)	39.5[ab] (1.97)	39.0[ab] (2.52)	37.2[a] (1.56)	38.5[a] (1.87)
Red Blood Cell (10^6/mL)	0.008	7.37[b] (0.17)	7.15[ab] (0.33)	7.06[ab] (0.49)	6.78[a] (0.33)	6.92[ab] (0.39)

[a,b,c]Dose groups with the same letter are not significantly different (α =0.05)

TABLE 4

FINDINGS OF THE 90-DAY CIS-1,2-DICHLOROETHYLENE TOXICITY STUDY IN MALE RATS

(4 pp.)

Table 4. Findings of the 90-Day cis-1,2-Dichloroethylene Toxicity Study in Male Rats

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 mM Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Body Weight						
Final (g)	NS	578 (62.0)	558 (75.1)	569 (55.7)	520 (46.6)	512 (55.1)
Gain (g)	0.025	180[a] (40.3)	157[a,b] (47.0)	162[a,b] (34.4)	131[a,b] (34.8)	114[b] (40.8)
Average Daily Consumption (ADC)						
Water ADC (g/day)	NS	49.3 (4.85)	50.1 (3.75)	53.6 (6.97)	51.3 (6.22)	52.2 (5.06)
Water ADC (% Body Weight)	<0.03	8.76[a] (1.08)	9.11[a,b] (1.24)	9.45[a,b] (0.89)	9.56[a,b] (1.00)	10.8[b] (1.61)
Food ADC (g/day)	NS	25.7 (1.92)	26.8 (2.19)	27.1 (2.98)	25.2 (2.10)	25.5 (2.77)
Food ADC (% Body Weight)	NS	4.49 (0.29)	4.86 (0.51)	4.78 (0.36)	4.78 (0.34)	5.08 (0.55)
Organ Weight						
Adrenal Weight (g)	NS	0.09 (0.03)	0.09 (0.03)	0.09 (0.02)	0.07 (0.01)	0.09 (0.01)
Adrenal (% Body Weight x 10)	NS	0.15 (0.05)	0.16 (0.05)	0.16 (0.04)	0.14 (0.02)	0.17 (0.03)

Table 4 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 mM Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Brain Weight (g)	NS	2.15 (0.10)	2.14 (0.13)	2.17 (0.15)	2.09 (0.09)	2.11 (0.08)
Brain (% Body Weight)	NS	0.37 (0.03)	0.39 (0.05)	0.38 (0.03)	0.41 (0.04)	0.42 (0.5)
Kidney Weight (g)	NS	4.02 (0.56)	4.40 (0.57)	4.70 (0.59)	4.30 (0.77)	4.58 (0.74)
Kidney (% Body Weight)	<0.001	0.70[a] (0.06)	0.80[b] (0.06)	0.83[b] (0.06)	0.83[b] (0.10)	0.89[b] (0.06)
Liver Weight (g)	NS	16.6 (3.07)	17.6 (3.12)	18.7 (2.09)	17.5 (3.71)	19.1 (1.92)
Liver (% Body Weight)	<0.001	2.85[a] (0.26)	3.15[ab] (0.27)	3.28[b] (0.18)	3.34[b,c] (0.44)	3.75[c] (0.20)
Spleen Weight (g)	NS	0.84 (0.13)	0.83 (0.13)	0.81 (0.14)	0.75 (0.15)	0.71 (0.12)
Spleen (% Body Weight)	NS	0.15 (0.02)	0.15 (0.02)	0.14 (0.02)	0.14 (0.03)	0.14 (0.02)
Testes Weight (g)	NS	3.51 (0.28)	3.67 (0.33)	3.63 (0.24)	3.49 (0.46)	3.45 (0.19)
Testes (% Body Weight)	NS	0.61 (0.06)	0.67 (0.09)	0.64 (0.08)	0.67 (0.06)	0.68 (0.07)

Table 4 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 m M Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Thymus Weight (g)	NS	0.42 (0.21)	0.44 (0.17)	0.45 (0.16)	0.36 (0.12)	0.37 (0.8)
Thymus (% Body Weight x 10)	NS	0.71 (0.28)	0.78 (0.21)	0.78 (0.23)	0.68 (0.19)	0.72 (0.17)
Clinical Chemistry						
AST (IU/L)	NS	35.7 (6.61)	34.4 (6.96)	32.2 (5.85)	35.4 (5.26)	26.1 (7.52)
Blood Urea Nitrogen (mg/dL)	<0.001	20.7[a] (3.07)	17.6[a] (1.90)	16.3[ab] (1.43)	16.1[ab] (2.92)	12.4[b] (1.42)
Calcium (mg/dL)	<0.001	10.4[a] (0.33)	11.2[bc] (0.39)	11.5[b] (0.41)	10.8[ac] (0.41)	11.0[ab,c] (0.17)
Creatinine (mg/dL)	0.002	0.62[a] (0.08)	0.58[a] (0.06)	0.52[ab] (0.08)	0.51[ab] (0.07)	0.42[b] (0.08)
Cholesterol (mg/dL)	NS	68.7 (12.4)	59.9 (25.6)	67.3 (12.7)	54.3 (19.6)	68.3 (21.9)
Phosphorous (mg/dL)	0.0035	8.02[b] (0.60)	6.91[a] (0.66)	7.48[a,b] (0.46)	7.77[ab] (0.73)	7.03[ab] (0.63)

Table 4 Cont.

Parameter Measured (Unit)	Significance p-Value	Control Mean (SD)	0.33 m M Mean (SD)	1 mM Mean (SD)	3 mM Mean (SD)	9 mM Mean (SD)
Hematology						
Hemoglobin (g/dL)	0.025	15.1[a] (0.65)	14.4[ab] (0.63)	14.6[ab] (0.59)	14.2[b] (0.44)	14.2[b] (0.58)
Hematocrit (%)	0.0025	41.4[a] (2.22)	38.9[ab] (1.82)	39.0[b] (1.58)	37.7[b] (1.08)	37.7[b] (2.51)
Red Blood Cell (10^6/mL)	NS	7.64 (0.04)	7.42 (0.38)	7.35 (0.23)	7.22 (0.21)	7.29 (0.38)

[a,b,c]Dose groups with the same letter are not significantly different (α =0.05)