Subchronic Inhalation Toxicity Study of 1,3-Dichloro-2-propanol in Rats

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The subchronic toxicity of 1,3-dichloro-2-propanol (1,3-DCP) was investigated in Fischer 344 rats after 13 weeks of repeated, whole-body inhalation exposure. Groups of 10 rats of each sex were exposed to 1,3-DCP vapor by whole-body inhalation exposure at concentrations of 0, 5, 20 or 80 ppm for 6 h/day, 5 days/week for 13 weeks. All of the rats were sacrificed at the end of the treatment period. During the test period, clinical signs, mortality, body weights, food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, gross findings, organ weights and histopathology were assessed. At 80 ppm, a decrease in the body weight gain, an increase in the urine protein and leukocyte counts and an increase in the liver and kidney weights were observed in both genders. Hematological and serum biochemical investigations revealed decreases in hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular HB, as well as increases in the platelet (PLT) count, serum aspartate aminotransferase and alanine aminotransferase. The number of white blood cells was significantly lower in males than in controls, but this was not the case in females. Histopathological alterations included an increase in the incidence of multifocal necrosis, inflammation, pigmentation, biliary hyperplasia and the foci of cellular alteration of the liver and chronic nephropathy and protein cast of the kidney. At 20 ppm, decreases in HCT and MCV and increases in the liver and kidney weights were observed in both genders. A decrease in the HB of females and an increase in the PLT count of females were also observed. Histopathological alterations included slight increases in the incidences of hepatic necrosis, hepatic inflammation and chronic nephropathy. At 5 ppm, we found decreases in the MCV of males and the HB of females, as well as an increase in the liver weight of both genders. In the present experimental conditions, the target organs were determined to be the liver, kidney and blood cells in rats. The no-observed-adverse-effect level was considered to be <5 ppm/6 h/day and the low-observed-adverse-effect level was believed to be 5 ppm/6 h/day in rats.

Keywords: 1,3-dichloro-2-propanol; industrial chemical; rats, subchronic inhalation toxicity

INTRODUCTION

Volatile organic compounds (VOCs) have high vapor pressures and are easily vaporized at ambient temperature and pressure. Most hydrocarbons, including nitrogenous, chlorinated and sulfurred organics, can be classed as VOCs. These compounds are usually utilized in industries that manufacture or use organic solvents, e.g. petrochemical, pulp or coating industries. Dichloropropanols are a family of chlorinated semi-VOCs that are used in industries such as hard resin production, water chlorination and paper fabrication (Garle et al., 1999; Hammond and Fry, 1999). 1,3-Dichloro-2-propanol (1,3-DCP) is used in high volume as an intermediate in the production of epichlorohydrin, the monomer used widely in the production of epoxy resin. Dehydration of 1,3-DCP with phosphoryl chloride forms 1,3-dichloropropene, a soil fumigant. Chlorination of 1,3-DCP with phosphorus pentachloride yields 1,2,3-trichloropropene. Hydrolysis of dichlorohydrins has been used in the

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production of synthetic glycerol. Therefore, workers may be exposed to 1,3-DCP during the manufacture and use of these chemicals. Exposure to 1,3-DCP may also occur from ingestion of food to which hydrochloric acid-hydrolyzed vegetable protein has been added or drinking water in which epichlorohydrin polyamine polyelectrolytes are used as flocculents and coagulants for water purification (Nyman et al., 2003).

