Thirteen-Week Inhalation Toxicity of N,N-Dimethylformamide in F344/N Rats and B6C3F1 Mice

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Male and female F-344 rats and B6C3F1 mice (10/sex/group) were exposed to N,N-dimethylformamide (DMF) by whole body inhalation exposure at 0, 50, 100, 200, 400, or 800 ppm, 6 h/day, 5 days/week, for 13 weeks. A concentration-dependent depression in body weight occurred in rats of both sexes at 400 (6–11%) and 800 ppm (20–22%). In contrast, all weight changes in both sexes of mice were within 10% of controls. No rats died, while 5 mice died from nonexposure-related causes. Relative liver weights were significantly increased at all DMF concentrations in both sexes and both species. Activities of serum sorbitol dehydrogenase (SDH) were statistically increased in male and female rats (200 to 800 ppm) on study days 4, 24, and 91 (13 weeks). Activities of alanine aminotransferase (ALT) and isocitrate dehydrogenase (ICD) were statistically increased in both sexes of rats exposed to 800 ppm DMF at all time points. Cholesterol (CHOL) levels were statistically increased in male and female rats (50–800 ppm) at all sampling time points. Levels of total bile acids (TBA) were statistically increased in both sexes of rats (400–800 ppm) on days 24 and 91. Centrilobular hepatocellular necrosis (minimal to moderate) was seen in rats of both sexes exposed at 400 and 800 ppm, with the lesions more severe in females. Centrilobular hepatocellular hypertrophy (minimal to mild) was found in all groups of DMF-exposed male mice, and in female male rats at 100–800 ppm. For male and female rats the no-observed-adverse-effect concentration (NOAEC) for microscopic liver injury was 200 ppm. The NOAEC was 50 ppm for female mice, but an NOAEC based upon the absence of microscopic liver injury was not determined in male mice.

Key Words: dimethylformamide; DMF; 13-week inhalation study; hepatotoxicity; centrilobular hepatocellular necrosis; centrilobular hepatocellular hypertrophy.

N,N-Dimethylformamide (dimethylformamide, DMF, CAS No. 68-12-2) is a colorless liquid, miscible with water, synthesized from the reaction of methyl formate with diethylamine. DMF is an excellent solvent due to its small molecular size, high dielectric constant, electron donating properties, its ability to form complexes, and its low volatility. This solvent action makes DMF useful in the manufacture of fibers and films, as a booster solvent in printing and adhesive formulations. DMF is used as a solvent for polyamides or polyurethanes used to produce synthetic leather, acrylic fibers, and wire enamels. DMF is used in many hydrocarbon separations such as the recovery or removal of acetylene and the extraction of butadiene from hydrocarbon streams (Du Pont, 1986; Trochimowicz et al., 1994). The National Institute for Occupational Safety and Health (NIOSH) estimates that more than 100,000 workers may be exposed to DMF in the United States (NIOSH, unpublished database, 1983). Currently, there are two domestic producers of DMF with an annual production capacity of about 48,000 metric tons (Marsella, 1994).

The toxicity of DMF has been studied in a variety of species employing numerous routes of administration (Kennedy, 1986, 2001). Hepatotoxicity has been reported in various animal species and humans following both acute and subchronic exposure (Scailteur and Lauwerys, 1987). The acute inhalation toxicity of DMF has been reasonably well characterized. Rats survived a single 4 h exposure to saturated vapors (nominal concentration approximately 5000 ppm) of DMF (Smyth and Carpenter, 1948). Roure et al. (1996) reported increased activities of serum sorbitol dehydrogenase in rats 24, 48, and 72 h after a single 4 h exposure to DMF (75 to 900 ppm). Inhalation of 2523 ppm DMF for 6 h/day for 5 days resulted in progressive weakness, discomfort, and weight loss, and 7 of 10 rats died (Kennedy and Sherman, 1986). Clayton et al. (1963) reported that two-week exposures of rats to DMF resulted in increased liver-to-body weight ratios in each of three different DMF exposure regimens. Exposures consisted of 91 ppm, 6 h/day, for 10 days; 1104 ppm, 0.5 h/day, for 10 days; or 91 ppm for 6 h/day, for 10 days, followed by a single exposure to 841 ppm for 0.5 h after the tenth exposure. These authors further investigated the effects of DMF administered at a concentration of 23 ppm for 5.5 h/day followed by 426 ppm during the final 30 min of each exposure day (58 total expo-
sures). Histopathologic changes (not defined) were observed in the livers of all species exposed to DMF (rats, rabbits, guinea pigs, mice, and dogs). Craig et al. (1984) exposed groups of male and female F344 rats and B6C3F1 mice to DMF at 0, 150, 300, 600, and 1200 ppm, 6 h/day, 5 days/week, for 12 weeks. Body weights were significantly reduced in rats exposed at 1200 ppm, but no body weight changes were observed in mice. Hepatic fibrosis was detected in male rats exposed at 1200 ppm DMF for 12 weeks, and hepatic cytomegaly around the central veins was seen in all groups of mice.

The hepatotoxicity of DMF is not confined to animals. There are several reports of hepatotoxicity in humans following occupational exposure to DMF following dermal as well as inhalation exposure. Toxicity was confirmed by increases in the activities of serum liver enzymes (Fiorito et al., 1988) or in some cases by histological changes (necrosis/fibrosis) detected by hepatic biopsies (Potter, 1973; Redlich et al., 1988, 1990).

