

**NTP Technical Report on**  
**Toxicity Studies of**  
**N,N-Dimethylformamide**  
(CAS NO: 68-12-2)

**Administered by Inhalation**  
**to F344/N Rats and B6C3F<sub>1</sub> Mice**

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The NTP Report on the toxicity studies of N,N-Dimethylformamide is based primarily on 13-week studies that began in January, 1989, and ended in May, 1989, at Battelle Memorial Laboratories, Columbus, OH.

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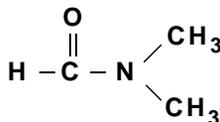
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## N,N-Dimethylformamide



**Molecular Formula:** C<sub>3</sub>H<sub>7</sub>NO

**CAS Number:** 68-12-2

**Molecular Weight:** 73.09

**Synonyms:** DMF, DMFA

### ABSTRACT

N,N-Dimethylformamide (DMF), a colorless liquid with a high boiling point, is a solvent used in a large number of industrial processes. Male and female F344/N rats (30/sex/group) and B6C3F<sub>1</sub> mice (10/sex/group) were exposed to DMF vapors at concentrations of 0, 50, 100, 200, 400, or 800 ppm, 6 hours/day, 5 days/week, for 13 weeks in whole body exposure inhalation studies. In addition to histopathology, sperm morphology, and vaginal cytology, which were evaluated in both species, the studies examined clinical pathology, cardiovascular, and renal function in rats only.

In genetic toxicity studies, DMF was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without S9 activation, nor did it induce germ cell mutations in male *Drosophila melanogaster* treated by feeding or injection. No induction of sister chromatid exchanges or chromosomal aberrations was noted in cultured Chinese hamster ovary cells treated *in vitro* with DMF, with or without an S9 metabolic activation system. In one laboratory, a marginal increase in mutant colonies was observed after treatment of mouse lymphoma L5178Y/TK<sup>+/-</sup> cells with DMF in the absence of S9; results from studies in 2 other laboratories were negative.

In the 13-week studies, all rats survived exposures to DMF. Body weight gains were reduced by 50-65% in rats exposed at 800 ppm and to a lesser extent in the 400 ppm group. Evidence of hepatocellular injury was noted as early as day 4, based on increases in activities of liver-specific enzymes in serum in rats of both sexes exposed at 200-800 ppm. Serum cholesterol levels were increased at all exposure concentrations. Relative liver weights were increased in male rats exposed at 100 ppm and higher concentrations, and in female rats at all concentrations. Minimal to moderate centrilobular hepatocellular necrosis was seen in rats of both sexes exposed at 400 and 800 ppm; the lesion was more severe in females.

There were no clear, adverse effects seen in urinalyses, in electrocardiographic studies, or in male reproductive system evaluations that could be related to DMF exposure. Hematologic studies showed mild hemoconcentration in males and females. Prolonged diestrus was observed in females exposed at 800 ppm.

Among mice exposed to DMF for 13 weeks, there was no chemically related mortality. Body weight gains were approximately 30% less than controls in females exposed at 800 ppm. Relative liver weights were increased in males and females at all exposure concentrations. Centrilobular hepatocellular hypertrophy (minimal to mild) was found in all groups of male mice exposed to DMF, and in female mice exposed at 100 ppm and higher concentrations. The length of the estrous cycle in mice increased with increasing DMF exposure.

In summary, DMF-related effects were seen in the liver of both rats and mice, with rats being more severely affected. For rats of both sexes, the no-observed-adverse-effect level (NOAEL) was 200 ppm, based on the absence of liver histopathology, although liver function assays and liver weights showed changes at all exposure levels (as low as 50 ppm). For mice, hepatocellular hypertrophy or increased liver weights occurred at all exposure concentrations.

## PEER REVIEW

### Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of N,N-Dimethylformamide on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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## Summary of Peer Review Comments

Mr. D. Lynch, NIOSH, introduced the draft report on the short-term toxicity studies of N,N-dimethylformamide (DMF) by reviewing the uses and rationale for study, experimental design, and results.

Mr. Beliczky, a principal reviewer, noted commented that the report was well-written. He noted that extra groups of rats were included for special studies of cardiovascular function and renal function as well as clinical pathology and asked why this was not done for mice. Mr. Lynch replied that the larger body size and base of experience for these studies in rats were the primary reasons for doing the studies in rats while cost was probably a reason for not doing the studies in mice. Mr. Beliczky asked whether the study had been initiated because of increased incidences of testicular cancers among aircraft maintenance workers and leather tanners. Mr. Lynch said that was certainly one rationale. Mr. Beliczky reported that he had heard that DuPont was conducting a 2-year bioassay and asked whether results were available. Mr. Lynch affirmed this and noted that the study was in rats; the in-life phase would be completed in December, 1991; and a report would be available in 1992.

Dr. Bailey, a second principal reviewer, said this was a good report, and the data presented supported the conclusions drawn. He commented that a recent report in the literature indicated that while the metabolite, AMCC (believed to be in the pathway leading to electrophilic products), was a minor metabolite in rodents it was of primary importance in humans. Thus, the risk of toxicity from exposure to DMF would appear to be higher in humans than in rodents. Mr. Lynch said he had not been aware of that reference and would add it.

Dr. Klaassen noted the increased serum cholesterol levels and wondered whether cholesterol was routinely measured. Dr. M. Thompson, NIEHS, said cholesterol wasn't routinely measured, but it seemed to be a fairly sensitive indicator of hepatocellular function, which in the current study would be consistent with the hepatotoxicity observed.

Seeing no objections, Dr. Klaassen accepted the report, with the suggested editorial and other changes, on behalf of the panel.

## INTRODUCTION

N,N-Dimethylformamide (DMF) is a clear, colorless liquid with a high boiling point (153°C), is miscible with water, and is synthesized by the reaction of methyl formate with diethylamine. DMF is an excellent solvent due to its small molecular size, high dielectric constant, electron-donating properties, and its ability to form complexes. Fibers, films, and surface coatings are processed from DMF solutions of high molecular weight polymers. Synthetic leather, acrylic fibers, polyurethanes, and wire enamels based on polyamides or polyurethanes, are produced from DMF solutions. DMF is used in many hydrocarbon separations, such as the recovery or removal of acetylene and the extraction of butadiene from hydrocarbon streams (DuPont, 1980; Sax and Lewis, 1987). The National Institute for Occupational Safety and Health (NIOSH) estimates that more than 120,000 workers are exposed to DMF in the United States (NIOSH, 1983). Currently, there is only one domestic producer of DMF, whose annual production is between  $50 \times 10^6$  and  $60 \times 10^6$  pounds, or 22 to  $27 \times 10^6$  kg (Porta, 1988).

DMF is absorbed readily following exposure by oral, dermal, or inhalation routes (IARC, 1989). Metabolism and excretion of DMF have been studied following a single i.p. injection of  $^{14}\text{C}$ -labeled chemical to male Sprague-Dawley rats. Within 72 hours, 90% of the injected radioactivity (100  $\mu\text{Ci}/\text{ml}$ , 1 ml/kg) was eliminated in the urine. Using gas chromatography/mass spectroscopy, about 50% of the dose was identified as N-hydroxymethyl-N-methylformamide (DMFOH); 15% as unmetabolized DMF; about 4% as N-methylformamide (NMF); and about 20% as unspecified metabolite(s) (Scailteur and Lauwerys, 1984).

When  $^{14}\text{C}$ -labeled DMF (0.74  $\mu\text{Ci}/\text{ml}$ , 6.8 mmol/kg) was administered i.p. to male CBA/CA mice, 83% of the label was recovered in the urine within 24 hours. Of the total radiolabel injected, 56% was recovered as the C-hydroxylated or N-demethylated derivative, 5% was unchanged DMF, and 3% was formamide (F) or N-(hydroxymethyl)formamide (NMFOH); unidentified metabolites comprised 18% (Brindley *et al.*, 1983).

In a follow-up study, the urine of CBA/CB mice dosed with  $^{14}\text{C}$ -methyl DMF (400 mg/kg, i.p.) was analyzed using high-field  $^1\text{H}$ -NMR and radio thin-layer chromatography. DMFOH (45% of the dose), monomethylamine (MA) (4%), and dimethylamine (DMA) (4%) were identified as urinary metabolites of DMF (Kestell *et al.*, 1986a). The appearance of metabolites in the urine of Sprague-Dawley rats following i.p. administration of DMF (1000 mg/kg) was followed for 72 hours using proton NMR spectroscopy (Tulip *et al.*, 1989). DMF, DMFOH, DMA, MA, and NMF were detected in the 0-24 hour urine sample; NMF was the prominent metabolite in the 24-48 hour sample; and N-acetyl-S-(N-methylcarbonyl)-cysteine (AMCC) was detected in the urine collected 48-72 hours after dosing. AMCC has previously been shown to be a urinary metabolite of NMF in male CBA/CA mice and male Wistar rats following i.p. administration as well as in humans treated with NMF (i.p. or oral) as an anticancer agent (Kestell *et al.*, 1986b). AMCC also has been detected in human urine following exposure to DMF (Mraz and Turecek, 1987). Mraz *et al.* (1989) compared the metabolism of DMF in BALB/c mice, Sprague-Dawley rats, and human volunteers. In the rodents exposed to DMF (0.1-7 mmol/kg i.p., or 7.3-512 mg/kg), between 1 and 5% of the administered DMF was excreted in the urine as AMCC. In the urine of the

volunteers exposed to DMF at 60 mg/m<sup>3</sup> (20 ppm) for 8 hours, the mean concentration of AMCC in the urine was 15% of the absorbed dose.