The most common adverse effects associated with 1,3-DCP in humans are hepatotoxicity, irritation of the mucous membranes, eyes and skin, as well as nausea and vomiting (Shiozaki et al., 1994). In Japan, 12 workers involved in the cleaning of a saponification tank that had contained epichlorohydrin were exposed to the compound by inhalation at an unknown concentration (Haratake et al., 1988). Of the 12 workers, five developed fulminant hepatitis and two of the five died of hepatic failure at 4 and 11 days, respectively, after carrying out the work. The potential toxicity of 1,3-DCP has been studied extensively over the past several decades using both short- and long-term animal tests. The acute oral LD$_{50}$ of 1,3-DCP ranged from 110 to 400 mg kg$^{-1}$ in rats and from 25 to 125 mg kg$^{-1}$ in mice, depending on the purity of 1,3-DCP and the rodent sex and strain used (RTECS, 2000; HSDB, 2002). The inhalation LC$_{50}$ of 1,3-DCP was 320–600 ppm (1700–3200 mg m$^{-3}$) following 1 to 5 days in mice. The 4-h LC$_{50}$ of 1,3-DCP for rats was found to be 125 ppm (Smyth et al., 1962; BIBRA International Ltd., 1999). The major adverse effects observed in humans exposed to 1,3-DCP were well demonstrated in experimental animal studies (Jersey et al., 1991; Hammond et al., 1996; Ohkubo et al., 1995) and was carcinogenic in both rats (Hercules Inc., unpublished results; Eder and Weinfurtner, 1994) and an in vitro system (Piascecki et al., 1990). It has been reported that the toxic mechanisms of 1,3-DCP are lipid peroxidation (Katoh et al., 1998; Kuroda et al., 2002), glutathione depletion (Katoh et al., 1998; Garle et al., 1999; Fry et al., 1999) and disruption of the mitochondrial membrane potential (Hammond et al., 1996). The metabolite 1,3-dichloroacetone (1,3-DCA) was identified to be responsible for necrosis of the liver and other disorders (Hahn et al., 1991; Shiozaki et al., 1994).

1,3-DCP is one of the high production volume chemicals that are manufactured in or are imported into the United States at quantities of 1 million pounds per year. Since industrial workers may be exposed to 1,3-DCP at a high concentration during the manufacture and use of epichlorohydrin, 1,3-dichloropropene, 1,2,3-trichloropropane and glycerol, it is important that the potential human health risks are assessed and that occupational exposures are managed accordingly. Based on its vapor pressure and occupational use, the inhalation of 1,3-DCP vapors represents the primary potential route of human exposure to 1,3-DCP. However, little is known about the delayed effects of occupational exposure to inhaled 1,3-DCP. Despite its widespread uses and exposure, there is no information regarding the potential subchronic toxicity of 1,3-DCP after inhalation.

The aim of this study was to determine the potential subchronic inhalation toxicity of 1,3-DCP via whole-body exposure in F344 rats. Inhalation and systemic exposure to 1,3-DCP are principal routes for humans because this chemical is volatile and can permeate the skin. In addition, the reactivity of 1,3-DCP is stable at normal temperature and humidity. Therefore, it is considered that whole-body inhalation exposure is more appropriate to evaluate the toxic potential of 1,3-DCP than nose-only inhalation exposure.

**MATERIALS AND METHODS**

**Animal husbandry and maintenance**

Forty-eight 5-week-old Fischer 344 rats of each gender were obtained from a specific pathogen-free colony at Charles River Japan Inc. (Kanagawa, Japan) and used after 10 days of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 22 ± 3°C and a relative humidity of 50 ± 20% with artificial lighting from 08:00 to 20:00 and with 12–15 air changes per hour (Industrial Chemicals Research Center, Korea Industrial Safety Corporation, Daejeon, Republic of Korea). The animals were housed individually in wire-bottomed stainless steel wire mesh cages that were placed in exposure chambers and were allowed sterilized tap water and commercial rodent chow (LabDiet 5002, PMI Nutrition, USA) ad libitum. The Institutional Animal Care and Use Committee approved the protocols used in this animal study, and animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

**Test chemical and exposure**

1,3-DCP was purchased from Acros Organics (Fisher Scientific Korea). Whole-body exposure chambers (Shibata Co., Japan) including a gas generator (Shibata Co.) were used to expose the rats to 1,3-DCP. The test animals were exposed to 5, 20 or 80 ppm 1,3-DCP or fresh air for 6 h/day, 5 days/week for 13 weeks. The inhalation exposure was carried out from 10:00 to 16:00 in a stainless-steel chamber (1000 l). The experimental design was based on the
usual working schedule for workers, as well as the major exposure route for the test chemical.

**Condition of chambers**

Temperature, relative humidity, pressure and air ventilation in the chambers were recorded using an environmental controller (Shibata Co.). The temperature and relative humidity were maintained at 23.3–23.7°C and 55.9–60.5%, respectively. The concentrations of 1,3-DCP in the chambers were calibrated with a standard chemical (Acros Organics Co., UK), air pump (Iwaki Co., Japan), gas meter (Shinagawa Co., Japan) and Teflon® bags. The conditions used for detecting 1,3-DCP by gas chromatography (Shimadzu Co., Japan) were as follows: detector, flame ionization detector; column, silicon DC-200 15% chromosorb with mesh of 80/100 and a 0.5-m length; detector temperature, 150°C; oven temperature, 100°C; injector temperature, 150°C; and injection volume, 1 ml of gas sample. The 1,3-DCP vapor concentrations in the chambers were measured every 15 min during exposure and were controlled to be within ±5% of the target concentration using a personal computer. The mean concentration measured every 30 min for 6 h was taken as the value on a given day. This was then averaged over the 13-week exposure period in order to obtain the mean and standard deviations, and the daily gas concentrations in the three chambers were measured at 5.3 ± 0.78, 20.8 ± 1.86 and 79.0 ± 4.53 ppm, respectively.