The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL), and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for DMF are both 10 ppm (30 mg/m³) as an 8 h time-weighted average (TWA) with a “skin” notation (ACGIH, 2001; OSHA, 1989). These exposure limits are based on the acute effects of DMF on the liver. DMF was selected for toxicologic evaluation by the National Toxicology Program because of (1) potential worker exposure due to its volatility and skin permeability; (2) its structural similarity to suspect carcinogens (dimethyl carbamyl chloride, dimethylhydrazine, and dimethyl sulfate); (3) a significant population of potentially exposed workers; and (4) high production volume.

Inhalation was chosen as the route of exposure because of the documented inhalation toxicity of DMF and because inhalation is a relevant route of human (worker) exposure. This 13-week DMF range-finding inhalation study was conducted to identify target organs, to characterize the concentration–response relationship, and to identify concentrations for use in possible subsequent chronic toxicity studies. Data from this study were used to help set concentrations used in the chronic toxicity/oncogenicity study of DMF conducted by Malley et al. (1994). This report also presents previously unpublished data on the effects of DMF exposure on cardiovascular function in rats and male reproductive effects in rats and mice.

MATERIALS AND METHODS

Chemical. Dimethylformamide was obtained in one lot (Lot 131046, Batch 02) from Chemical Dynamics Corporation (South Plainfield, NJ). This batch was identified as DMF by spectroscopy (infrared, ultraviolet/visible, and nuclear magnetic resonance). Combined analytical data (Karl Fischer titration, nonaqueous amide functional group titration, and two gas chromatographic systems) indicated a purity of greater than 99%.

Vapor generation system. DMF was generated from the liquid state as a vapor by counter-current distillation with a fresh aliquot of test chemical used each day. Liquid DMF was pumped from the glass reservoir of the generation system with a metering pump (Model RP-SY, Fluid Metering, Inc., Oyster Bay, NY) through Teflon lines to a counter-current distillation column. The column consisted of 110 mm diameter glass tubing, approximately 1 m long, packed with four stainless steel, expanded mesh, Koch-Sulzer distillation column packing units (17 cm long × 10 cm diameter, Koch Engineering Co., New York, NY). Primary dilution air supplied to the column for vaporization was drawn from conditioned room air through activated charcoal and high efficiency particulate air (HEPA) filters. The test atmosphere was generated at the highest exposure concentration, delivered to the exposure chamber with a common distribution manifold, and subsequently diluted (secondary dilution) at each chamber to target concentrations. Air was drawn by exhaust blowers into the delivery system from the air in the exposure room thus maintaining a negative pressure (relative to room) in all transport lines, plenum, and exposure chambers. All of the dilution air inlets were combined in a single manifold that drew air from one point in the room.

Vapor concentration monitoring. A Miran-980 infrared spectrometer (Foxboro Co., Norwalk, CT) with the following instrument settings was used for monitoring the DMF chamber concentrations: path length 3.75 m, slit width 1.0 mm, wavelength 3.518 μm. The exposure chambers, room, and exhaust manifold were sampled hourly. The average of three successive absorbance readings at each time point was used to calculate the respective DMF concentration. A low range calibration curve was used to measure DMF in the control chamber, room, and exhaust manifold, while a high range curve was used to monitor concentrations in the other chambers. An extensive multipoint calibration of the infrared analyzer was performed just prior to the initiation of the animal exposures. During the study the analyzer was challenged daily with a zero and three calibration standards (5.6, 56, and 896 ppm DMF).

Calibration of the infrared analyzer was based on the injection of measured quantities of neat liquid DMF into the calibration loop of the infrared cell. Because of slight nonlinearity observed over the intended range of 0–896 ppm, separate calibration curves were constructed, one for the low range (0–56 ppm) and one for the high range (56–896 ppm). Based on this calibration, the following sensitivity parameters were obtained: decision limit = 1.33 ppm, minimum detectable level = 2.66 ppm, minimum level of quantification = 4.43 ppm, and minimum quantifiable standard = 5.60 ppm.

Chamber characterization. Chemical decomposition studies of the neat DMF and test samples of the chamber atmosphere using cryogenic preconcentration techniques and a gas chromatograph with parallel flame ionization mass spectrometric detectors demonstrated that no degradation of liquid DMF occurred during the residence time in the reservoir. In addition, the vapor generation process did not cause decomposition of the test material prior to introduction into the chambers, and the DMF did not degrade after introduction into the chambers.

The uniformity of the DMF vapor concentration in each exposure chamber with animals present was measured by infrared spectroscopy. The between-port variability, expressed as a percentage relative deviation, did not exceed 5%. The time to reach 90% of the target concentrations (T90) ranged from 3–6 min, and decay times to 10% of the target concentration (T10) ranged from 18–28 min.

Study design. Groups of 30 rats and 10 mice of each sex were exposed to vapor concentrations of DMF at 0 (chamber controls), 50, 100, 200, 400, or 800 ppm, 6 h (plus T90) per day, 5 days/week, for 13 weeks. Each group of 30 rats was subdivided into a base study group (10) and two additional subgroups of 10 for evaluations of cardiac physiology and renal function, respectively. Male and female F344/N rats and B6C3F1 (C57B1/6N, female × C3H/HeN Mtv-, male) mice used in these studies were produced under strict barrier conditions at Taconic Farms, Inc. (Germantown, NY). Animals were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats and mice were shipped to the study laboratory at four weeks of age, quarantined at the study laboratory for two weeks, randomized to study group, and placed on study at six weeks of age. Both species were individually housed in multicompartment stainless steel wire mesh cages inside 2000-l volume stainless steel and glass inhalation chambers (Hazleton 2000, Harford Systems/Lab Products, Inc., Aberdeen, MD) during the entire study. Cage positions within the chambers were rotated on a weekly
basis. NIH-07 rat and mouse ration diet (Zeigler Bros., Inc., Gardners, PA) was used during this study.