AMCC is a cleavage product of an NMF glutathione conjugate (S-(N-methylcarbamoyl) glutathione, or SMG). This mercapturate has been postulated to arise from the P-450 catalyzed oxidation of the formyl moiety of N-alkylformamides leading to electrophilic (hepatotoxic) products, possibly methyl isocyanate (MIC) (Kestell *et al.*, 1987; Mraz *et al.*, 1989; Cross *et al.*, 1990). AMCC has recently been identified in the urine of Sprague-Dawley rats following i.p. injection of 30 mg/kg MIC (Slatter *et al.*, 1991), which supports the speculation that MIC may be the ultimate hepatotoxic DMF metabolite. It appears that there are two major metabolic pathways of N-alkylformamide metabolism: hydroxylation at the alpha carbon of the N-alkyl group, which probably constitutes a detoxication reaction; or metabolic oxidation of the formyl group, which leads to formation of hepatotoxic metabolites (Kestell *et al.*, 1987).

The toxicity of DMF has been studied in many species, by various administration routes with generally similar results (Kennedy, 1986; IARC, 1989). Hepatotoxicity has been reported in most species studied, including humans, following both acute and subchronic exposure (Scailteur *et al.*, 1987). The toxicity of DMF following inhalation exposure has been reasonably well characterized. Rats survived a single 4-hour exposure to saturated vapors of DMF, with a nominal concentration of approximately 5000 ppm (Smyth and Carpenter, 1948). To determine sensory irritation, Kennedy and Sherman (1986) exposed mice to DMF by nose-only exposure for 10 minutes at concentrations ranging from 55 to 2110 ppm. An RD<sub>50</sub> (the exposure concentration leading to a 50 percent decrease in respiratory rate) could not be predicted, and the maximum decrease in respiration rate was 28% at 210 ppm DMF. These researchers also exposed rats to DMF (6 hours/day for 5 days at 2523 ppm); 7/10 rats died, and acute liver necrosis was observed in the exposed animals.

Clayton *et al.* (1963) reported that 2-week DMF inhalation exposures of an unspecified strain of rats resulted in increased liver-to-body-weight ratios at all exposure levels investigated. Exposures consisted of 91 ppm, 6 hours/day for 10 days; 1104 ppm, 30 minutes/day for 10 days; and 91 ppm for 6 hours/day for 10 days, followed by a single exposure to 841 ppm for 30 minutes after the tenth exposure. This study further investigated the effects of DMF administered at a concentration of 23 ppm for 5.5 hours/day, followed by 426 ppm during the final 30 minutes of each exposure day (58 total exposures). Histopathologic changes (not defined) were observed in the livers of all species exposed to DMF (rats, rabbits, guinea pigs, mice, and dogs), but no significant effects on body weights were observed.

Craig *et al.* (1984) exposed groups of F344 rats and B6C3F<sub>1</sub> mice of both sexes to DMF at 0, 150, 300, 600, and 1200 ppm, 6 hours/day, 5 days/week for 12 weeks. By the end of the study, body weights of rats exposed to 1200 ppm DMF were about 75-80% of controls; one male and one female rat in the highest exposure concentration died as did one male exposed to 300 ppm. Liver lesions in rats included necrosis, fibrosis, hypertrophic hepatocytes, accumulation of yellow-brown pigment, and increased mitotic figures. The deaths of 8 of the 20 mice exposed to 1200 ppm and 2 of 20 exposed to 600 ppm, were attributed to DMF exposure. Liver lesions seen at the higher exposure concentrations included necrosis and pigment accumulation; cytomegaly around the hepatic central veins was seen in all exposed groups of mice, with the extent related to the exposure concentration.

Inhalation exposure to as little as 10.7 mg/m<sup>3</sup> (3.6 ppm) DMF, 4 hours/day for 20 days, has been reported to alter the length of the estrous cycle in nonpregnant rats and to be fetotoxic to pregnant rats; but similar exposure to concentrations as high as 600 mg/m<sup>3</sup> (200 ppm) for 8 days did not affect the rat testis (Sheveleva *et al.*, 1979). Numerous studies have been conducted by various routes of exposure to evaluate the teratogenicity and developmental toxicity of DMF (IARC, 1989). Malformations were seen in mice given DMF by i.p. exposure, and in rabbits following oral exposure. The offspring of Long-Evans rats exposed by inhalation to 172 ppm DMF, 6 hrs/day, on days 6 through 15 of gestation, showed no malformation attributable to exposure, although fetal growth was retarded (Kimmerle and Macherer, 1975). Hellwig *et al.* (1991) exposed Sprague-Dawley rats to DMF at 287 ppm for 1-8 days during gestation. No malformations were observed, but fetal weight and length were reduced. As part of the same study Chbb:HM rabbits were exposed by inhalation to DMF at 50, 150, or 450 ppm, 6 hours/day, on days 7-19 of gestation. At 450 ppm, maternal body weight gain and fetal weights were decreased, and an increased incidence of hernias (7/86 fetuses, 4/15 litters) was observed.

Cardiac abnormalities consisting of alterations in sarcomeric structure were produced in rabbits exposed to DMF vapors at a concentration of 40 ppm, 8 hours/day, for 50 days (Arena, *et al.*, 1983). Rats exposed to a DMF aerosol (concentration unspecified) for one-half hour per day for 30 days exhibited left ventricular hypertrophy, vascular formations in arterial walls, and cardiac muscle edema (Santa-Cruz and Maccioni, 1978). Blood pressure changes were observed in rats exposed to 300 mg/m<sup>3</sup> DMF (100 ppm) in 15-minute sessions with 40-minute interruptions, for a total of 4 hours/day. Exposures continued for 27 days; the weighted DMF concentration was 130 mg/m<sup>3</sup> (43 ppm) (Germanova *et al.*, 1979)

Rats exposed to DMF aerosols (concentration unspecified) for 1 hour/day for 15 days showed renal abnormalities consisting of tubular degeneration in the proximal tract and marked glomerular loop wall thickening (Costa *et al.*, 1978). Kidney toxicity also has been reported in gerbils and guinea pigs given repeated large doses of DMF (17000 ppm and higher in drinking water, or 10 ml as the neat chemical) (Llewellyn *et al.*, 1974; Martelli, 1960).

DMF (10<sup>-2</sup>M) induced a slight increase in unscheduled DNA synthesis in primary F344 rat hepatocyte cultures in one study (Williams, 1977), but not in two others (Williams and Laspia, 1979; Ito *et al.*, 1982). In other studies, negative results were obtained with DMF (10<sup>-1</sup> 10<sup>-2</sup> M) in the hepatocyte primary culture/DNA repair assay using B6C3F<sub>1</sub> mouse or Syrian hamster hepatocytes (McQueen *et al.*, 1983; Klaunig *et al.*, 1984).

The potential carcinogenicity of DMF recently has been evaluated (IARC, 1989). It was determined that DMF may be carcinogenic to humans (Group 2B), based on findings from epidemiological studies of an excess risk for testicular germ cell cancers and cancer of the buccal cavity among workers exposed occupationally to DMF and other materials. The available studies of the carcinogenicity of DMF in animals were considered inadequate to make an evaluation.

The Occupational Safety and Health Administration's (OSHA) permissible exposure limit (PEL) for DMF, the NIOSH recommended exposure limit (REL), and the threshold limit value (TLV) of the American Conference for Governmental Industrial Hygienists (ACGIH), are all 10 ppm (30

mg/m<sup>3</sup>) as an 8-hour time-weighted average (TWA) with a notation that skin absorption can be significant (OSHA, 1989; NIOSH, 1988; ACGIH, 1986). These exposure limits are based on the previously described hepatotoxic effects of DMF.

DMF was nominated by NIOSH and NCI for toxicologic and carcinogenic evaluation because of potential worker exposure due to its volatility and skin permeability; the large population of potentially exposed workers; the chemical's large production volume; its structural similarity to dimethyl carbamyl chloride, a rodent carcinogen; and the lack of adequate DMF carcinogenicity studies in animals. Inhalation was chosen as the route of exposure because of the documented toxicity of DMF to animals exposed by this route, and because inhalation is a relevant route of human (worker) exposure. This report summarizes the results of 13-week inhalation studies with F344/N rats and B6C3F<sub>1</sub> mice, including assessments of the reproductive system, clinical pathology, electrocardiography, and urinalysis, as well as *in vitro* studies of the genotoxicity of DMF in *Salmonella typhimurium*, in mouse lymphoma L5178Y/TK<sup>±</sup> cells, in Chinese hamster ovary (CHO) cells, and in studies of sex-linked, recessive mutations in *Drosophila melanogaster*. The 12-week DMF inhalation studies by Craig *et al.* (1984) were used as a basis for the selection of exposure concentrations for the current studies.

## MATERIALS AND METHODS

### Procurement and Analysis of Dimethylformamide

Dimethylformamide was obtained from Chemical Dynamics Corp., South Plainfield, NJ (Lot 131046, Batch 02). Spectroscopic examination by infrared, ultraviolet/visible, and nuclear magnetic resonance was used to identify the chemical as DMF; its purity was determined as greater than 99%, according to Karl Fischer water analysis, nonaqueous amide functional group titration, and two gas chromatographic systems. The chemical was stored in amber glass bottles with Teflon-lined lids at room temperature; subsequent chemical reanalyses by gas chromatography and non-aqueous titration showed purity levels exceeding 99% through the course of the studies.