**Experimental groups and selection of concentrations**

Prior to testing, rats were evaluated by clinical observations and body weight determinations during a 7-day quarantine period to assure freedom from potential confounding variables. Forty males and 40 females were randomly assigned to four experimental groups: three treatment groups receiving 5, 20 and 80 ppm 1,3-DCP and a vehicle control group. Each group consisted of 10 rats of each gender. All of the rats were sacrificed after treatment for 13 weeks. The experimental concentrations were selected based on the results of a preliminary dose-range finding study. Groups of five rats of each gender were exposed to 1,3-DCP via whole-body inhalation at concentrations of 10, 33 and 100 ppm for 2 weeks. Both males and females showed a moderate-to-severe reduction in body weight gain and food intake at 100 ppm. Accordingly, 80, 20 and 5 ppm were selected as the high, medium and low doses, respectively, using a scaling factor of ×4.

**Clinical observation and mortality**

All animals were observed twice daily (before and after exposure) throughout the study period for any clinical signs of toxicity, and mortality.

**Body weights and food consumption**

The body weights of each rat and food consumption were measured at the beginning of exposure and once a week during the exposure period. The amounts of food were calculated before they were supplied to each cage, and their remnants were measured on the next day in order to calculate the difference, which was regarded as daily food consumption (g/rat/day).

**Ophthalmoscopy**

External eye examination on all males and females was carried out shortly before the beginning of the experiments and in the last week of the exposure period. The ocular fundus was examined during the last week of the exposure period using an indirect binocular ophthalmoscope (IO-H, Neitz Instruments Co., Japan). The conjunctiva, sclera, cornea, lens and iris of each eye were also examined.

**Urinalysis**

During the last week of exposure, urinalysis for five males and five females per group was carried out with fresh urine to determine the specific gravity, pH, protein, glucose (GLU), ketone body, occult blood, bilirubin, urobilinogen, nitrite and leukocyte contents by using a Uriscan S-300 urine chemistry analyzer (Yeongdong Electronic Co., Republic of Korea).

**Hematology**

The animals were fasted overnight prior to necropsy and blood collection. Blood samples were drawn from the posterior vena cava by using a syringe with a 24-gauge needle under sodium pentobarbital anesthesia. The blood samples were collected into test tubes (Green Cross Medical Industry, Republic of Korea) and analyzed within 20 min using an automatic hematology analyzer (Hemavet 850, CDC Technology, USA). The following parameters were determined: red blood cell (RBC, erythrocyte) count, hemoglobin concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLTs), white blood cell (WBC, leukocyte) count and differential WBC count.

**Serum biochemistry**

Blood samples were centrifuged at 3000 rpm for 10 min within 1 h after collection. The sera were stored at −80°C in a freezer prior to analysis. The following serum biochemistry parameters were evaluated using an autoanalyzer (Shimadzu CL-7200, Shimadzu Co.): aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine
(CRTN), GLU, lactate dehydrogenase (LDH), total cholesterol (T-CHO), total bilirubin (T-BIL), total protein (TP) and creatine phosphokinase (CPK).

**Gross findings**

At the end of experiments, all surviving animals were anesthetized by an intraperitoneal administration of sodium pentobarbital (50 mg kg\(^{-1}\) body weight) for blood sample collection. The rats were then sacrificed by exsanguination from the abdominal aorta. Complete gross postmortem examinations were performed on all terminated animals.

**Organ weights**

The absolute and relative (organ-to-body weight ratios) weights of the following organs were measured: liver, right kidney, lung, heart, thymus, right testis and/or right ovary.