Clinical examinations, supplemental studies, pathology. During exposure weeks 1 (study day 4), 3 (study day 24), and 13 (study day 91) rats were anesthetized with a CO/ O gas mixture and blood samples were collected from the retroorbital sinus for serum chemistry and hematologic evaluations (10 rats/sex/concentration). In addition, serum samples were collected from five animals of each sex and each species during quarantine, and from the same number of animals assigned to the control group at scheduled necropsy for viral titer analyses (Microbiological Associates, Bethesda, MD). Serological analyses were negative for significant infectious pathogenic diseases.

Hematologic analyses included leukocyte, lymphocyte, band and segmented neutrophils, monocyte, basophil, eosinophil, erythrocyte, reticulocyte, and platelet counts; hemoglobin concentration; mean corpuscular hemoglobin; mean corpuscular hemoglobin concentration; and mean cell volume. All data except those for reticulocyte and differential counts were obtained using an Ortho ELT-8 hematologist analyzer. Leukocyte differential counts were performed manually on blood smears stained with Wright-Giemsa. Reticulocyte preparations were made using equal volumes of whole blood and New Methylene Blue. Smears of these preparations were evaluated using a Miller disk.

Serum chemistry analyses, urinalyses, renal function, and cardiac physiology studies were performed only in rats. Serum chemistry analyses included sorbitol dehydrogenase (SDH), creatine kinase (CK), creatinine (CREA), albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bile acids (TBA), total bilirubin (TB), cholesterol (CHOL), total protein (TP), urea nitrogen (UN), and isotc dehydrogenase (ICD). Assays were performed on a Hitachi 704™ chemistry analyzer using standard methods: SDH-enzymatic assay; CK-kinetic, spectrophotometric; CREA-kinetic, spectrophotometric; ALB-bromocresol green; ALT-kinetic, spectrophotometric; ALP-kinetic, spectrophotometric; TBA-spectrophotometric; TB-diazobilirubin; CHOL-enzymatic assay; TP-modified biuret; UN-modified urease; and ICD-kinetic, spectrophotometric.

Urinalysis/renal function studies were conducted on five rats/sex from the 0, 50, 200, and 800 ppm treatments following 13 weeks of exposure. Rats were placed individually in metabolism cages for 16 h without food but with access to water. Evaluations included volume, appearance, specific gravity, glucose, urine creatinine, urine total protein, sodium, urine osmolality, and microscopic examination. Assays were performed on a Hitachi 704™ chemistry analyzer using standard methods. Blood pressures and electrocardiograms were measured within 24 h of the last DMF exposure in animals anesthetized with urethane (ip, 1 g/kg). A catheter was inserted into the left carotid artery and four standard limb leads and chest leads V3 and V10 were collected simultaneously with the animals in right lateral recumbency. An Electronics for Medicine (White Plains, NY) VR12 system was used to amplify and observe signals and a Kyowa Dengoo FM tape system recorded all signals and stored raw data. Analog electrocardiographic recordings and digitized high speed paper tracings were analyzed by a veterinary cardiologist without knowledge of the animal group assignment. Assessments of cardiovascular function were included in this study because of limited reports in the literature of structural and functional cardiac abnormalities in rats and rabbits following DMF inhalation exposure (Arena et al., 1982; Germanova et al., 1979; Santa-Cruz and Maccioni, 1978). Sperm morphology and vaginal cytology evaluations were performed on rats and mice exposed at 0, 50, 200, or 800 ppm DMF according to Morrissey et al. (1988). Daily smears for vaginal cytologies were made during the 12 consecutive days prior to necropsy. Sperm motility studies were performed during the scheduled necropsy.

Rats and mice were killed by asphyxiation with CO, and a complete necropsy was performed on all animals. Organs and tissues were examined for gross lesions. The weights of the liver, thymus, right and left kidneys, right and left testicles, heart, and lungs were recorded. Tissues were preserved in 10% neutral-buffered formalin and routinely processed for preparation of histologic sections and stained with hematoxylin and eosin for microscopic examination. Lungs were inflated with fixative. Tissues examined microscopically from all control and 800 ppm DMF exposed animals included: adrenal glands, brain, bronchial lymph nodes, cecum, colon, duodenum, epididymis/semenal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), gallbladder (mouse), gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, larynx, liver, lungs and main bronchi, mammary gland, mandibular and mesenteric lymph nodes, mediastinal lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pharynx, pitiitary gland, preputial or clitoral gland (rats), rectum, salivary glands, skin, spleen, sternum including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder. The following organs and tissues were also examined: bone marrow in both sexes of rats assigned to the 400 and 200 ppm groups, hearts (cardiac physiology studies) from both sexes of rats from all treatment groups, and kidneys (five rats/sex) from the 0, 50, 200, and 800 ppm groups (renal function studies). Livers were examined in rats and mice of both sexes from all treatment groups. Details of pathology and review procedures have been described by Maronpot and Boorman (1982). Selected liver sections from control and treated animals were also stained with periodic-acid-Schiff with and without diastase pretreatment or with Perls’ iron stain for hemosiderin.