### Vapor Generation System

A counter-current distillation system was used to generate vapors from liquid-state dimethylformamide. A fresh aliquot of liquid DMF was taken daily from the glass reservoir of the generation system, using a metering pump and teflon lines to transport the chemical to the counter-current distillation column. This column consisted of a glass tube, 110 mm in diameter x 1 m long, packed with four stainless steel, expanding-mesh Koch-Sulzer distillation column packing units, each 17 cm long x 10 cm diameter (Koch Engineering Inc., New York, NY). Conditioned room air, drawn through activated charcoal and high-efficiency particulate air (HEPA) filters, supplied primary dilution air for the distillation column. The test vapor was generated at the highest exposure concentration (800 ppm), delivered to the exposure chamber through a common distribution manifold, then diluted to target concentrations at each exposure chamber. Test atmosphere was conveyed to exposure chambers through stainless steel or Teflon-lined flex hose to minimize chemical interaction with the test atmosphere. Negative air pressure was maintained in the delivery system.

### Concentration Monitoring

DMF concentrations in the exposure chambers were monitored with a Miran-980 infrared spectrometer (Foxboro Co., Norwalk, CT) at the following settings: path length, 3.75 m; slit width, 1 mm; wavelength, 3.518  $\mu\text{m}$ . Exposure chambers, the exposure room, and the exhaust manifold were sampled hourly; the average of three successive absorbance readings at each time point was used to calculate the respective DMF concentration. Levels in the control chamber, exposure room, and exhaust manifold were measured on a low-calibration curve, while a high-calibration curve was used to monitor concentrations in other chambers. Extensive, multipoint calibration of the infrared analyzer was performed immediately before initiating animal exposures; during the study the analyzer was challenged daily with a zero and three calibration standards (5.6 ppm, 56 ppm, and 896 ppm DMF). Chamber concentrations were under manual

operator control and were maintained within 10% of the target concentrations throughout the studies (Table 1).

**TABLE 1 Mean Chamber Concentrations of N,N-Dimethylformamide in the 13-Week Inhalation Studies**

Target Concentration (ppm)	Analytical Concentration <sup>a</sup> (ppm)	Maximum Concentration <sup>b</sup> (ppm)	Minimum Concentration <sup>b</sup> (ppm)	Percent of Sample Within 10% of Target Concentration <sup>a</sup>
0	0 <sup>c</sup>			
50	50.2 ± 1.68 <sup>d</sup>	57.0	46.9	98.8
100	99.6 ± 2.17	106.1	90.6	100
200	199.1 ± 5.84	212.8	180.9	100
400	401.3 ± 10.13	441.6	374.6	100
800	804.6 ± 19.69	845.3	757.6	100

a Infrared analysis.

b Daily mean concentrations.

c All analyses were below the detection limit of 1.33 ppm.

d Mean ± SD.

### Chamber Characteristics

No degradation of the liquid dimethylformamide occurred during its storage in the system reservoir during vapor generation or in the chambers. Cryogenic preconcentration techniques, a gas chromatograph with parallel-flame ionization, and mass spectrometric detectors were used to test the liquid DMF. Infrared spectroscopy was used to measure the uniformity of the DMF vapor concentration in each exposure chamber with animals present. The variability between ports did not exceed 3%. Times required to reach 90% of the target concentrations ( $T_{90}$ ) ranged from 3 to 6 minutes; decay times to 10% of the target concentration ( $T_{10}$ ) ranged from 18 to 28 minutes.  $T_{10}$  times were 2 to 3 times longer with animals in the chamber than without animals.

### Study Design

Male and female F344/N rats and B6C3F<sub>1</sub> mice used were produced under strict barrier conditions at Taconic Farms, Inc. (Germantown, NY). The animals were offspring of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. The rats were received by the laboratory at 32 days of age, quarantined for 20 days in the study lab, and received first exposure at 51 days. Mice were received at the lab at the age of 32 days, quarantined for 11 days, and received first exposure at 46 days of age. At the end of the quarantine period, 5 males and 5 females of each species were randomly selected, killed, necropsied, and examined by a staff veterinarian. No gross evidence of disease was detected, nor were there significant serum levels of antibodies found for viruses tested (Boorman *et al.*, 1986; Rao *et al.*, 1989; 1989a).

Animals were randomized to exposure and control groups from stratified weight groups using a Xybion<sup>®</sup> computer program (Xybion Medical Systems Corp., Cedar Knolls, NJ). Rats and mice were housed individually in multicompartments, stainless steel wire mesh cages during the entire study; cage positions within the chambers were rotated on a weekly basis. NIH-07 rat

and mouse ration diet (Ziegler Bros., Inc., Gardners, PA) was provided *ad libitum* during the quarantine and non-exposure periods; water from the City of Columbus was provided *ad libitum* at all times with no further treatment by the laboratory.

Groups of 30 rats of each sex 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 hours (plus T<sub>90</sub>)/day, 5 days/week for 13 weeks. Rats were subdivided into 3 study groups, 10 of each sex, for each exposure level: a base study group, a cardiovascular group, and a renal function (urinalysis) group. Animals were observed twice daily for morbidity and moribundity; body weights were measured weekly and at necropsy.

### **Clinical Pathology Studies, Cardiovascular Study, and Pathology**

Clinical pathology studies were performed on cardiovascular study rats at 4 and 23 days, and on base-study rats at 13 weeks. Rats were anesthetized with CO<sub>2</sub>, and blood samples were collected from the retroorbital sinus using heparinized capillary tubes. Blood was placed in plastic tubes containing potassium EDTA anticoagulant for hematologic analyses (~0.5 mL), and in tubes without anticoagulant for biochemical determinations (~1.0 mL). The latter samples were allowed to clot at room temperature, centrifuged, and the serum removed. All hematologic and biochemical analyses were performed on the day the samples were collected.

Using an Ortho ELT-8 (Ortho Diagnostic Systems, Inc., Raritan, NJ), automated hematologic determinations were performed to measure or calculate the following variables: erythrocyte (RBC), leukocyte, and platelet counts, hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH). Leukocyte differentials were determined from the microscopic analysis of blood smears stained with Wright Giemsa. Blood smears were made from preparations of equal volumes of new methylene blue and blood that had been incubated for at least 20 minutes. The smears were examined microscopically using a Miller disc for the quantitative determination of reticulocytes.

Biochemical analyses were performed using a Hitachi 704 clinical chemistry analyzer (Hitachi Boehringer Mannheim Diagnostics, Inc., Indianapolis, IN). Reagents and methods obtained from the manufacturer were used for the following assays: alanine aminotransferase (ALT), alkaline phosphatase (AP), creatine kinase (CK), total protein (TP), albumin (ALB), creatinine, cholesterol, and urea nitrogen (UN). Diagnostic kits for sorbitol dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), and total bile acids were obtained from Sigma Chemical Co. (St. Louis, MO) and adapted for the clinical chemistry analyzer.

At the end of the study, urinalysis endpoints were measured in 5 male and 5 female rats in the 0, 50, 200, and 800 ppm exposure groups. Rats were placed individually in metabolism cages for 16 hours without food but with access to water. Collection tubes were immersed in ice/water baths during the collection period. In addition to volume, appearance, specific gravity, pH, and microscopic evaluation, concentrations of creatinine, sodium, glucose, and protein were

measured with the Hitachi 704 chemistry analyzer, using standard methods. Urine osmolality was measured by freezing point depression.

Blood pressures and electrocardiograms were measured within 24 hours of the last DMF exposure in cardiovascular group rats anesthetized with urethane (i.p., 1 g/kg). A catheter was inserted into the left carotid artery and blood pressure measured with a Gould Statham P23-ID pressure transducer (Gould, Inc., Medical Products Division, Oxnard, CA). Four standard limb leads and chest leads V3 and V10 were attached to each animal using 1-inch, 18-gauge needle electrodes. ECGs and blood pressure readings were collected simultaneously with the animals in right lateral recumbency. A VR12 system (Electronics for Medicine, White Plains, NY) was used to amplify and observe signals; a Kyowa Dengo FM tape system (Kyowa Electronic Instruments Co., Ltd., Tokyo) was used to record all signals and store the data. Following data collection, each rat was killed (0.5 ml sodium pentobarbital administered through the catheter); the heart was removed and prepared for microscopic examination. Analog electrocardiographic recordings were qualitatively analyzed by a veterinary cardiologist, without knowledge of the animal group assignment; the recordings were quantitatively analyzed from high-speed paper tracings using an electromagnetic digitizing board (SPD Series Glass Tablet, Scriptel Corp., Columbus, OH) interfaced to an IBM PC. Blood pressure data and the following segments of the ECG waveforms were analyzed statistically: P duration, P-Q interval, QRS duration, QT interval, R amplitude (Lead 1, Lead aVF, and Lead V10), T amplitude (Lead 1, Lead aVF, and Lead V10), R angle, and T angle.

At study termination, rats in the core and renal function groups and mice were killed with CO<sub>2</sub>; complete necropsies were performed on all animals. Organs and tissues were examined for gross lesions, and the weights of the liver, thymus, both kidneys, both testicles, heart, and lungs were recorded. Protocol-required tissues (Table 2) were preserved in 10% neutral-buffered formalin and routinely processed for preparation of hematoxylin and eosin-stained histologic sections for microscopic examination. Special stains were performed on selected sections. Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed; the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of contractor pathologists and the PWG. Details of pathology and review procedures are as in Maronpot and Boorman (1982) and Boorman *et al.* (1985).

