National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

DRAFT

NTP-CERHR REPORT on the REPRODUCTIVE and DEVELOPMENTAL TOXICITY of DIMETHYL METHYLPHOSPHONATE

SEPTEMBER 2004 **NTP-CERHR-XXXXX-00**

DRAFT EXAMPLE

NTP-CERHR Executive Summary

Dimethyl methylphosphonate (DMMP)

DMMP is a clear liquid used in a variety of applications including its use in flame retardants, textile conditioning, hydraulic fluids, gasoline additives, and in the testing of protective equipment by the military. No information was located on human exposure levels but it is anticipated that production sites and military installations would be primary locations for human exposures. While information is generally lacking on levels of DMMP in the environment, it has been detected in ground water at the Rocky Mountain Arsenal in Colorado at levels as high as 1.3 milligrams per liter. Assuming the contaminated water was used as drinking water, an adult would consume as much as 2.6 milligrams per day of DMMP.

No studies were available on the reproductive or developmental effects of DMMP in humans. There were no data available to evaluate the possible reproductive effects of DMMP in female laboratory animals. There were not sufficient data available to evaluate the developmental effects of DMMP in laboratory animals. However, sufficient data were available to evaluate the reproductive effects of DMMP in male rats and mice.

When male rats or mice were exposed orally to DMMP and then mated to untreated females, the number of dead embryos observed in the uterus increased as the exposure level increased. This effect is attributed to DMMP-induced genetic damage in the male germ cells that results in early death of the conceptuses soon after they implant in the uterine wall. Male mice appear to be less sensitive to such effects than male rats; such effects were observed at exposures of 500 mg/kg bw/day and higher in male mice while the effects were observed at 250 mg/kg bw/day and higher in male rats.

The highest level of DMMP reported for ground water is 1.30 milligrams/liter, at the Rocky Mountain Arsenal in Colorado. If drinking water contained this level of DMMP, a person would be exposed to approximately 2.6 milligrams per day, or 0.037 milligrams/ kilogram body weight/ day. In this worst-case exposure scenario, human exposure levels would be less than $1/1000^{th}$ the level that results in reproductive toxicity in male rodents.

Based on these data, the NTP concludes that DMMP induces reproductive toxicity in male rats and mice. While high levels of DMMP exposure might lead to similar effects in humans, the NTP concludes that there is minimal concern for human male reproductive toxicity at the relatively low estimated human exposure level of 2.6 milligrams per day.

DRAFT EXAMPLE

Preface

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

Dimethyl methylphosphonate (DMMP) was nominated by an NIEHS scientist in November, 1999, based on the possibility of widespread exposure due to numerous uses including as a flame retardant, an additive for gasoline, an antifoam agent, a plasticizer and stabilizer, a textile conditioner, an antistatic agent, and an as additive to solvents and low-temperature hydraulic fluids. Because the relevant data base on the reproductive and developmental effects of DMMP was evaluated as too small for consideration by an Expert Panel, DMMP was assigned to an alternate review process; specifically, this report was prepared by CERHR staff scientists and evaluated by external peer reviewers. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is intended to (1) interpret the strength of scientific evidence that DMMP is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk.

The report on DMMP will be a central part of the subsequent NTP CERHR Monograph. The monograph will include the NTP CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the website (http://cerhr.niehs.nih.gov) or from:

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Note to Reader:

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (http://cerhr.niehs.nih.gov/). The format for this report follows that of CERHR Expert Panel Reports including synopses of studies reviewed, and an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the CERHR Scientists and are prepared according to the NTP/NIEHS guidelines. In addition, the report includes comments or notes limitations of the study in the synopses. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by CERHR.

Abbreviations

BMD_{10}	benchmark dose corresponding to a 10% effect level
BMDL	lower limit of the 95% confidence interval around the benchmark dose
CAS RN	Chemical Abstracts Service Registry Number
CERHR	Center for the Evaluation of Risks to Human Reproduction
CHO	Chinese hamster ovary
DMMP	dimethyl methylphosphonate
EPA	Environmental Protection Agency
g	gram(s)
GD	gestation day
GDH	glycerolphosphate dehydrogenase
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HSDB	Hazardous Substances Data Bank
kg	kilogram(s)
K_{ow}	octanol–water partition coefficient
L	liter(s)
LD_{10}, LD_{50}	lethal dose affecting 10, 50% of the population
LOAEL	lowest observed adverse effect level
m^3	cubic meters
mg	milligram(s)
mL	milliliter(s)
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
S.	*Salmonella*
SD	standard deviation
SDH	succinic dehydrogenase
SEM.	standard error of the mean
U.S.	United States
UV	ultraviolet
µg	microgram(s)
ºC	degrees centigrade

Table of Contents

Tables

Figures

1.0 CHEMISTRY, USE, AND HUMAN EXPOSURE

As noted in the CERHR Expert Panel Guidelines, Section 1 is initially based on secondary sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews.