**Histopathology**

The following tissues were obtained from all animals: abnormal lesions, skin, mammary gland, spleen, pancreas, jejunum, stomach, duodenum, ileum, cecum, colon, mesenteric lymph node, salivary gland, submandibular lymph node, ovaries, uterus, vagina, urinary bladder, epididymides, prostates, seminal vesicles, rectum, kidneys, adrenal glands, liver, sternum, thymus, heart, lung, trachea, esophagus, thyroid (including parathyroids), tongue, aorta, sciatic nerve, skeletal muscle, femur, thoracic spinal cord, Harderian glands, brain, pituitary gland, eyes, testes, nasal cavity, nasal turbinates and Zymbal glands. Eyes and testes were preserved in Davidson’s fixative and Bouin’s fixative, respectively. Other tissues were fixed with a 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin and sectioned at 3–5 \(\mu\)m. The sections were stained with hematoxylin–eosin stain for microscopic examination. The nasal passages and nasal turbinates were decalcified prior to being embedded and sectioned. The nasal cavity was sectioned at levels posterior to the upper incisors, the incisive papilla, the second palatine ridge and the first molar teeth (Young, 1981). All organs and tissues taken from all animals in the vehicle control and the high-dose groups were examined microscopically. All gross lesions, as defined by the study pathologist, were also included in the examination.

**Statistical analysis**

Data are presented as means ± SDs. The variance of numerical data was checked using Barlett’s (1937) test. If the variance was homogeneous, data were subjected to one-way analysis of variance (ANOVA), and if the variance was not homogenous, data were analyzed by the multiple comparison procedure of the Dunnett (1964) post-hoc test. Clinical signs, necropsy findings and histopathological findings were represented as frequencies and were subjected to Fisher (1970) exact probability test when necessary. All statistical analyses were conducted with Statistical Analysis Software (SAS, NC, USA). The significance of the differences from the control group was estimated at the probability levels of 1% and 5%.

**RESULTS**

**Clinical signs and mortality**

No treatment-related toxic symptoms or mortality were observed in any of the animals treated with 1,3-DCP during the study period (data not shown).

**Body weight changes and food consumption**

As shown in Fig. 1, the body weight gain of male rats was statistically significantly suppressed in the 80-ppm group on Days 7 through 90 of treatment compared with that of the control group. As presented in Fig. 2, the body weight gain of female rats was also significantly lower in the 80-ppm group from Day 7 of treatment to termination compared with the control group. Although the difference was not statistically significant between the groups, food consumption was also slightly lower in the 80-ppm groups of both genders from Day 1 of the test to termination compared with that of the control group (data not shown).

**Ophthalmoscopy**

Ophthalmologic examinations did not show any treatment-related ocular lesions in any of the animals (data not shown).

![Fig. 1. Mean body weights for male rats exposed to 1,3-DCP at concentrations of 0 (filled circles), 5 (open circles), 20 (filled inverted triangles) and 80 (open squares) ppm. Values are presented as means ± SDs. **Indicates significant difference at \(P < 0.01\) compared with the control group.](https://example.com/figure1.png)
Urinalysis

The results of urinalysis are presented in Table 1. In the male 80-ppm group, a statistically significant increase in urine protein was observed. In the female 80-ppm group, significant increases in urine protein and leukocyte counts were observed. The other urinary parameters tested in both sexes were not significantly different between the treatment groups and controls.

Hematology

Table 2 summarizes the hematological findings for the male and female rats obtained during the study. In males, MCV was significantly decreased in the 5-ppm group when compared with the control group. HCT and MCV were significantly decreased in the 20-ppm group, and MCHC was significantly increased in the same dose group in comparison with that of the control group. Hemoglobin (HB), HCT, MCV, MCH and WBC were significantly decreased, while the PLT count was significantly increased in the 80-ppm group when compared with that of the control group. In females, HB was significantly decreased in the 5-ppm group compared with the control group. RBC, HB, HCT and MCV were significantly decreased, while MCHC and PLT count were significantly increased in the 20-ppm group when compared with the control group. The 80-ppm group showed statistically significant decreases in the HB, HCT, MCV and MCH and a statistically significant increase in the PLT count compared with the controls.