## **Reproductive System Evaluations**

Sperm morphology and vaginal cytology evaluations were performed on rats and mice exposed to 0, 50, 200, or 800 ppm DMF. To screen for potential reproductive toxicity, epididymal sperm motility was evaluated at necropsy, and vaginal cytology was evaluated on animals during the 2 weeks just preceding necropsy, using procedures outlined by Morrissey *et al.* (1988). For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrous cycle.

Sperm motility was evaluated at necropsy as follows: The left cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, and a small cut was made in the distal cauda epididymis. The sperm that extruded and the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field were counted. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS) and minced. The solution was mixed gently and heat-fixed at 65°C. Sperm density was subsequently determined using a hemocytometer.

To quantify spermatogenesis, the left testis was weighed, frozen, and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis, and per gram of testis.

## **Genetic Toxicity**

### **Mutagenicity Studies**

Mutagenicity studies of DMF in *Salmonella typhimurium* were conducted as described by Mortelmans *et al.* (1986). Briefly, DMF was supplied as a coded aliquot, and was tested for mutagenicity in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, using the preincubation assay in both the absence and presence of Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. DMF was tested at doses up to 10,000 µg/plate. All doses were tested in triplicate, and all tests were repeated. A positive response is defined in this assay as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment.

### **Drosophila Sex-Linked Recessive Lethal Assay**

The assay was performed as described by Zimmering *et al.* (1985). DMF was supplied as a coded aliquot. Toxicity tests were used to set concentrations of study chemical at a level which would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Exposure by feeding was done by allowing Canton-S males (10-20 flies/vial) to feed for 72 hours on a solution of DMF in 5% sucrose.

Because no positive response was obtained in the feeding experiment, the chemical was retested by injection into adult males. In the injection experiments, 24-72 hours-old Canton-S males were injected with DMF (dissolved in distilled water) and allowed to recover for 24 hours. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; successive matings were used to sample sperm treated at successively earlier post-meiotic stages. F<sub>1</sub> heterozygous females were allowed to mate with their siblings, then placed in individual vials. F<sub>1</sub> daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a single, spontaneous,

premeiotic mutation event results in a number of mutants from a given male; the cluster is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). If a cluster was identified, all data from the male in question were discarded. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males. A minimum of 2 experiments was performed.

### **Mouse Lymphoma Assay**

The experimental protocols and statistical methods are presented in detail by McGregor *et al.* (1988), Mitchell *et al.* (1988), and Myhr and Caspary (1988). DMF was supplied to the laboratory as a coded aliquot. Mouse lymphoma L5178Y/TK<sup>±</sup> cells were maintained at 37°C as suspension cultures in Fischer's medium. All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Incubation with DMF was for 4 hours; the medium plus chemical then was removed, and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 2-day expression period, cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of TFT-resistant cells (TK<sup>-/-</sup>); cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO<sub>2</sub> for 10 - 12 days.

### **Chinese Hamster Ovary Cytogenetics Assays**

DMF was supplied as a coded aliquot, and testing was performed as reported by Galloway *et al.* (1985, 1987). Briefly, Chinese hamster ovary cells were incubated with DMF or solvent (dimethylsulfoxide) for induction of sister chromatid exchanges and chromosomal aberrations, both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Additional details are provided in Appendix D.

## **Statistical Methods**

### **Analysis of Continuous Variables**

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ, body weight, blood pressure, and electrocardiographic data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-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 dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value, or at most, half of the next smallest value.

### **Analysis of Vaginal Cytology Data**

Since the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

### **Analysis of Mouse Lymphoma Assays**

Data were evaluated statistically for both trend and peak response (Myrh and Caspary, 1991). Both responses had to be significant ( $P < 0.05$ ) for a chemical to be considered capable of inducing mutation; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

### **Analysis of *Drosophila* Assays**

Sex-linked recessive lethal data were analyzed by the normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P value was less than 0.01 and the mutation frequency in the treatment group was greater than 0.10%, or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.05 and 0.01, but the frequency in the treatment group was between 0.10% and 0.15%, or (b) the P value was between 0.10 and 0.05, but the frequency in the treatment group was greater than 0.10%. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

### **Analysis of CHO Cytogenetics Assays**

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. A single increased dose was considered weak evidence for a positive response (W+); > 20% increases at 2 or more doses was considered a positive response (+).

Chromosomal aberration data are presented as the percentage of cells with aberrations. As with SCE, both the dose-response curve and individual dose points were statistically analyzed. For a single chromosomal aberration trial, a statistically significant ( $P < 0.05$ ) difference for one dose point and a significant trend ( $P < 0.015$ ) were considered weak evidence for a positive response (W+); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

### **Quality Assurance**

The studies of DMF were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58, 1978). The Quality Assurance Unit of Battelle Columbus Laboratory performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

**TABLE 2 Experimental Designs and Materials and Methods  
in the 13-Week Inhalation Studies of N,N-Dimethylformamide**

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**EXPERIMENTAL DESIGN**

<b>Study Laboratory</b>	Battelle Memorial Laboratories, Columbus, Ohio
<b>Date of Studies</b>	January-May, 1989
<b>Animal Species and Strains</b>	F344/N Rats, B6C3F <sub>1</sub> mice
<b>Animal Source</b>	Taconic Farms, Germantown, Pa.
<b>Size of Study Groups</b>	Rats--30/sex/concentration; mice--10/sex/concentration
<b>Route of Administration</b>	Whole body inhalation
<b>Exposure Concentrations</b>	Rats and mice--0, 50, 100, 200, 400, or 800 ppm
<b>Duration of Exposure</b>	Rats, mice--13 weeks, 6 h/day plus T <sub>90</sub> , 5 days per week
<b>Method of Animal Distribution</b>	Animals assigned to exposure and control groups by computer generated tables of random numbers, using sex and body weight as blocking variables
<b>Animal Chamber Environment</b>	Temp--75 ± 3°F; relative humidity--55 ± 15%; fluorescent light 12 h/d; 10-15 air changes/h. Animal were individually caged.
<b>Diet</b>	NIH-07, available <i>ad libitum</i> , except during exposure periods; water available at all times except for animals tested for renal function.
<b>Time Held Before Study</b>	Rats--20 days; mice--11 days
<b>Age When Placed on Study</b>	Rats--51 days; mice--46 days
<b>Age When Killed</b>	Rats: Renal function group--138 days; male rats in the cardiovascular group--143 or 144 days; female rats in the cardiovascular group--145 or 146 days; male rats in the base group--144 days; female rats in the base group--145 days. Mice: males--139 days; females--138 days
<b>Type and Frequency of Observation</b>	Observed 2 x d for mortality and moribundity; body weights and clinical observations were measured weekly and at necropsy
<b>Necropsy and Histologic Examinations</b>	Necropsy was performed on all animals; the following tissues were examined microscopically from all control and high dose groups from the base study: adrenal glands, brain, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur with marrow, gallbladder (mice), gross lesions and tissue masses with regional lymph nodes, heart and aorta, intestines (duodenum, jejunum, ileum, cecum, colon, rectum), kidneys, larynx, liver, lungs with mainstem bronchi, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland and adjacent skin, nasal cavity and turbinates (3 sections), pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial /clitoral glands (rats), salivary glands, spleen, skeletal muscle (thigh), stomach (including forestomach and glandular stomach), thymus, thyroid gland, trachea, urinary bladder, and vagina (SMVCE females). The target organ (liver) was examined in all lower dose groups. Microscopic examination was also performed on the following: hearts from the cardiovascular study (10 rats/sex exposed to 0, 50, 100, 200, 400, and 800 ppm DMF); kidneys from the renal function study (5 rats/sex exposed to 0, 50, 200, and 800 ppm DMF). Weights (to the nearest mg) of the following organs were obtained from all core study animals: liver, thymus, kidneys, testes, heart and lungs.
<b>Supplemental Evaluations</b>	Clinical Chemistry (rats only): Hematologic and serum chemistry analyses, and urinalysis were performed Cardiovascular Study (rats only): Blood pressure and electrocardiograms determined. Sperm morphology/vaginal cytology: Sperm morphology/vaginal cytology was evaluated in rats and mice exposed to 0, 50, 200, and 800 ppm.

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## RESULTS

### F344/N Rats

All rats survived to the end of the 13-week study. DMF was mildly irritating at 400 and 800 ppm, as evidenced by occasional nasal and ocular discharges; no other adverse clinical signs were attributed to DMF exposure. A concentration-dependent depression in body weight gain occurred at 400 ppm and 800 ppm in both sexes (Table 3; Fig. 1).

**TABLE 3** Survival and Weight Gain of F344/N Rats in the 13-week Inhalation Studies of N,N-Dimethylformamide

Exposure Concentration (ppm)	Survival <sup>a</sup>	Mean Body Weights			Final Weights Relative to Controls (%) <sup>d</sup>
		Initial	Final <sup>b</sup>	Change <sup>c</sup>	
<b>MALE</b>					
0	10/10	150.6	349.4	198.8	
50	10/10	160.3	353.0	192.7	101
100	10/10	151.2	342.8	191.6	98
200	10/10	157.2	358.5	201.3	103
400	10/10	154.0	330.7	176.7	95
800	10/10	163.5	268.8	105.3	77
<b>FEMALE</b>					
0	10/10	118.6	193.0	74.4	
50	10/10	116.3	201.6	85.3	104
100	10/10	112.9	206.9	94.0	107
200	10/10	116.7	193.7	77.0	100
400	10/10	113.9	175.0	61.1	91
800	10/10	120.3	146.2	25.9	76

<sup>a</sup> Number surviving at 13 weeks/number of animals per dose group.