1.1 Chemistry

1.1.1 Nomenclature

The CAS RN for dimethyl methylphosphonate is 756-79-6.

Synonyms for dimethyl methylphosphonate include:

> DMMP; Fyrol DMMP; dimethyl methanephosphonate; dimethoxymethylphosphine oxide; methylphosphonic acid dimethyl ester (9CI); methanephosphonic acid, dimethyl ester; phosphonic acid, methyl-, dimethyl ester; NCI-C54762

In this report, the term DMMP will be used.

1.1.2 Formulae and Molecular Mass

DMMP has a molecular mass of 124.09 and a molecular formula of $C_3H_9O_3P$. The structure for DMMP is shown in Figure 1

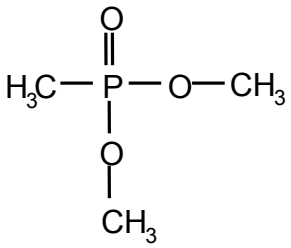


Figure 1. Structure of dimethyl methylphosphonate

1.1.3 Chemical and Physical Properties (Table 1)

DMMP is a low-viscosity, colorless liquid that is miscible in a variety of industrial solvents and miscible to soluble in water and contains high levels of phosphorus by weight (25%) [*1*].

Table 1. Chemical and Physical Properties of Dimethyl methylphosphonate

Property	Value
Physical state	colorless liquid
Melting point	<50˚ C
Boiling Point	181˚ C
Specific Gravity/Density	1.145 g cm-3
Solubility in Water	Substantial. Hydrolyzes slowly in contact with water.
Solubility in solvents	Miscible in alcohol, ether, benzene, acetone, and carbon tetrachloride.
Vapor Pressure	1.2 mm Hg at 25 C
Flash point	43°C
Refractive index	1.411
Stability	Combustible
Reactivity	Incompatible with strong oxidizing agents, strong bases. May soften some rubbers or plastics.
Log K_{OW}	-1.88

Reviewed in HSDB [*2*] and Rowland et al. [*1*].

1.1.4 Technical products and impurities

No data were located.

1.2 Use and human exposure

1.2.1 Production

The United States produced between 0.2–2 million pounds (91,000–910,000 kg) of DMMP in 1977 from two producers, Mobile Oil Corporation and Stauffer Chemical Company [*2*]. DMMP is manufactured by reacting trimethylphosphite or the sodium salt of dimethyl hydrogenphosphite with methyl chloride [*1*] [*2*]. The information on the manufacturers of DMMP is inconsistent. HSDB lists Akzo Chemicals and Tenneco Inc. as the only manufacturers and lists production volume as probably greater than 454 kg in 1972 and 1975, but this volume does not seem accurate. NIOSH National Occupational Exposure Survey in 1983 lists a total of 5 industries with 53 facilities for DMMP. Rowland et al [*1*] states that Mobile Chemical Company is the only U.S. manufacturer of DMMP listed in the literature.

1.2.2 Use

DMMP is used as a flame retardant, solvent, hydraulic fluid, antifoam agent, plasticizer and stabilizer, textile conditioner and antistatic agent, a preignition additive for gasoline, and an additive for solvents and low-temperature hydraulic fluids. DMMP is also used in the military to simulate nerve gas during testing of protective equipment. DMMP is also listed as a chemical weapons precursor and is regulated by the Chemical Weapons Convention.

1.2.3 Occurrence

No information on the possible exposure of the public to DMMP through contact with air, drinking water, food, or consumer products has been identified. DMMP has high water solubility and is not expected to bioconcentrate in organisms or adsorb to soils or sediments. Leaching into groundwater is a possible route of exposure for DMMP and DMMP is expected to be moderately persistent in the environment [*1*].