Serum biochemistry

Table 3 shows the results of serum biochemistry tests obtained in the study for male and female rats. In males, statistically significant decreases in AST, ALP, BUN, LDH and CPK were observed in the 5-ppm group in comparison with the control group. The 20-ppm group showed statistically significant decreases in ALP, BUN, T-BIL and CPK and statistically significant increases in serum GLU and TP when compared with the control group. The serum levels of ALP, BUN, CRTN and T-CHO were significantly decreased, while AST, ALT and TP were significantly increased in the 80-ppm group as compared with those of the control group. In females, statistically significant decreases in ALP and BUN were observed in the 5-ppm group compared with those of the control group. The 20-ppm group exhibited statistically significant decreases in ALP, BUN, CRTN and

![Fig. 2.](image_url) Mean body weights for female rats exposed to 1,3-DCP at concentrations of 0 (filled circles), 5 (open circles), 20 (filled inverted triangles) and 80 (open squares) ppm. Values are presented as means ± SDs. **Indicates significant difference at P < 0.01 compared with the control group.

Table 1. Urinary analysis in male and female rats after inhalation of 1,3-DCP for 13 weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose (ppm)</th>
<th>0</th>
<th>5</th>
<th>20</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketone (mg per 100 ml)</td>
<td>10.00 ± 0.00</td>
<td>6.00 ± 5.48</td>
<td>9.00 ± 2.24</td>
<td>8.00 ± 4.47</td>
<td></td>
</tr>
<tr>
<td>Protein (mg per 100 ml)</td>
<td>22.00 ± 10.96</td>
<td>36.00 ± 37.15</td>
<td>24.00 ± 13.42</td>
<td>86.00 ± 31.30**</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>6.70 ± 0.27</td>
<td>6.70 ± 0.27</td>
<td>6.80 ± 0.45</td>
<td>6.50 ± 0.00</td>
</tr>
<tr>
<td>Leucocytes (WBC l⁻¹)</td>
<td>19.00 ± 8.22</td>
<td>19.00 ± 8.22</td>
<td>32.00 ± 24.90</td>
<td>130.00 ± 207.97</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketone (mg per 100 ml)</td>
<td>7.00 ± 4.47</td>
<td>4.00 ± 5.48</td>
<td>9.00 ± 2.24</td>
<td>2.00 ± 4.47</td>
<td></td>
</tr>
<tr>
<td>Protein (mg per 100 ml)</td>
<td>8.00 ± 13.04</td>
<td>2.00 ± 4.47</td>
<td>0</td>
<td>20.00 ± 14.14*</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>6.10 ± 0.22</td>
<td>6.60 ± 0.22</td>
<td>6.50 ± 0.5</td>
<td>6.50 ± 0.35</td>
</tr>
<tr>
<td>Leucocytes (WBC l⁻¹)</td>
<td>7.00 ± 10.95</td>
<td>17.00 ± 11.51</td>
<td>12.00 ± 12.55</td>
<td>65.00 ± 22.36**</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means ± SDs.

*Indicates significant difference at P < 0.05 compared with the control group.

**Indicates significant difference at P < 0.01 compared with the control group.
T-BIL compared with those of the control group. The 80-ppm group showed statistically significant decreases in ALP, BUN and CRTN and statistically significant increases in AST, ALT and TP compared with the findings for the control group.

**Gross findings**

At the scheduled necropsy, there were no treatment-related gross findings in any of the treated animals.

**Relative organ weights**

In males (Table 4), the liver weight in all of the treatment groups and kidney weight in the 20- and 80-ppm groups were significantly increased in a dose-dependent manner compared with those of the control group. The weights of testis and heart in the 80-ppm group were also significantly increased in comparison to the control group. In females, the liver weight in all of the treatment groups and the kidney weight in the 20- and 80-ppm groups were significantly increased in a dose-dependent manner compared with those of the control group. Heart weight in the 80-ppm group was also significantly heavier than that of controls.

**Histopathological findings**

The results of histopathological examination are presented in Table 5. In males, 10 cases of multifocal necrosis, 4 cases of inflammation, 10 cases of pigmentation, 8 cases of biliary hyperplasia and 1 case of the foci of cellular alteration were observed in the livers of the 80-ppm group. Ten cases of chronic nephropathy were also found in the 80-ppm group. One case each of multifocal necrosis and inflammation was found in the 20-ppm group. Chronic nephropathy was noted in the 80-ppm group. In females, 10 cases of multifocal necrosis, 7 cases of inflammation, 10 cases of pigmentation, 1 case of biliary hyperplasia and 6 cases of the foci of cellular alteration were observed in the liver of the 80-ppm group. Four cases of chronic nephropathy and 2 cases of renal protein cast were also observed in the 80-ppm group. Hepatic inflammation occurred in 2 cases in the 20-ppm group. The incidences of these findings were significantly higher in the 80-ppm group than those in the control group, but not in the 20-ppm group. The severity of the histopathological changes was slight in the 20-ppm group, but moderate-to-severe in the 80-ppm group. The other histopathological findings observed in both genders of the treatment groups were also found in the control group or were determined to be spontaneous changes without any dose–response relationship.