Statistical methods. Organ and body weight data were analyzed using the parametric multiple comparisons procedure of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data were analyzed using the nonparametric multiple comparison procedures of Dunn (1964) and Shirley (1977) to assess the significance of pairwise comparisons between exposed and control groups. Jonckheere’s test (Jonckheere, 1954) was used to evaluate the significance of concentration-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic concentration-response (Dunnett, Dunn). If the p-value from Jonckheere’s test was greater than or equal to 0.01, Dunn’s or Dunnett’s test was used rather than Shirley’s or Williams’ test. The proportion of time spent in each stage of the estrus cycle was compared by applying a multivariate ANOVA (Morrison, 1976), which was performed after an arcsine transformation of the data. Histopathologic incidence data were compared using Fisher’s exact test.

RESULTS

Chamber atmospheres. Mean ± SD concentrations in ppm for each of the DMF exposures were: 50.2 ± 1.68, 99.6 ± 2.17, 199.1 ± 5.84, 401.3 ± 10.13, and 804.6 ± 19.69, respectively, based on infrared analysis. Daily mean chamber concentrations were within ± 10% of target concentrations on more than 98% of exposure days.

Thirteen-Week Studies—Rats

Clinical signs and survival. All rats survived the 13-week study. DMF was mildly irritating to rats exposed at 400 and 800 ppm, evidenced by occasional nasal and ocular discharges.

Body weights. Mean body weights from the base study animals are presented in Figure 1. A concentration-dependent depression in body weight gain occurred in rats exposed at 400 (6–11%) and 800 ppm (20–22%) over the duration of the study. The percent weight depression was greater in exposed females at 400 ppm while at 800 ppm the depression was greater in males. The effect of the 800 ppm DMF exposure on the body weight of the female rats was observed early in the study as this group of rats did not gain any weight during the first week of exposure.

Organ weights. Group mean absolute lung weights were significantly lower for all DMF exposed rats compared to
control rats. Lung-to-body weight ratios were significantly lower in male (50 to 200, and 800 ppm) and in female (50 to 400 ppm) rats. Absolute right kidney weights were significantly lower in males (800 ppm) and females (400 and 800 ppm) exposed to DMF. Kidney-to-body weight ratios were significantly increased in males (100 to 800 ppm), but significantly increased in females only at the highest DMF exposure group. Absolute heart weights were significantly decreased in the highest exposure group of male rats, and in the highest two exposure groups in females. Absolute thymus weights were significantly reduced in males (400 and 800 ppm) and females (800 ppm), while relative thymus weights were significantly reduced only in the 800 ppm males. Right testis weight relative to body weight was significantly increased in the 400 and 800 ppm DMF groups, while absolute testis weights were not affected by exposure to DMF. None of the preceding organ weight changes were correlated with any gross or histopathologic lesions.

Absolute liver weights were significantly increased in males (100 to 400 ppm) and females (50 to 200 ppm) exposed to DMF, while at 800 ppm liver weights were significantly lower than the respective controls of both sexes. All DMF-exposed groups had significantly higher liver-to-body weight ratios. Liver weights from male and female rats exposed to DMF for 13 weeks are presented in Table 1.

**Hematology.** Significant increases in erythrocyte count, hemoglobin concentration, and hematocrit, were observed in rats of both sexes exposed at 800 ppm DMF for 13 weeks. Erythrocyte counts were also increased on study day 24 in male and female rats exposed at 800 ppm, while hemoglobin concentration and hematocrit were also increased in males exposed at 800 ppm on study day 24 and in female rats exposed at 800 ppm on study days 4 and 24. Mean corpuscular hemoglobin was significantly decreased in males (800 ppm) and females (400 and 800 ppm) exposed to DMF on study day 24. Following 13 weeks of exposure, mean corpuscular hemoglobin levels were significantly decreased in both sexes exposed at 200–800 ppm. Reticulocyte counts were significantly decreased in rats of both sexes exposed at 800 ppm on study day 4, but these counts were significantly increased in rats exposed at 800 ppm on study day 24. Statistically significant changes in hematology measurements observed following 13 weeks of exposure to DMF at 800 ppm are consistent with a mild dehydration and

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**TABLE 1**

**Absolute and Relative Liver Weights in Rats Exposed to Inhaled DMF for 13 Weeks**

<table>
<thead>
<tr>
<th>DMF concentration (ppm)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>Relative</td>
</tr>
<tr>
<td>0</td>
<td>13.28 ± 0.43</td>
<td>3.80 ± 0.073</td>
</tr>
<tr>
<td>50</td>
<td>14.30 ± 0.40</td>
<td>4.05 ± 0.09*</td>
</tr>
<tr>
<td>100</td>
<td>15.16 ± 0.34**</td>
<td>4.43 ± 0.12**</td>
</tr>
<tr>
<td>200</td>
<td>16.62 ± 0.50**</td>
<td>4.63 ± 0.11**</td>
</tr>
<tr>
<td>400</td>
<td>14.98 ± 0.35*</td>
<td>4.53 ± 0.09**</td>
</tr>
<tr>
<td>800</td>
<td>10.79 ± 0.34**</td>
<td>4.02 ± 0.09**</td>
</tr>
</tbody>
</table>

**Note.** Absolute weights are given in g; mean ± SE; 10 animals/group. Relative weights are organ weight/body weight × 100; mean of individual ratios.

*Significantly different from control, p < 0.05.