DMMP is subject to hydrolysis and UV photolysis and will hydrolyze to the half ester and methanol with an estimated half-life of 13.2 years at 20˚C. The average half-life in soil is estimated to average 12 days

with a range of 0.2–60 days and the half-life in muddy water is 1–30 weeks, depending on temperature and initial concentration [*2*]. The atmospheric vapor phase half-life is estimated to be 1.6 months.

Military testing sites are potential sources for exposure to DMMP in soil and buildings and contamination of groundwater from improper disposal methods.

Methylphosphonic acid, produced by hydrolysis of flame retardants containing dimethyl methyl phosphonate, has been measured in surface water samples from the Netherlands, Belgium, Germany, Finland, Sweden, Canada, and the U.S. [*3*]. Samples were collected from industrially polluted sources and from relatively clean sources. DMMP was detected in 25 samples of water characterized as industrially polluted and 3 waste water samples with a mean methylphosphonic acid concentration of 10 μg/L. The authors concluded that the occurrence of methylphosphonic acid was correlated with the degree of industrial pollution.

In 1989, DMMP was detected in groundwater at Rocky Mountain Arsenal, Colorado at concentrations ranging from 6.5–1300 μg/L [*1*]. Samples taken at two sites at the Arsenal contained DMMP at concentrations of 490 and 760 μg/L. Analytical techniques for the analysis of DMMP in contaminated groundwater were determined by Tomkins et al. [*4*]. Using these methods, groundwater samples from the Rocky Mountain Arsenal were analyzed for DMMP. Recovery was about 46% and DMMP was not observed to be above the method recovery limit of 0.19 μg/L in any of four samples of highly contaminated groundwater.

DMMP was identified in a liquid waste lagoon at the Aberdeen Proving Ground [*5*]. Soil and concrete samples from Rocky Mountain arsenal in Colorado were tested for organophosphonates commonly used as simulants using experimental extraction and analytical methods [*6*]. DMMP recovery was erratic and the chromatography was not satisfactory, but the method reporting limit in soil was 12.6 μg/g and the method detection limit in soil was about 5 μg/g. In concrete, the DMMP method reporting limit was 37.4 μg/g and the method detection limit was 7.5 μg/g respectively. The methods were not considered successful in this study, but it does appear that DMMP was present at detectable levels at the Rocky Mountain Arsenal and this arsenal was a site of potential exposure.

1.2.4 Human exposure

No information was found on human exposure to DMMP in occupational or environmental settings. Rowland et al [*1*] suggest that because of low acute toxicity and irritancy and high vapor pressure, there may be little warning of exposure. High water solubility and volatility and slow evaporation from aqueous solutions may lead to exposures in the air and water.

Exposure may occur at production sites, sites where DMMP is used as a flame retardant and viscosity depressant in polyester and epoxy resins, and military sites where DMMP is or has been used as a nerve gas simulant [*2*]. NIOSH Occupational Hazard Surveys in 1974 and 1983 estimated an increase in employees exposed to DMMP of 204 to 2134 over 10 years with 763 female empoyees in 1983 [*7*]. The occupations most likely to be exposed included assemblers, machine operators, and mechanics [*8*].

1.3 Utility of data

Data were identified on occupational scenarios entailing likely exposure to DMMP, although quantitative exposure assessments in workers were not available. Measurement of DMMP in groundwater at some military installations has been reported and can be used to estimate worst-case scenario exposures of people residing in the vicinity.

1.4 Summary of human exposure data

DMMP is used as a flame retardant, solvent, hydraulic fluid, antifoam agent, plasticizer and stabilizer, textile conditioner and antistatic agent, a preignition additive for gasoline, and an additive for solvents and low-temperature hydraulic fluids. DMMP is also used in the military to simulate nerve gas during testing of protective equipment and is listed as a chemical weapons precursor. Current production volume is not known but in 1977, as much as 910,000 kg may have been produced in the U.S. No human exposure information has been identified, but it is anticipated that exposure would be restricted to production sites and military installations. The highest concentration of DMMP in an environmental matrix is 1300 µg/L in groundwater at Rocky Mountain Arsenal, Colorado, although typical concentrations identified in ground water have been 1 to 2 orders of magnitude lower. Assuming the use for drinking of water (2 L/day) with the highest reported level of contamination, DMMP would be ingested at a level of 2.6 mg/day (37 µg/kg/day for a 70-kg individual).