<sup>b</sup> At necropsy.

<sup>c</sup> Mean weight change of the animals in each dose group.

<sup>d</sup> (Dosed group mean/Control group mean) x 100.

In male rats in the 800 ppm group, there were increases in HCT, HGB concentration, and erythrocyte count (also in the 400 ppm group) at 24 and/or 91 days. Groups exposed to several DMF concentrations had decreases in MCV, MCH, and platelet count at 24 and 91 days. HCT, HGB, and erythrocyte counts were increased at 4, 24, and 91 days in female rats in the 800 ppm group. As with the male rats, mild to moderate decreases occurred in MCV and MCH in female rats in several exposed groups at 24 and 91 days.

In male rats, increases in concentrations of total bile acids occurred in the 400 and 800 ppm groups at all time points and in ICDH activity in the 800 ppm group at all time points. Concentrations of cholesterol were increased in all exposed animals at all time points, and activities of ALT were increased in all animals in the two highest exposure groups. Decreased concentrations of total protein and albumin occurred at day 4, but concentrations at day 24 of the two analytes were increased in the 400 (except for total protein) and 800 ppm groups. Concentrations of serum albumin remained higher in the 800 ppm group at day 91. The remaining consistent change in male rats was decreased activity of AP in the 800 ppm group at

all time points, and at lower exposure levels (200 and 400 ppm) at 24 days (Appendix B, Table B1).

In female rats, at 4, 24, and 91 days, concentrations of cholesterol were increased in all exposure groups, and the activity of SDH in serum was increased in the 200, 400, and 800 ppm groups. Activities of ALT and ICDH, and concentrations of total bile acids, were increased at all time points in the 800 ppm group and at lower exposure groups at days 24 and 91. AP was decreased and CK was increased at all time points in the 800 ppm group. Occasional significant changes occurred in these two variables at lower exposure levels. Concentrations of total protein and albumin were decreased in female rats at 4 (800 ppm), 24 (400 ppm), and 91 days (all exposure groups except 50 and 100 ppm for albumin). Other statistically significant changes were scattered and were not considered important (Appendix B, Table B1).

Absolute lung weights were decreased significantly for all DMF-exposed groups, compared to control rats. Lung-weight-to-body-weight ratios were significantly lower in males in the 50, 100, 200, and 800 ppm exposure groups and in females in the 50-400 ppm groups (Appendix A, Table A1). No concentration-dependent relationship with absolute or relative lung weights was noted.

Liver-to-body-weight ratios were mildly increased in all exposed groups except male rats at 50 ppm (Appendix A, Table A1). Kidney-weight-to-body-weight ratios were increased in the base-study males in the 100 to 800 ppm groups and in females in the 800 ppm group. Right and left testis weights, relative to body weight, were significantly increased in the 400 and 800 ppm DMF groups. Relative heart weights of the base-study and special cardiovascular-study rats were not affected by DMF exposure in either sex (Appendix A, Tables A1 and 2).

Urine chemistry and microscopic examination of urine sediment revealed minimal effects after 13 weeks of exposure to DMF; in both males and females, the differences resulted from values contributed by one male and one female rat with high urine volumes and low values for specific gravity, osmolarity, and various solute concentrations (Appendix B, Table B2). No exposure-related lesions were observed in kidneys of rats of either sex exposed to DMF.

Cardiovascular evaluations revealed no DMF-related heart rate or blood pressure effects (data not shown). Analyses of electrocardiograms (ECG) revealed that eight of the 109 rats tested had qualitatively abnormal ECG waveforms. All eight were in DMF-exposed groups. Two animals (a 50 ppm male and a 200 ppm male) had premature ventricular depolarization, which was not considered exposure-related. Six animals (a 50 ppm male, a 50 ppm female, 3 females and 1 male in the 400 ppm groups, and an 800 ppm female) had increased amplitudes of the T-wave, reflecting a primary repolarization change. Statistical evaluations of the ECG waveforms revealed a quantitative decrease in the R wave amplitude and an increase in the R wave angle, as measured by lead 1 in high exposure concentration male rats. Further, the T wave amplitude, as measured in lead aVF, was increased in high-concentration female rats, but not in the 400 ppm group, where most of the apparent T wave increases were seen subjectively. Overall, the electrocardiographic changes were subtle, were not increased in incidence or severity by DMF exposure, and were not accompanied by any gross or microscopic evidence of cardiotoxicity, although they occurred in groups that also showed increases in serum CK activity.

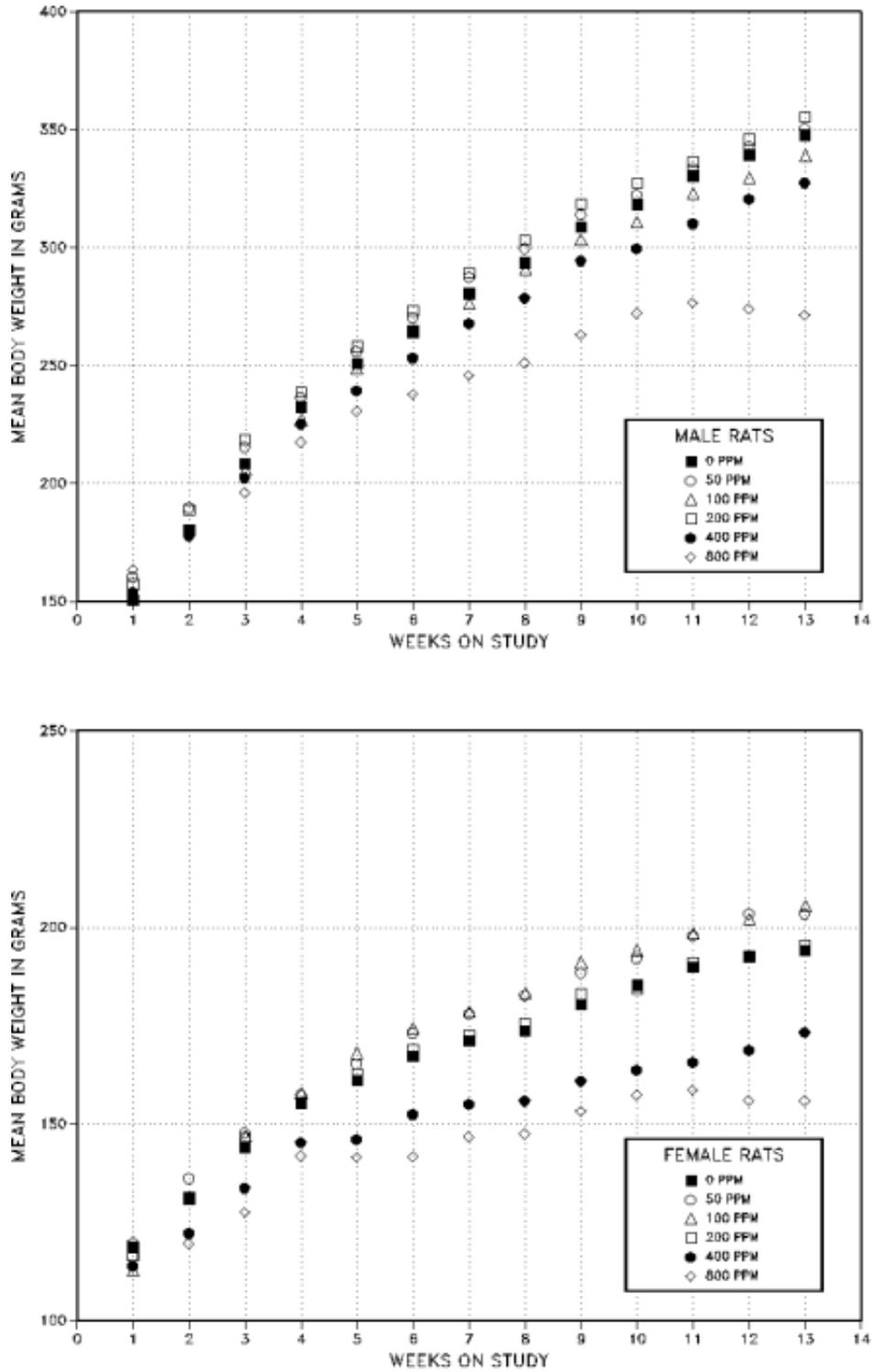


Figure 1 Body Weights of F344/N Rats Administered N,N-Dimethylformamide by Inhalation for 13 Weeks

Sperm density and sperm motility were not affected by DMF treatment. The average spermatid count (50, 200, and 800 ppm groups), the number of spermatid heads per testis (50, 200, and 800 ppm groups), and the number of spermatid heads per gram of testis (800 ppm group) were increased in the DMF-exposed male rats (Appendix C, Table C1). Testis, epididymis, and left caudal weights were not affected by DMF treatment.

Female rats exposed to 800 ppm DMF differed significantly from controls in the length of time spent in the different phases of the estrous cycle (Appendix C, Table C2). The length of the estrous cycle could not be defined, or was longer than 12 days, in 7/10 animals. In the remaining 3 animals, the cycle length was 5.3 days vs. 5.0 days in the controls.