**Significantly different from control, p < 0.01.
ALP activities were significantly decreased in rats exposed at lower DMF concentrations following 13 weeks of exposure. SDH (200 and 400 ppm) were also significantly increased at DMF for 13 weeks. TBA (400 ppm), ALT (400 ppm), and ICD and SDH (200 and 400 ppm) were also significantly increased in male rats exposed at 800 ppm DMF for 13 weeks. TBA levels were also statistically increased on study days 4 (800 ppm) and 24 (50, 200, and 800 ppm). SDH activities were significantly increased in rats exposed at 800 ppm on study days 4 and 24. CHOL concentrations were increased at all DMF exposure concentrations and at all time points. TP (50–800 ppm) and ALB (200–800 ppm) levels and the activities of ALP (800 ppm) were significantly decreased in rats exposed for 13 weeks. TBA levels were also statistically increased on study days 4 (800 ppm) and 24 (200 to 800 ppm). ALT activities were significantly increased on study days 4 (800 ppm) and 24 (100 to 800 ppm). Activities of ICD were significantly increased on study days 4 (800 ppm) and 24 (50, 200, and 800 ppm). SDH activities were significantly increased in rats exposed at 200 to 800 ppm on study days 4 and 24. Statistically significant differences in group mean TP and ALB concentrations also occurred in DMF exposed rats of both sexes, primarily in the 800 ppm groups. The direction of the alteration was not consistent. Selected serum chemistry data from female rats exposed to DMF are presented in Table 3.

No changes attributable to DMF exposure were observed.

Blood pressure and electrocardiographic studies. Cardiovascular evaluations revealed no DMF-related heart rate or blood pressure effects. Analysis of electrocardiograms (ECG) revealed a small shift of electrical axis in the 800 ppm males.
only. Increased T wave amplitudes were seen only in the 800 ppm females. These ECG changes were subtle and were not accompanied by any gross or microscopic evidence of cardio-toxicity.

**Sperm morphology and vaginal cytology.** In male rats, sperm density and sperm motility were not affected by DMF treatment. The average spermatid count (50, 200, 800 ppm), the number of spermatid heads per testis (50, 200, 800 ppm), and the number of spermatid heads per gram of testis (800 ppm) were significantly increased in the DMF exposed rats. Left caudal epididymis and left testis weights were not affected to toxicity.

**Selected Clinical Chemistry Results from Female Rats Exposed to Inhaled DMF for Up to 13 Weeks**

<table>
<thead>
<tr>
<th>Analyte (units)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDH (IU/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>23 ± 0</td>
<td>24 ± 1</td>
<td>23 ± 1</td>
<td>28 ± 1**</td>
<td>40 ± 3**</td>
<td>103 ± 24**</td>
</tr>
<tr>
<td>Day 24</td>
<td>21 ± 1</td>
<td>19 ± 1</td>
<td>22 ± 1</td>
<td>29 ± 2**</td>
<td>30 ± 2**</td>
<td>53 ± 5****</td>
</tr>
<tr>
<td>Day 91</td>
<td>26 ± 2</td>
<td>26 ± 1</td>
<td>29 ± 2</td>
<td>40 ± 3**</td>
<td>48 ± 5**</td>
<td>171 ± 18**</td>
</tr>
<tr>
<td><strong>ALT (IU/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>42 ± 2</td>
<td>41 ± 1</td>
<td>40 ± 1</td>
<td>41 ± 1</td>
<td>46 ± 2</td>
<td>172 ± 39**</td>
</tr>
<tr>
<td>Day 24</td>
<td>32 ± 1</td>
<td>35 ± 2</td>
<td>36 ± 1*</td>
<td>38 ± 1**</td>
<td>44 ± 3**</td>
<td>98 ± 8****</td>
</tr>
<tr>
<td>Day 91</td>
<td>54 ± 4</td>
<td>52 ± 3</td>
<td>60 ± 5</td>
<td>49 ± 2</td>
<td>66 ± 6</td>
<td>319 ± 31****</td>
</tr>
<tr>
<td><strong>ICD (IU/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>11.9 ± 12.7</td>
<td>12.2 ± 2.3</td>
<td>12.2 ± 3.5</td>
<td>15.4 ± 3.5</td>
<td>13.5 ± 1.3</td>
<td>30.2 ± 5.4**</td>
</tr>
<tr>
<td>Day 24</td>
<td>7.5 ± 13.8</td>
<td>9.3 ± 1.7</td>
<td>11.3 ± 1.3**</td>
<td>11.1 ± 1.4</td>
<td>22.3 ± 2.6****</td>
<td></td>
</tr>
<tr>
<td>Day 91</td>
<td>4.3 ± 6.9</td>
<td>5.7 ± 0.7</td>
<td>10.1 ± 1.7**</td>
<td>5.7 ± 0.8*</td>
<td>66.4 ± 12.0****</td>
<td></td>
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<tr>
<td><strong>CHOL (mg/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>97 ± 2</td>
<td>120 ± 2**</td>
<td>137 ± 4**</td>
<td>152 ± 6**</td>
<td>141 ± 3**</td>
<td>138 ± 4****</td>
</tr>
<tr>
<td>Day 24</td>
<td>89 ± 2</td>
<td>106 ± 2**</td>
<td>106 ± 2**</td>
<td>117 ± 2**</td>
<td>111 ± 2**</td>
<td>117 ± 4****</td>
</tr>
<tr>
<td>Day 91</td>
<td>97 ± 3</td>
<td>109 ± 2**</td>
<td>129 ± 2**</td>
<td>115 ± 2**</td>
<td>137 ± 3**</td>
<td>136 ± 4****</td>
</tr>
<tr>
<td><strong>TBA (µM/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>15.0 ± 1.0</td>
<td>16.5 ± 2.2</td>
<td>16.0 ± 1.6</td>
<td>16.2 ± 0.8</td>
<td>18.7 ± 1.6</td>
<td>34.8 ± 4.3**</td>
</tr>
<tr>
<td>Day 24</td>
<td>9.6 ± 1.5</td>
<td>12.7 ± 2.9</td>
<td>11.6 ± 1.5</td>
<td>15.7 ± 2.0*</td>
<td>23.8 ± 3.7**</td>
<td>67.2 ± 13.2****</td>
</tr>
<tr>
<td>Day 91</td>
<td>8.5 ± 1.1</td>
<td>7.9 ± 1.5</td>
<td>13.9 ± 2.1</td>
<td>12.3 ± 2.1</td>
<td>27.6 ± 2.7**</td>
<td>37.5 ± 4.0****</td>
</tr>
</tbody>
</table>