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL PARAMETERS

As noted in the CERHR Expert Panel Guidelines, Section 2 is initially based on secondary sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews.

2.1 Toxicokinetics

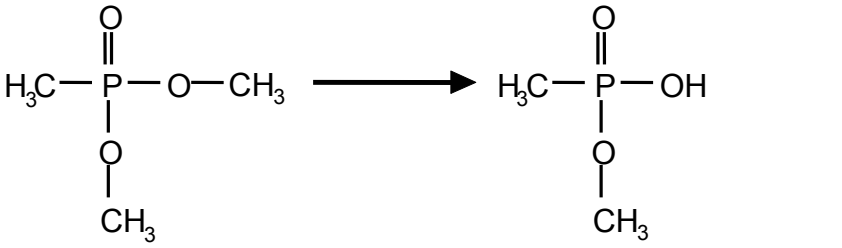
2.1.1 Human data

No information was found on the toxicokinetics (absorption, distribution, metabolism, and excretion) of DMMP in humans.

2.1.2 Experimental animal data

The biotransformation and renal toxicity of DMMP in rats was investigated by Blumbach et al. [*9*]. After single oral doses of 50 and 100 mg/kg DMMP, unchanged DMMP was excreted primarily in urine and the only metabolite was methyl phosphonate (Figure 2). DMMP was rapidly absorbed and excreted within 24 hours with a half-life of elimination between 3 and 6 hours. Parent compound and metabolite were undetectable at 36 hours after oral administration. Recovery of metabolite in urine was lower in male rats than in female rats 24 hours after administration. Comparison of metabolism in male and female rats indicated that there was no gender difference in formation or excretion of metabolites. The study also investigated α-2u-globulin accumulation in the kidney of male and female rats dosed by gavage with 500 or 1000 mg/kg DMMP for 5 days. Relative kidney weight was increased and a dose-dependent increase in α-2u-globulin was observed in male but not female rats and was accompanied by protein droplet formation in the proximal tubules of the kidney. The authors concluded that biotransformation occurs mainly by hydrolysis most likely catalyzed by esterases and hypothesized that the gender differences in metabolite recovery may stem from increased retention of DMMP in the kidneys of male rats.

Figure 2. Hydrolysis of DMMP to methyl methylphosphonate. From Blumbach et al. [*9*].



DMMP Methyl methylphosphonate

2.2 General toxicity

2.2.1 Human data

No information was found on the toxicity of DMMP in humans.

2.2.2 Experimental animal data

DMMP exhibits little toxicity to rats and mice by the oral route of administration. Oral LD_{50} values are 10,190 mg/kg in rats and >6,810 mg/kg in mice [*1*].

The National Toxicology Program (NTP) [*5*] performed a series of studies to examine the acute and chronic toxicity of DMMP (>98% purity) in rats and mice, summarized in Table 2.

Acute studies in F344/N rats and $B6C3F_1$ mice were conducted on groups of 5 males and 5 females of each species. DMMP was administered by gavage in corn oil at doses of 1470, 2150, 3160, 4640, or 6810 mg/kg and animals were observed for 14 days. DMMP produced no treatment-related deaths at doses up to 6810 mg/kg, but treatment-related clinical signs including transitory (1-4 hours after dosing) inactivity, unsteady gait, and prostration were seen in all dose groups above 1470 mg/kg.

Subacute (15-day) studies in F344/N rats and $B6C3F_1$ mice were conducted in groups of 5 males and 5 females of each species administered DMMP by gavage in corn oil at doses of 0, 1250, 2500, 5000, 10,000, or 15,000 mg/kg/day for 15 consecutive days. In mice the 15,000 mg/kg/day dose was administered neat (without vehicle). All animals were observed twice daily, body weight was measured on days 0 and 15 for mice and day 0 for rats, and necropsies were performed on all animals. In addition, histopathologic examination was performed on the stomachs of all mice. Results indicated that survival was decreased in mice in the 10,000 and 15,000 mg/kg/day dose groups and was accompanied by inactivity, prostration, and shallow breathing. There were stomach lesions (gastropathy, gastritis, heperkeratosis, or epithelial ulceration) in female mice in the 5000, 10,000, and 15,000 mg/kg dose groups and squamous atrophy, gastropathy, or gastritis in male mice in all dose groups. Rats experienced increased mortality at doses of 5000 mg/kg/day or greater. Inactivity occurred at doses of 2500 mg/kg/day or greater and unsteady gait at 5000 or 10,000 mg/kg/day.