No lesions that were considered related to DMF exposure were observed grossly at necropsy. Microscopically, lesions associated with exposure to DMF were found in the liver of both sexes of rats. Liver lesions consisted of minimal to moderate necrosis of individual hepatocytes around central veins, associated with the presence of varying numbers of macrophages containing golden-brown pigment. Affected hepatocytes were typically rounded and shrunken with nuclear pyknosis or karyorrhexis (Plate 1). Pigment within macrophages stained intensely for iron (Plate 2) and was therefore interpreted to be hemosiderin. The pigment was also diastase-resistant, PAS-positive, suggesting the presence of lipofuscin as well. Kupffer cells in affected centrilobular areas also contained iron-positive and diastase-resistant, PAS-positive intracellular pigment. A few mixed inflammatory cells were associated with these lesions, as was a slightly increased amount of hepatocyte mitotic activity. Liver lesions were limited to the 400 and 800 ppm treatment groups in both sexes and generally were more severe in females at the highest dose level. Incidence and severity data for liver lesions in rats are presented in Table 4.

**TABLE 4** Liver Lesions in F344/N Rats in the 13-Week Inhalation Studies of N,N-Dimethylformamide<sup>a</sup>

Exposure Concentration (ppm)	0	50	100	200	400	800
<b>MALE</b>						
Hepatocyte necrosis	0/10	0/10	0/10	0/10	10/10 (1.0)	10/10 (1.7)
Macrophage pigment	0/10	0/10	0/10	0/10	0/10	10/10 (1.0)
<b>FEMALE</b>						
Hepatocyte necrosis	0/10	0/10	0/10	0/10	8/10 (1.3)	10/10 (2.8)
Macrophage pigment	0/10	0/10	0/10	0/10	0/10	10/10 (2.0)

<sup>a</sup> Incidence and 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.

**B6C3F<sub>1</sub> Mice**

Five male mice died of undetermined causes during the study (Table 5); 3 of these were in the lowest exposure group, suggesting that exposure to DMF was not involved. All female mice survived the 13-week exposure period. No exposure-related clinical signs were observed in any of the DMF-exposed mice.

**TABLE 5 Survival and Weight Gain of B3C6F<sub>1</sub> Mice in the 13-Week Inhalation Studies of N,N-Dimethylformamide**

Exposure Concentration (ppm)	Survival <sup>a</sup>	Mean Body Weight (grams)			Final Weight Relative to Controls (%) <sup>d</sup>
		Initial	Final <sup>b</sup>	Change <sup>c</sup>	
<b>MALE</b>					
0	10/10	26.2	34.0	7.8	
50	7/10	25.4	33.5	8.1	99
100	9/10	26.2	30.6	4.4	90
200	9/10	26.2	34.3	8.1	101
400	10/10	26.7	33.2	6.5	98
800	10/10	24.6	30.9	6.3	91
<b>FEMALE</b>					
0	10/10	21.1	25.2	4.1	
50	10/10	21.4	26.3	4.9	104
100	10/10	22.0	27.2	5.2	108
200	10/10	21.2	28.6	7.4	114
400	10/10	20.8	27.0	6.2	107
800	10/10	21.7	24.6	2.9	98

a Number surviving at 13 weeks/number of animals per dose group.

b At necropsy.

b Mean weight change of the animals in each dose group.

c (Dosed group mean/Control group mean) x 100.

A reduced body weight gain was noted in male mice exposed at 800 ppm (Table 5). As is evident in Figure 2, a transient loss of several grams occurred in the group mean body weight of several exposure groups. There was no indication of a problem involving access to food or water; in most cases body weights of the affected mice appeared to rebound during the next weighing period to values similar to those in the respective controls. Relative and/or absolute kidney and lung weights were variably increased in all exposed groups of females (Appendix A, Table A4). Both absolute and relative thymus weights in male mice exposed at 50 ppm were decreased compared to controls; this finding was not considered biologically significant. Absolute liver weights were moderately increased in males (200-800 ppm) and females (50-800 ppm) exposed to DMF. Relative liver weights were increased in both sexes at all exposure levels (Table 6).

No significant changes in reproductive system evaluations were observed in male mice exposed to DMF (Appendix C, Table C3). In females, there was a significant trend toward an increase in the estrous cycle length (Appendix C, Table C4). The animals in the 200 ppm group spent significantly more time in the stages of estrus and diestrus than did the controls.

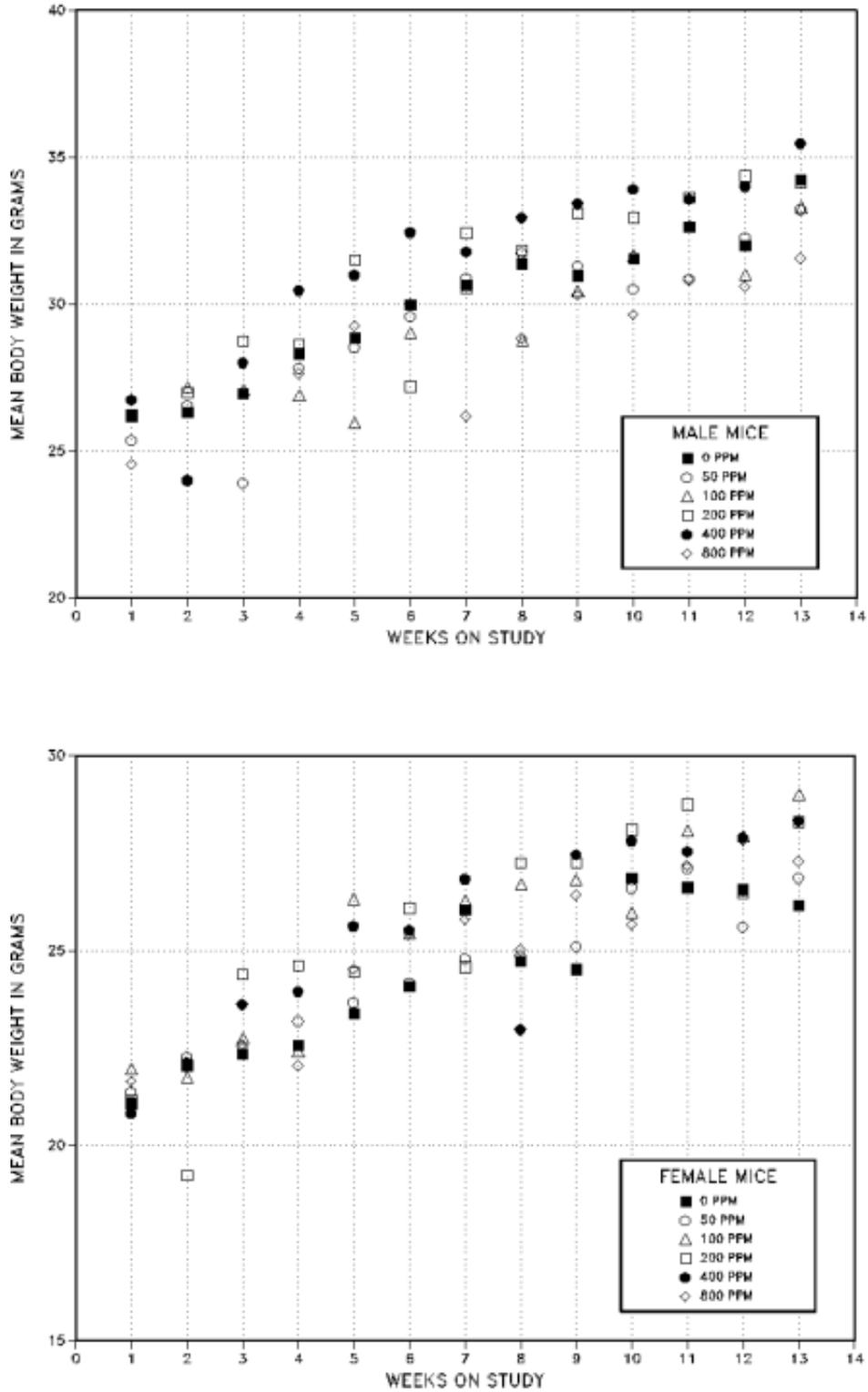


Figure 2 Body Weights of B3C6F<sub>1</sub> Mice Administered N,N-Dimethylformamide by Inhalation

**TABLE 6** Mean Absolute and Relative Liver Weights of B3C6F<sub>1</sub> Mice in 13-Week Inhalation Studies of N,N-Dimethylformamide<sup>a</sup>

Exposure Concentration (ppm)	0	50	100	200	400	800
<b>MALE</b>						
Necropsy body weight	33.97	33.51	30.61	34.28	33.18	30.87
Absolute	1.668	1.907	1.574	2.074**	2.020**	1.940**
Relative	49.13	56.94*	51.26*	60.53**	60.74**	62.40**
<b>FEMALE</b>						
Necropsy body weight	25.20	26.26	27.20	28.60**	27.02	24.62
Absolute	1.171	1.306*	1.477**	1.756**	1.699**	1.514**
Relative	46.41	49.73*	54.23**	61.44**	62.92**	61.55**

<sup>a</sup> Mean organ weights and body weights are given in grams; organ-weight-body-weight ratios are given in mg mean organ weights/g mean body weight.

\* Significantly different from control using Dunnett's test (P 0.05).

\*\* Significantly different from control using Dunnett's test (P 0.01).

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. Microscopic change attributed to DMF exposure was found only in the liver, and was diagnosed as centrilobular hepatocellular hypertrophy. This lesion was characterized by minimal to mild enlargement of hepatocytes surrounding central veins. The cytoplasm of affected cells was increased in amount and stained homogeneously, in contrast to the more typical granulovacuolar cytoplasm of periportal hepatocytes (Plate 3). 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 (Plate 4). Occasional apoptotic bodies were seen in the areas of hypertrophy, but overt hepatocellular necrosis was not seen in DMF-treated mice. Liver lesions were present in all exposure groups except the lowest concentration (50 ppm) females. Incidence and severity data for the liver lesion in mice are shown in Table 7.