*Note.* Values are mean ± SE; 10 animals/group except where indicated.

*Significantly different from control, p < 0.05.

**Thirteen-Week Studies—Mice**

**Clinical signs and survival.** No treatment related clinical signs were observed in any of the DMF-exposed mice. Five male mice (three 50 ppm, one 100 ppm, and one 200 ppm) died of undetermined causes during the study. The fact that the deaths occurred at the lower exposure concentrations and no
female mice died suggests that exposure to DMF was not involved.

Body weights. Final mean body weights for the male DMF-exposed mice ranged from 90–101% of controls, while final mean body weights for female mice ranged from 98–114% of controls (data not shown).

Organ weights. In males, right kidney weight was significantly decreased in the 800 ppm group, but the right kidney

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**FIG. 2.** Centrilobular area of the liver from a rat exposed at 800 ppm DMF for 13 weeks. Several hepatocytes around the central vein are in various stages of degeneration and necrosis (arrows) (H & E, original magnification ×320).

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**TABLE 4**

Incidence of Liver Lesions in Rats Exposed to Inhaled DMF for 13 Weeks

<table>
<thead>
<tr>
<th>DMF concentration (ppm)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
</table>

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte necrosis</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10** (1.0)*</td>
<td>10/10** (1.7)</td>
</tr>
<tr>
<td>Macrophage pigment</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10** (1.0)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocyte necrosis</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>8/10** (1.3)</td>
<td>10/10** (2.8)</td>
</tr>
<tr>
<td>Macrophage pigment</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10** (2.0)</td>
</tr>
</tbody>
</table>

*(Severity score) based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

**Significantly different from control, $p < 0.01$.**
weight in females was significantly increased in all but the highest dose group. Relative kidney weights were significantly increased in the 50, 100, and 800 ppm females. Among the females, absolute lung weight was significantly increased in all of the DMF groups, and relative lung weight was increased in the 800 ppm group. Absolute lung weights were not affected in males, and relative lung weights were significantly increased in the 800 ppm group. Absolute and relative thymus weights were significantly decreased in the 50 ppm males. None of the preceding organ weight changes were correlated with any gross or histopathologic lesions.

Absolute liver weights were significantly increased in males (200 to 800 ppm) and females (50 to 800 ppm) exposed to DMF. Relative liver weights were significantly increased in both sexes at all exposure concentrations (Table 5).

Sperm morphology and vaginal cytology. No significant changes in organ weights, spermatid or spermatozoal measurements were observed in male mice exposed to DMF. In females, there were no statistically significant group differences in estrous cycle length, however, there was a significant trend toward an increase in estrous cycle length (controls 4.15, 50 ppm 4.05, 200 ppm 4.55, and 800 ppm 4.80 days; \( p = 0.035 \)) in the DMF-exposed groups. No anestrous cycles were observed in any exposed or control mice.

Gross and histopathology. Gross necropsy findings in mice that may have been exposure-related were limited to tan foci of the liver noted in one male mouse each in the 400 and 800 ppm exposure groups. The only microscopic change attributable to DMF exposure was found in the liver of both sexes and diagnosed as centrilobular hepatocellular hypertrophy. This lesion was characterized by minimal to mild enlargement of hepatocytes surrounding central veins (Fig. 3). The cytoplasm of affected cells was increased in amount and stained homogeneously, in contrast to the more typical granular cytoplasm of periportal hepatocytes. The nuclei of these hypertrophic cells also were enlarged. In some cases where lesions were minimal, enlargement of hepatocytes was not significant, but tinctorial change and nuclear enlargement were prominent. PAS staining of the livers of selected 800 ppm animals demonstrated sharply demarcated centrilobular areas of glycogen-depleted hepatocytes, corresponding to the areas of hepatocellular hypertrophy. Occasional apoptotic bodies were seen in the areas of hypertrophy, but overt hepatocellular necrosis was not seen in DMF-exposed mice. Liver lesions were present in all exposure groups except the lowest concentration (50 ppm) females. Lesion severity reached a maximal level in the mice exposed to DMF at 200 ppm. Incidence and severity data for the liver lesion observed in mice are presented in Table 6.

**DISCUSSION**

The liver was the primary site of toxicity in rats and mice exposed to inhaled DMF for 13 weeks. Centrilobular hepatocellular necrosis seen in exposed rats was accompanied by increased activities of hepatic intracellular enzymes in the serum, and also by increases in relative liver weights. The centrilobular hepatocellular hypertrophy seen in exposed mice was accompanied by increased absolute and relative liver weights. No histopathological changes were observed in any other organs or tissues from either species.