In 13-week studies, F344/N rats and $B6C3F_1$ mice in groups of 10 males and 10 females were administered 0, 250, 500, 1000, 2000, 4000, or 8000 mg/kg/day DMMP in corn oil by gavage 5 days per week. Excessive mortality occurred in the first experiment with male and female F344/N rats at doses of 2000 mg/kg/day and above and in $B6C3F_1$ mice at doses of 4000 and 8000 mg/kg/day. Rats in the 4000 mg/kg/day group had decreased body weight and at 8000 mg/kg/day had rough hair and decreased activity. In mice, DMMP had no effect on body weight, food consumption, clinical signs, gross pathology, or histopathology, and no stomach lesions were observed.

The 13-week study was repeated in rats because of dosing errors in the first study. Groups of 10 males and 10 females were administered 0, 250, 500, 1000, 2000, or 4000 mg/kg/day DMMP in corn oil by gavage 5 days per week for 13 weeks as in the first study. Mortality of all rats occurred in the 4000 mg/kg/day group in the first week and 6 of 10 males and 3 of 10 females in the 2000 mg/kg/day group. In the 2000 mg/kg/day group, final body weight was decreased in males (6%) and females (7%) compared to the control group and relative liver weight was increased. No clinical signs of toxicity were observed. DMMP-treated male rats had an increased incidence of kidney nephrosis and hyaline droplet degeneration. The testis showed hyperspermatogenesis in tubules at doses higher than 1000 mg/kg/day, but the effect was not dose-dependent. Some male and female rats in the higher dose groups had inflammation of the salivary gland.

Two-year studies of DMMP oral toxicity in F344/N rats and $B6C3F_1$ mice were conducted on groups of 50 male and 50 female animals. Rats were administered doses of 0, 500, or 1000 mg/kg by gavage and mice were administered 0, 1000, or 2000 mg/kg by gavage 5 days/week for 103 weeks. Animals were observed twice a day, clinical signs were recorded weekly, and body weight was measured weekly for 13 weeks and monthly thereafter. Necropsy and histopathologic examination was performed on all animals

at the end of the study. Decreased survival occurred in male rats at both doses of DMMP and in females at the high dose. Mean body weights of male rats were decreased 5-10% up to week 76 and 10-24% up to week 104 and body weights of female rats were decreased 8-12% after week 80 at 1000 mg/kg/day. Kidney lesions occurred in male rats consisting of increased calcification of renal papilla, cortical tubular cell hyperplasia, and pelvic epithelial hyperplasia. The 2-year toxicity study in mice was compromised by high mortality in males and females accompanied by decreased body weight and kidney lesions in males.

The EPA Health Advisory for DMMP [*1*] describes additional unpublished studies by Ciba-Geigy and Stauffer Chemical Company for acute and subacute toxicity of DMMP.

An acute oral toxicity study administered groups of 5 male and 5 female Tif:RAIf rats a single dose by gavage of 1000, 3000, 4500, 6000, 8000, 10,000, or 15,000 mg/kg undiluted DMMP (Stauffer, 1983 cited in Rowland [*1*]). Mortality occurred at doses of 8000 and above and all doses produced ataxia, muscular hypotonia, prostration, hypoventilation, and reduced spontaneous motor activity that lasted for >6 hours. Clinical signs were not noticeable 24 hours after dosing. Cyanosis accompanied other clinical signs in the high dose group.

The toxicity of DMMP following intravenous injection was studied in Tif:MAGf mice and Tif:RAIf rats (Ciba-Geigy, 1977 cited [*1*]). Groups of 5 male and 5 female mice were given a single injection of 100, 300, 600, 800, 900, 910, 920, or 930 mg/kg DMMP in distilled water. Mice exhibited ataxia, rough coat, restlessness, humpback, tremors, ventricumbency, labored respiration, muscular hypertonia, exophthlamos, twitching muscles, salivation, lacrimation, convulsions, and reduced spontaneous motor activity. Groups of 5 male and 5 female rats were given a single injection of 600, 800, 1000, 1200, or 1500 mg/kg DMMP in distilled water. Mortality was observed at all doses except 600 mg/kg and clinical signs of toxicity at all doses were similar to those observed in mice.