**TABLE 7** Liver Lesions in B3C6F<sub>1</sub> Mice in the 13-Week Inhalation Studies of N,N-Dimethylformamide<sup>a</sup>

Exposure Concentration (ppm)	0	50	100	200	400	800
<b>MALE</b>	0/10	4/10 (1.8)	9/10 (1.3)	10/10 (2.0)	10/10 (2.0)	10/10 (2.0)
<b>FEMALE</b>	0/10	0/10	10/10 (1.3)	10/10 (1.9)	10/10 (2.0)	10/10 (2.0)

<sup>a</sup> Incidence and severity score ( ) based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions from groups of 10.

## Genetic Toxicity Studies

Dimethylformamide was essentially nonmutagenic and nontoxic in all *in vitro* short term tests for genotoxicity. DMF (100 - 10,000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested by a preincubation protocol in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Mortelmans *et al.*, 1986; Appendix D, Table D1). The chemical was tested in 3 laboratories for induction of gene mutations in mouse lymphoma L5178Y/tk<sup>+/-</sup> cells (Appendix D, Table D2). In 2 of the laboratories, DMF, tested up to a maximum dose of 5 µg/ml, was non-toxic and non-mutagenic with or without induced S9 (Mitchell *et al.*, 1988; Myhr and Caspary, 1988). Results from the third laboratory showed a marginal mutagenic response at the highest dose tested (5000 mg/ml) in all three trials conducted without S9 (McGregor *et al.*, 1988). Evidence of toxicity, in the form of reduced relative total growth, occurred at 5000 mg/ml, the dose which consistently showed a small increase in the number of mutant colonies. In cytogenetic studies with Chinese hamster ovary cells, DMF (concentrations up to 5000 mg/ml) did not induce sister-chromatid exchanges (Appendix D, Table D3) or chromosomal aberrations (Appendix D, Table D4), with or without Aroclor 1254-induced male Sprague-Dawley rat liver S9. DMF did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered either by feeding or by injection (Appendix D, Table D5).



**Plate 1.** Centrilobular area of the liver from a female rat exposed to 800 ppm DMF. Several hepatocytes around the central vein are in various stages of degeneration and necrosis (arrows). (H&E, 320X)

**Plate 2.** Centrilobular area of the liver from a female rat exposed to 800 ppm DMF. There is an accumulation of iron-positive pigment in macrophages around the central vein. (Perl's stain, 200X)

**Plate 3.** Liver from a male control (a) and 800 ppm DMF dose group (b) mouse. Centrilobular hepatocytes in the treated animal (arrows) are hypertrophic with increased amounts of more darkly staining cytoplasm and enlarged nuclei. (H&E, 130X)

**Plate 4.** Liver from a male control (a) and 800 ppm DMF dose group (b) mouse. Centrilobular hepatocytes (arrows) are clearly delimited to show loss of PAS-positive intracellular glycogen. (PAS/hematoxylin stain, 80X)

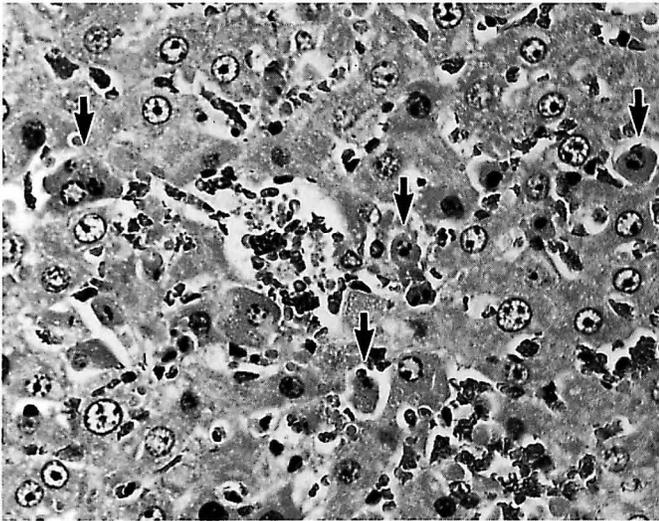


PLATE 1

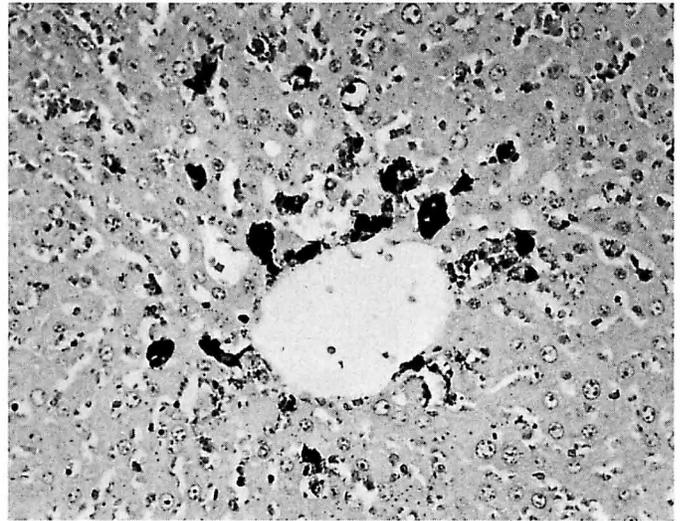


PLATE 2

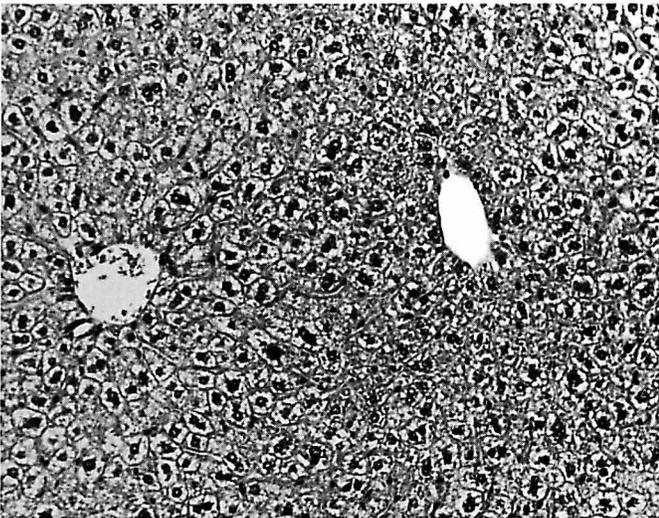


PLATE 3 (a)

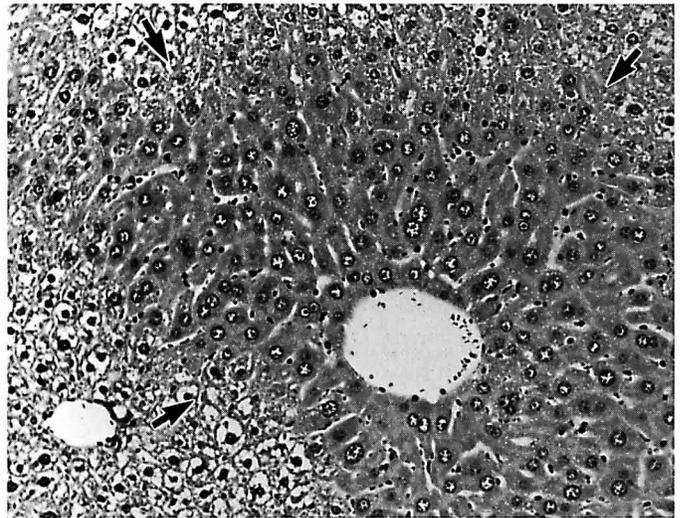


PLATE 3 (b)

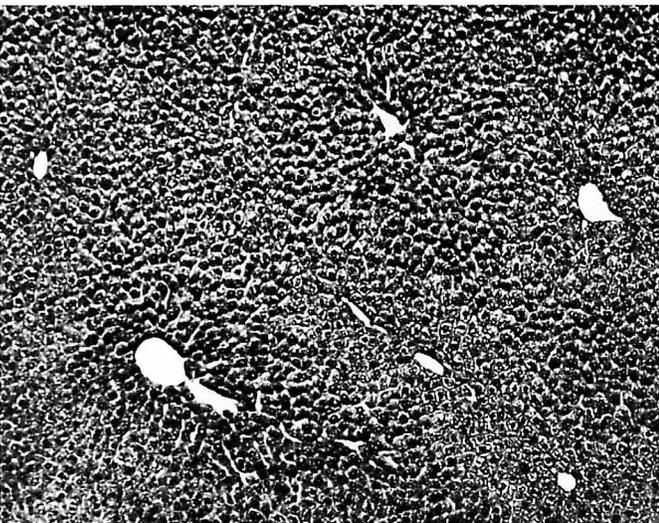


PLATE 4 (a)

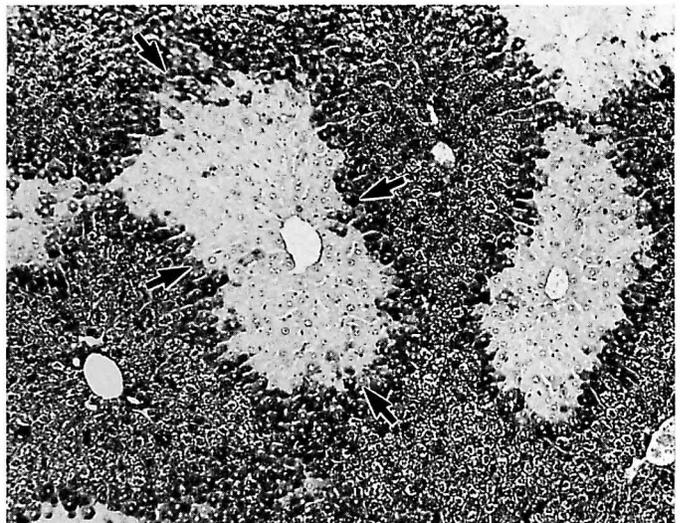


PLATE 4 (b)



## 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 enzymes in the serum and by changes in liver weights. The centrilobular hepatocellular hypertrophy in exposed mice also was reflected in increased liver weights.