**DISCUSSION**

The present study was conducted to investigate the potential subchronic toxicity of 1,3-DCP in Fisher
Table 4. Relative organ weight of male and female rats after inhalation of 1,3-DCP for 13 weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose (ppm)</th>
<th>0</th>
<th>5</th>
<th>20</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>2.69 ± 0.15</td>
<td>3.12 ± 0.18</td>
<td>3.54 ± 0.21</td>
<td>4.26 ± 0.48**</td>
</tr>
<tr>
<td>Kidney: right</td>
<td></td>
<td>0.29 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.35 ± 0.04**</td>
<td>0.41 ± 0.04**</td>
</tr>
<tr>
<td>Testis: right</td>
<td></td>
<td>0.48 ± 0.02</td>
<td>0.48 ± 0.03</td>
<td>0.50 ± 0.03</td>
<td>0.56 ± 0.03**</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>0.43 ± 0.06</td>
<td>0.39 ± 0.04</td>
<td>0.41 ± 0.03</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.29 ± 0.02</td>
<td>0.31 ± 0.01</td>
<td>0.32 ± 0.03</td>
<td>0.34 ± 0.03**</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td>0.09 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>2.49 ± 0.21</td>
<td>2.92 ± 0.14**</td>
<td>3.37 ± 0.16**</td>
<td>4.98 ± 0.35**</td>
</tr>
<tr>
<td>Kidney: right</td>
<td></td>
<td>0.31 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.36 ± 0.02**</td>
<td>0.42 ± 0.02**</td>
</tr>
<tr>
<td>Ovary: right</td>
<td></td>
<td>0.03 ± 0.006</td>
<td>0.03 ± 0.006</td>
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<td>0.03 ± 0.006</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>0.51 ± 0.12</td>
<td>0.50 ± 0.07</td>
<td>0.57 ± 0.07</td>
<td>0.59 ± 0.07</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.34 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>0.39 ± 0.03**</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td>0.14 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

Values are presented as means ± SDs (%).

*Indicates significant difference at $P < 0.05$ compared with the control group.

** Indicates significant difference at $P < 0.01$ compared with the control group.
344 rats following 13 weeks repeated whole-body exposure at concentrations of 0, 5, 20 and 80 ppm. The results of this study showed that the whole-body inhalation exposure to 1,3-DCP for 13 weeks resulted in various adverse effects on body weight, liver, kidney and blood cells at concentrations >5 ppm in rats.

No treatment-related clinical signs were observed at any of the doses examined. Some adverse clinical signs on the skin, eyes and respiratory systems were expected in the animals because 1,3-DCP is a chemical that acts as a skin and eye irritant (Smyth et al., 1962; RTECS, 2000). However, in the study no irritant effects were observed in any animal at doses of 1,3-DCP up to 80 ppm. The significant suppression of body weight gain observed in the 80-ppm groups of both genders was attributed to the exposure to the test chemical, which is consistent with the slightly decreased food consumption observed in the group during the study period. This is a clear indication of the general toxicity induced by 1,3-DCP, which suggests that inhalation exposure to this chemical causes mild anorexia, followed by the suppression of body weight gain in rats. The suppression of body weight gain was consistently observed until the end of the experimental period in the 80-ppm group. These findings agree with observations following repeated oral intakes of 1,3-DCP. According to the report of Jersey et al. (1991), repeated oral administration of 1,3-DCP caused a significant decrease in body weight gain and food consumption when it was administered to rats by gavage at 10 mg kg⁻¹ day⁻¹ for 13 weeks.

The significant increase of urine protein observed in the 80-ppm groups of both genders was considered to be related to the 1,3-DCP treatment because it was observed in both genders at the higher concentrations and was associated with microscopic pathological changes. The significant increase of the urine leukocyte count observed in the female 80-ppm group was also considered to be related to the 1,3-DCP treatment, since the increment was remarkable compared with the control group and because a tendency to increase was also found in the male 80-ppm group. These findings suggest that inhalation exposure to 1,3-DCP at 80 ppm caused an increase in glomerular permeability for normally non-filtered plasma macromolecules such as albumin (ALB) due to chronic nephritis, which was also observed in the histopathological examination.