Minimal to moderate necrosis of individual hepatocytes was observed in male and female rats exposed to DMF at 400 or 800 ppm in the current study. Hepatic necrosis was also reported in rats that died following short-term, high level exposures (2523 ppm, 6 h/day for 5 days) to DMF (Kennedy and Sherman, 1986). Massmann (1956) exposed rats to DMF at 100, 230, and 450 ppm (8 h/day, 6 days/week) for 120 days and reported liver necrosis in rats exposed at 450 ppm. Craig et al. (1984) reported hepatic fibrosis in male Fischer 344 rats exposed at 1200 ppm DMF (6 h/day, 5 days/week) for 12 weeks but not in rats exposed at lower concentrations (150, 300 or
600 ppm). The more severe hepatic lesion observed at 1200 ppm may be due to the higher exposure concentration used in this study. Malley et al. (1994) reported centrilobular hepatocellular hypertrophy and hepatic single cell necrosis in male and female rats (Crl:CD BR) exposed to DMF at 100 or 400 ppm (6 h/day, 5 days/week) for 24 months.

Minimal to mild centrilobular hepatocyte hypertrophy was found in male mice exposed to DMF at all exposure concentrations (50–800 ppm) and in female mice exposed to DMF at concentrations greater or equal to 100 ppm in the current study. In B6C3F1 mice exposed to DMF (150 to 1200 ppm; 6 h/day, 5 days/week, for 12 weeks) hepatic cytomegaly (hypertrophy) around central veins was seen in all exposed groups, and the incidence and severity were directly related to exposure concentration (Craig et al., 1984). Malley et al. (1994) also reported hepatocellular hypertrophy in male and female mice exposed to DMF at 50–800 ppm.

![FIG. 3. Liver from a mouse exposed at 800 ppm DMF for 13 weeks. Centrilobular hepatocytes (arrows) are hypertrophic with increased amounts of more darkly staining cytoplasm and enlarged nuclei (H & E, original magnification ×130).](image)

### TABLE 6
**Incidence of Liver Lesions Observed in Mice Exposed to Inhaled DMF for 13 Weeks**

<table>
<thead>
<tr>
<th>DMF concentration (ppm)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrilobular hepatocellular hypertrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0/10</td>
<td>4/10* (1.8)*</td>
<td>9/10** (1.3)</td>
<td>10/10** (2.0)</td>
<td>10/10** (2.0)</td>
<td>10/10** (2.0)</td>
</tr>
<tr>
<td>Females</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10** (1.3)</td>
<td>10/10** (1.9)</td>
<td>10/10** (2.0)</td>
<td>10/10** (2.0)</td>
</tr>
</tbody>
</table>

*Severity score based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

**Significantly different from control, p < 0.01.**
(Crl:CD-1 (ICR)BR) exposed to DMF at 25, 100, or 400 ppm (6 h/day, 5 days/week) for 18 months and hepatic single cell necrosis in all groups, including controls, also after 18 months.

The hepatotoxic effects of inhaled DMF were more pronounced in rats (necrosis) than mice (hypertrophy) in the current study. This finding is supported by Craig et al. (1984) who reported fibrosis in rats and necrosis/cytomegaly in mice following inhalation exposure to DMF. This discordance between rats and mice may be due to species differences in the metabolism of DMF (Mraz et al., 1989) or possibly to differences in the delivered DMF dose. Mraz et al. (1989) have suggested that metabolism of DMF to a toxic intermediate is critical for the occurrence of hepatotoxicity. These authors reported higher urinary levels of N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC), a metabolic end product formed from the oxidation of the formyl moiety of DMF, in rats (1.7–5.2% of the administered dose; 7–512 mg/kg, ip) compared to 1.1–1.6% of the same administered dose in mice. Malley et al. (1994) reported hepatocellular hypertrophy and hepatic single cell necrosis in both sexes of rats and mice exposed to DMF at 100 or 400 ppm for 24 and 18 months, respectively. This lack of species difference in the severity of the observed hepatotoxicity may be due to the much longer exposure duration for mice used in this study. Surprisingly, and in marked contrast to the effects observed in rodents, no evidence of hepatotoxicity was reported in cynomolgus monkeys exposed to DMF at 30, 100, or 500 ppm (6 h/day, 5 days/week) for 13 weeks (Hurtt et al., 1992). This latter finding may be due to the reported differences in the metabolism of DMF between rodents and nonhuman primates (Hundley et al., 1993a,b). These authors reported that the plasma concentration curve (AUC) values and peak plasma levels for DMF in rats and mice following a single 6 h exposure at 500 ppm were substantially greater than levels formed in monkeys exposed under identical conditions. They also reported enhanced metabolism of DMF in rats and mice but not in monkeys following repeated 6 h/day exposures to 500 ppm DMF.