DMF-induced hepatotoxicity has been reported in a variety of animal species and in humans, following both short- and long-term exposures by various routes (Kennedy, 1986; IARC, 1989). Wistar rats given 1139 mg/kg DMF i.p. showed centrilobular coagulative necrosis 48 hours after injection (Mathew *et al.*, 1980). Hepatocellular fatty change, centrilobular necrosis, and elevated levels of serum sorbitol dehydrogenase were reported in rats 21 hours following i.p. injection of DMF at 240 and 479 mg/kg (Lundberg *et al.*, 1981). Scailteur *et al.* (1981) reported increased levels of sorbitol dehydrogenase (over 800% of controls) in male Sprague-Dawley rats that received 4 i.p. injections (949 mg/kg) of DMF.

In longer-term studies, rats were exposed at 100, 230, and 450 ppm DMF by inhalation, 8 hours/day, 6 days/week for 120 days (Massmann, 1956). Rats grew normally at all exposure concentrations, but liver necrosis was reported in the 450 ppm group. Becci *et al.* (1983) reported on the toxicity of DMF incorporated into the feed of CD-1 mice at 160, 540, and 1850 ppm for 119 days. Absolute and relative liver weights were increased in both sexes in the 1850 ppm groups, but no differences in body weight gain nor in food consumption were reported. Slight hepatocytomegaly was seen in a majority of the mice in the 1850 ppm group and was considered by the authors to be a normal adaptive change reflecting the biotransformation of DMF.

In the current study, biochemical evidence of mild to moderate hepatocellular damage was present in the serum as early as 4 days into the study and at all time points evaluated in both male and female rats. Elevated serum cholesterol was seen in all exposed rats at all time points. Other changes indicative of hepatocellular damage included increased activities of ALT, SDH, and ICDH, and increased concentrations of total bile acids. Most of these changes occurred in the 200 to 800 ppm groups. Comparing the 3 time points (4, 24, and 91 days), the magnitudes of the increases generally were similar, with those in the female rats being slightly higher at 91 days. There was little evidence to suggest that animals developed tolerance to DMF or, conversely, that the severity of hepatotoxicity increased during the 13-week study.

The hepatotoxic effects of inhaled DMF were more pronounced in rats (necrosis and pigment accumulation) than in mice (hypertrophy) in the current study. This finding is in agreement with the results of an earlier study by Craig *et al.* (1984), who reported more severe hepatotoxicity in rats (fibrosis) compared to mice (necrosis/cytomegaly), following inhalation exposure to DMF concentrations as high as 1200 ppm for 12 weeks. This discordance between rats and mice may be due in part to differences between the species in the rate of metabolism of DMF. Mraz *et al.* (1989) suggest that metabolism of DMF to a toxic intermediate is critical for hepatotoxicity, and that the mercapturate, AMCC, may result from reaction of the intermediate with glutathione. These researchers also postulate that since thiocarbamates such as AMCC are carbamoylating agents, they may contribute to the toxicity of DMF. In mice and rats treated with 0.1-7 mmol/kg

(7-512 mg/kg) i.p., the amount of DMF excreted in the urine as AMCC ranged from 1.1-1.6% of the administered dose in mice and from 1.7-5.2 % in rats. Even at the highest dose in mice (7 mmol/kg), AMCC was excreted rapidly and could not be detected beyond 24 hours. In rats receiving 7 mmol/kg DMF, excretion of DMF metabolites was delayed (Mraz *et al.*, 1989). Such variances in metabolism may contribute to the observed species differences in the hepatotoxicity of inhaled DMF in the current study. It should be noted that humans occupationally or intentionally exposed to DMF appear to produce a higher percentage of the AMCC mercapturic acid metabolite than do rodents (Mraz *et al.*, 1989). Since the hepatotoxicity of the alkylformamides has been linked to the production of mercapturic acid metabolites, this finding suggests that humans may be at greater risk to the effects for DMF exposure than was suggested by studies in rodents (Mraz *et al.*, 1991).

There are several reports of toxicity in humans following occupational exposure to DMF. Potter (1973) reported on a worker at a fabric coating plant who was accidentally splashed with DMF over 20% of his body. Three days later, the worker experienced epigastric pain; a blood sample revealed elevated serum AST, ALT, and conjugated and total bilirubin. Eleven days following the accident, a biopsy showed minimal hepatic septal fibrosis. Redlich *et al.* (1988) reported that 36/58 (62%) of workers using DMF at a polyurethane-coated fabrics plant had increased AST or ALT, and 35/46 (76%) of production workers with the greatest contact with DMF showed abnormal increases in serum of these 2 liver enzymes. Exposure to DMF occurred by dermal contact as well as inhalation. In a follow-up of a selected group of these workers, liver biopsies revealed focal hepatocellular necrosis. In 3 workers exposed to DMF for less than 3 months, microvesicular steatosis was found, while macrovesicular steatosis was seen in 4 workers with 1-10 years of exposure to DMF (Redlich *et al.*, 1990). Wang *et al.* (1991) reported multiple zonal necrosis and elevated levels of ALT and AST in a worker exposed to DMF while employed at a factory producing synthetic leather and polyurethane-coated fabrics. In a follow-up of 183 other workers at the same plant with airborne DMF exposure ranging from 9-60 ppm, 8 additional workers (all negative for hepatitis B) had abnormal liver function tests.

Clayton *et al.* (1963) reported degenerative changes in heart muscle in 4 dogs exposed to 58 aerosolized doses of 23 ppm DMF for 5.5 hours, followed by 30 minutes of 426 ppm. Other reports of DMF-induced cardiotoxic effects were mentioned previously. To further evaluate the possibility of cardiotoxicity, blood pressure and ECG measurements were recorded in rats, and serum CK activities were monitored. No effects of DMF exposure on heart rate or blood pressure were found. In electrocardiographic studies, subtle changes in ECG waveforms were discerned only in a few DMF-exposed rats; these changes could not be attributed definitely to myocardial injury. Changes in the T wave, seen primarily in female rats, are indicative of an alteration in repolarization and could be due to ischemia or autonomic factors as well as cardiotoxicity. The changes in R wave amplitude and angle, primarily noted in males, signify a slight right axis deviation in the direction of depolarization of the left ventricle. The magnitude of the R wave alterations was within the normal range, and these changes may have no clinical significance. The results of the serum CK analyses were in substantial agreement with the ECG findings in terms of affected groups, because increases in CK activities occurred in female rats at all time points in the 800 ppm group, in animals in several groups at lower exposure concentrations, and in male rats at one time point (800 ppm, 24 days). Increased activities of CK in serum are generally of muscular origin (skeletal and/or cardiac). Causes of increased serum activities include trauma, physical exertion, tissue hypoxia, high body temperature, some

infectious diseases, and degeneration or catabolism of muscular tissue. In the current study, although the ECG and serum CK data could indicate myocardial injury, there was no histopathologic evidence of muscle damage caused by DMF. Another factor that hinders interpretation of these findings is the reduction in body weight gain in rats in the higher exposure groups. In an unpublished study from the clinical pathology laboratory at the National Institute of Environmental Health Sciences, dietary restrictions for 3 days of 50 and 75% compared to control rats produced increases in CK activities of 1.8 and 2.8 fold, respectively. Although the mechanism for the increases in the two studies is unclear, the cause may be related to decreased food intake (although this was not specifically measured in these studies) rather than a direct cardiotoxic effect of DMF.

Occupational exposure to DMF has been associated with an increase in testicular cancer among aircraft maintenance workers and leather tanners (Ducatman *et al.*, 1986; Levin *et al.*, 1987). In the current study, testicular toxicity was not observed in rats or mice, and no adverse effects on sperm density or motility were observed in either species. These findings are consistent with other findings in animals, as the testis has never been identified as a target organ for DMF toxicity (summarized by Kennedy, 1986). However, the structurally related chemicals, formamide and monomethylformamide, have been shown to produce reversible testicular atrophy in rodents following single i.p. injections (Chanh *et al.*, 1971).

Effects on the estrous cycle of both rats and mice exposed to DMF were noted in the present study. Sheveleva *et al.* (1979) exposed nonpregnant rats to 3.6 or 0.77 ppm DMF by inhalation for 4 hours/day for 20 days. Rats exposed to 3.6 ppm showed a significant lengthening of the inter-estrous interval compared to concurrent controls. A similar effect was noted in rats in the current study, although this prolonged diestrus was seen only in rats exposed to 800 ppm DMF, a concentration that produced hepatotoxicity and reduced body weight gain.

In summary, under these exposure conditions, inhalation of DMF produced hepatotoxicity in both rats and mice. For rats of both sexes, the no-observed-adverse-effect level was 200 ppm, based on the absence of liver histopathology, although liver function assays and liver weights showed changes at all exposure levels (as low as 50 ppm). For mice, hepatocellular hypertrophy, or increased liver weights, occurred at all exposure concentrations.



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