### Table 5. Number of cases of histopathological findings in male and female rats after inhalation of 1,3-DCP for 13 weeks

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th></th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (ppm)</td>
<td>0</td>
<td>5</td>
<td>20</td>
<td>80</td>
<td>Dose (ppm)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>No. of rats</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Multifocal necrosis</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>10*</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td></td>
<td>0</td>
<td>0</td>
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<tr>
<td>Pigmentation</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
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<tr>
<td>Biliary hyperplasia</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8*</td>
<td></td>
<td>0</td>
<td>0</td>
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<tr>
<td>Foci of cellular alteration</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic nephropathy</td>
<td></td>
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<td>0</td>
<td>4</td>
<td>10*</td>
<td></td>
<td>0</td>
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<td>Protein cast</td>
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<td></td>
<td></td>
<td>0</td>
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<tr>
<td>Heart</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Pituitary gland</td>
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<td>0</td>
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<td>Submandibular lymph node</td>
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<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Focal hyperplasia</td>
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<td>0</td>
<td>2</td>
<td>1</td>
<td></td>
<td>0</td>
<td>1</td>
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<tr>
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<td></td>
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<tr>
<td>Vacuolation</td>
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<td>0</td>
<td></td>
<td>1</td>
<td>1</td>
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<tr>
<td>Ovary</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiectasis</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Luteal cyst</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clitorial gland</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Indicates significant difference at $P < 0.05$ compared with the control group.
The significant decrease of HCT observed for both genders in the 20- and 80-ppm groups was considered to be a treatment-related effect, since this finding exhibited a clear-cut dose–response relationship and was consistent with the decreased MCV observed in these groups. The treatment-related reduction of MCV was observed even in the male 5-ppm group. However, the RBC count was not affected by treatment with 1,3-DCP. Therefore, it was thought that the significant decrease of HCT observed in this study mainly resulted from the reduction of MCV. The significant decrease of HB observed in the male 80-ppm group and all of the female groups was considered to be a treatment-related effect, since it was observed in both sexes, and the female treatment groups showed a clear-cut dose-dependent reduction. The significant decrease of WBC found in the 80-ppm groups of both genders was associated with a decreased HB concentration. The significant increase of PLTs observed in the male 80-ppm group and the female 20- and 80-ppm groups and the significant decrease of MCH were also considered to be due to the 1,3-DCP treatment, because these changes were remarkable and showed a clear-cut dose–response relationship. On the other hand, the significant decrease of RBC observed in the female 20-ppm group and the significant increase of MCHC found in the 20-ppm groups of both genders were not considered to be treatment related. This is because the changes did not exhibit a dose–response relationship and were within the limits of normal biological variations (Wolford et al., 1986; Kang et al., 1995; Petterino and Argentino-Storino, 2006). The hematological findings obtained in the present study are consistent with the results of the previous reports, which indicated that the 13-week oral repeated dose of 1,3-DCP to rats caused significant alterations in hematological parameters (Jersey et al., 1991; Lym et al., 2003).

Serum aminotransferase activities have long been considered to be sensitive indicators of hepatic injury (Molander et al., 1955). Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells (Zimmerman and Seef, 1970). Therefore, the marked release of AST and ALT into the circulation indicates severe damage to hepatic tissue membranes during intoxication. The significant increase of serum AST and ALT activities observed in the 80-ppm groups of both genders was considered to be treatment related because the increment was remarkable (45–559%) and showed a clear-cut dose–response relationship. The hepatotoxic effects induced by 1,3-DCP administration were confirmed by both increased liver weight and histopathological alterations. The other serum biochemical changes observed in the treatment groups were of no toxicological significance because they were slight and/or did not show a dose–response relationship. Moreover, they were within the limits of normal biological variations (Wolford et al., 1986; Kang et al., 1995; Petterino and Argentino-Storino, 2006) and were unaccompanied by correlated histopathological findings.