Korsrud et al. (1973) reported that increased SDH activity in serum is the most sensitive indicator of hepatocellular injury in rats. Travlos et al. (1996) reported an association between treatment-related increases in ALT and SDH activities and the occurrence of histopathological changes in the rat liver. Results from the current study are in agreement with these reports. Elevated serum SDH activities were detected on study days 4, 24, and 91 in both sexes of rats exposed to DMF at 200, 400, and 800 ppm. Mean SDH activities were positively correlated with DMF exposure concentrations in both sexes at all three sampling times. These increased activities of hepatic enzymes at all time points indicate the sustained toxic insult of DMF on the rat liver throughout the 13-week exposure period. Similarly, enzyme activities were statistically increased in rats of both sexes exposed to 400 (ALT) and 800 ppm DMF (ALT; ICD) at all sampling times. These increased activities of liver enzymes and the statistically increased levels of TBA (400–800 ppm, both sexes on days 24 and 91) are consistent with the increased liver weights and with the hepatic necrosis observed in rats exposed to DMF at 400 and 800 ppm in the current study. The statistically increased levels of serum CHOL (50–800 ppm) found in both sexes and at all sampling points are indicative of a disruption in hepatic function. In female rats exposed to 800 ppm DMF for 13 weeks, the activities of ICD and ALT were elevated 15.4- and 5.9-fold, respectively, compared to controls and were of a greater magnitude than the increases in enzyme activities observed in males exposed at 800 ppm. These findings agree with the more severe microscopic liver lesions found in the female rats. Brondeau et al. (1983) reported statistically significant increases in SDH and ALT activities in male Sprague-Dawley rats exposed at 126 ppm (6 h/day for four days). Tanaka (1971) reported increases in AST and ALT activities in three-week-old female SD rats exposed to 200 ppm DMF (8 h/day for four weeks). Results from the latter two studies are consistent with the results in the current study. Craig et al. (1984) reported a statistically significant increase in ALT activity in female rats exposed to 1200 ppm DMF for 12 weeks. Malley et al. (1994) also reported statistically significant increases in SDH activities in male and female rats exposed to DMF at 100 or 400 ppm for 3, 12, and 18 months or in males only after 24 months.

Relative liver weights were statistically increased in all groups of male and female rats exposed to DMF for 13 weeks in the current study. Malley et al. (1994) reported increased relative liver weights in male and female rats exposed at 100 or 400 ppm for 12 months (interim sacrifice) and in both sexes exposed to 400 ppm for 24 months.

In the current study, relative liver weights were significantly increased in all groups of male and female mice exposed to DMF. The elevated liver weights are consistent with the centrilobular hepatocellular hypertrophy observed in the exposed mice. Clayton et al. (1963) reported statistically significant increased liver weights in female mice that received 58 exposures to DMF (23 ppm for 5.5 h/day followed by 426 ppm for 0.5 h/day). Malley et al. (1994) also reported statistically significant increased absolute and relative liver weights in mice exposed at 100 ppm (males) and 400 ppm DMF (both sexes) for 18 months.

A concentration-dependent depression in body weight gain was reported in male and female rats exposed to DMF at 400 and 800 ppm in the current study. Craig et al. (1984) also reported decreased body weight gain in male and female rats exposed to DMF at 1200 ppm for 12 weeks. Malley et al. (1994) reported decreased body weight gain in male and female rats exposed to DMF at 400 ppm and in male rats exposed to 100 ppm for 24 months.

Body weights of female mice exposed to DMF at 800 ppm were significantly decreased compared to control mice in the current study. In contrast, Craig et al. (1984) reported no differences in body weight in male and female mice exposed up to 1200 ppm DMF for 12 weeks. Malley et al. (1994)
reported that body weight gain was increased in male and female mice exposed to DMF at 400 ppm for 18 months. The increased body weight gain may reflect the larger sample size of the mouse groups used in the latter study, which may make it easier to detect small weight differences.

Among female rats exposed at 800 ppm in the current study, the length of the estrous cycle could not be defined or was longer than 12 days in 7/10 animals. The estrous cycle length of the remaining three rats was 5.3 days versus 5.0 days in the controls; however, body weight was statistically depressed at the 800 ppm concentration. In DMF-exposed female mice, there was a significant trend toward an increase in estrous cycle length. In contrast to the findings in the current study, Malley et al. (1994) reported no DMF-related effects on estrous cycles of rats or mice (evaluated from test day 107 through test day 131) exposed to DMF at 400 ppm during their chronic toxicity/oncogenicity study. Use of different rodent strains and assessments of effects at different DMF exposure concentrations may account for these observed differences.

In summary, subchronic inhalation exposure to DMF produced hepatotoxicity characterized by statistically significant increased liver weights at all exposure concentrations (rats and mice), statistically significant increased activities of serum enzymes and other markers of liver function (rats), and histopathological changes in the liver (rats and mice) with rats exhibiting more severe lesions. The liver was the only target organ identified and no histopathological changes were observed in any other organs or tissues in either species. No treatment-related hematological or renal function changes were observed in rats and no adverse effects on male reproductive endpoints were found in either species. No evidence of cardiotoxicity was observed in rats exposed to DMF, which does not support the limited evidence of DMF-induced cardiotoxicity in the literature. Adverse effects on the estrous cycle were observed in female rats exposed at 800 ppm but not at lower concentrations nor in any of the female mice exposed to DMF. For rats of both sexes the no-observed-adverse-effect concentration (NOAEC) for microscopic liver lesions was 200 ppm based on the absence of microscopic liver lesions at this and lower exposure concentrations, although liver enzymes and liver weights were increased at all DMF exposure concentrations (50–800 ppm). For female mice the NOAEC for microscopic liver lesions was 50 ppm, however increased liver weights were observed at this concentration. A NOAEC could not be defined in male mice, as centriflobular hepatocellular hypertrophy and increased liver weights were observed at all DMF exposure concentrations (50–800 ppm).

ACKNOWLEDGMENTS

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