It is well known that body weight and organ weight are sensitive indicators of potentially toxic chemicals in general toxicity studies (Andersen et al., 1999; Kim et al., 2004). As described above, inhalation exposure of rats to 1,3-DCP caused a significant suppression in body weight gain in the 80-ppm groups of both genders. The suppressed body weight in the group affected the relative weights of some organs such as the testis and heart in the high-dose group. However, the weight changes in the above organs are of uncertain toxicological significance because they are considered to be the result of a reduction in body weight and because there was no corresponding pathology that accompanied these differences in organ weight. On the contrary, the increased relative liver weight observed in all of the treatment groups of both genders and the increased relative kidney weight found in the 20- and 80-ppm groups of both genders indicate that these findings are closely related to the exposure of 1,3-DCP, since correlated histopathological changes such as inflammation, necrosis and hyperplasia in the liver and nephropathy in the kidney were detected in the groups at high frequencies.

The principal histopathological findings observed in the present study included multifocal necrosis, inflammation, pigmentation, biliary hyperplasia and foci of cellular alteration in the liver and chronic nephropathy and protein cast in the kidney. The dose-dependent increase in both the incidence and severity of these findings with increasing dose indicates that these findings were caused by exposure to 1,3-DCP. Previous studies have also demonstrated that 1,3-DCP has severe adverse effects on various major organs, such as the liver and kidney, in humans and experimental animals (Haratake et al., 1988; Jersey et al., 1991; Lym et al., 2003). The other histopathological changes observed in the treatment groups were not considered to be treatment-related effects, because they occurred at a low incidence and did not exhibit a dose–response relationship. Moreover, these findings are well known to occur commonly in normal Fischer rats (Boorman et al., 1990; Greaves, 1990; Haschek and Rousseaux, 1998; Kim et al., 2006).

Limited data have been reported on the potential repeated dose toxicity of 1,3-DCP. Furthermore, these reported toxic effects of 1,3-DCP have not been fully documented, as these reports were published as abstract reports. According to the abstract report of Jersey et al. (1991), 13 weeks of oral repeated doses of 1,3-DCP to rats caused decreased body weight...
gain and feed consumption, altered hematological parameters, increased liver and kidney weights, alterations in serum chemistry and urinary parameters, gross pathological changes in the stomach and histopathological changes in the stomach, kidney, liver and nasal tissue at 100 mg kg\(^{-1}\) day\(^{-1}\); it also caused increased liver weights and histopathological changes in the stomach, kidneys and liver at 10 mg kg\(^{-1}\) day\(^{-1}\). In a more recent study (Lym et al., 2003), Sprague-Dawley rats given 1,3-DCP at 15, 30 or 60 mg kg\(^{-1}\) day\(^{-1}\) by daily gavage for 13 weeks exhibited dose-dependent increases in liver and kidney weights. In males only, an increase in ALB and dose-dependent decreases in WBCs, MCV, MCH and basophils were observed. In females, RBC, HB, HCT, MCH concentration and neutrophils were slightly decreased, while PLTs and T-CHO were increased. A carcinogenicity study showed that treatment-related non-neoplastic lesions were observed in the liver, kidney and thyroid of male and female Wistar rats given 1,3-DCP via their drinking water for 104 weeks at the dose levels of 6.3 mg kg\(^{-1}\). Statistically significant dose-related increases in the combined incidences of the following tumors were observed in male and female rats: in the liver, hepatocellular adenoma and carcinoma; in the tongue/oral cavity, squamous cell papilloma and carcinoma; and in the thyroid, follicular cell adenoma and carcinoma. The results reported by the above researchers and the present study clearly show that 1,3-DCP is hepatotoxic, nephrotoxic and hematotoxic in experimental animals.

In conclusion, the 13-week repeated inhalation exposure of rats to 1,3-DCP resulted in decreases in HB and MCV and an increase in the relative liver weight at >5 ppm; decreases in the HCT and PLTs and increases in the relative kidney weight and histopathological alterations including hepatic necrosis, hepatic inflammation and chronic nephropathy at >20 ppm; as well as decreases in the body weight gain, MCH and WBC and increases in the urine protein, leukocyte, AST, ALT, histopathological alterations such as multifocal necrosis, inflammation, pigmentation, biliary hyperplasia and foci of cellular alteration of the liver, and chronic nephropathy and protein cast of the kidney at 80 ppm. The target organs were determined to be the liver, kidney and blood cells in rats. The no-observed-adverse-effect level was considered to be <5 ppm/6 h/day, and the low-observed-adverse-effect level was believed to be 5 ppm/6 h/day in rats. The present results are expected to provide some information on the general toxic effects and target organ toxicity of 1,3-DCP via repeated inhalation exposure, which can aid in the process of risk assessment.

REFERENCES


REFERENCES