The toxicity of DMMP following inhalation exposure was studied in groups of 10 male and 10 female Tif:RAIf rats. Nose-only exposure to DMMP aerosol concentrations of 1355 (± 107) or 2589 (± 176) mg/m^3 occurred over 4 hours and the animals were observed for 14 days **[the given uncertainty figures were not defined. Assuming an inhalation factor of 1 m^3/kg/day in rats [*10*], these concentrations would produce inspired doses of 22 and 432 mg/kg over the 4-hour period]**. No mortality was observed by inhalation to these concentrations, but convulsions, exophthalmos, lateral or ventral position, and ruffled fur were observed and were more pronounced at the higher concentration. Examination of the lungs and "congested organs" revealed hemorrhages.

The dermal toxicity of DMMP was examined in 5 male and 5 female Tif:RAIf rats administered a single 24-hour dermal application of undiluted DMMP (4.0 mL/kg **[4580 mg/kg]**) onto the shaved back (Ciba-Geigy, 1976 cited in Rowland [*1*]). During 8 days of observation, no mortality, local effects, or systemic effects were seen. Transient irritation of abraded skin was observed in 3 male and 3 female Russian rabbits given DMMP 0.5 mL **[278–337 mg/kg calculated by Rowland [*1*]]** by occluded patch for 24 hours (Ciba-Geigy, 1976 cited in Rowland [*1*]; weight adjusted calculation by Rowland).

A primary skin irritation test in albino rabbits examined the effects of dermal application of 0.05 mL DMMP at concentrations of 1, 10, 20, or 50% in tapwater (Ciba-Geigy, 1976 cited in Rowland [*1*]). Test locations were scored at 24 and 72 hours following treatment. DMMP elicited no irritation reaction at concentrations of 1, 10, or 20% while slight irritation was observed at 50% concentration at 24 hours, but was no longer apparent at 72 hours after exposure. In a skin sensitization test, 10 albino guinea pigs were exposed to 0.5 mL of a 10% (w/v) aqueous solution of DMMP for 5 hours (Ciba-Geigy, 1976 cited by Rowland [*1*]). A challenge dose of DMMP was given after 2 weeks elapsed and indicated that DMMP was not a skin sensitizer.

Table 2. Summary of NTP Studies on DMMP [*5*]

Study design	Species	DMMP dose/route	Endpoints	Results	Effect Levels
Acute toxicity	Rat-F344/N (5 male; 5 female)	1470, 2150, 3160, 4640, or 6810 mg/kg/day in corn oil, gavage	Observation-14 days, necropsy	Transitory inactivity, unsteady gait, prostration at all doses except 1470 mg/kg	
	Mouse-B6C3F$_1$ (5 male; 5 female)	1470, 2150, 3160, 4640, or 6810 mg/kg/day in corn oil, gavage	Observation-14 days, necropsy	Mortality 2/5 at 6810 mg/kg. Transitory inactivity at 4640 and 6810 mg/kg	
15-day Study	Rat-F344/N (5 male; 5 female)	0, 1250, 2500, 5000, 10,000, or 15000 mg/kgday in corn oil, gavage	Observation, body weight, necropsy	Treatment related deaths >5000 mg/kg. Inactivity, unsteady gait	Cannot be established.
	Mouse-B6C3F$_1$ (5 male; 5 female)	0, 1250, 2500, 5000, 10,000, or 15,000 mg/kg/day in corn oil, gavage	Observation, body weight, necropsy, histopathology of stomach	Mortality 4/5 males and 5/5 females at 10,000 mg/kg and all mice at 15,000 mg/kg/day. Increased stomach lesions in all dose groups	LOAEL: 1250 mg/kg (male); 5000 mg/kg (female)
13-week Study	Rat-F344/N (10 male; 10 female)	0, 250, 500, 1000, 2000, or 4000 mg/kg/day in corn oil, gavage, 5 days/week	Observation, necropsy and histology	Mortality 10/10 rats at 4000 mg/kg in 1 week, 6/10 males and 3/10 females at 2000 mg/kg died. Increased liver/body weight. Hyaline droplet formation in kidneys of all treated males	NOAEL: 1000 mg/kg/d
2-year Study	Rat-F344/N (50 male; 50 female)	0, 500, or 1000 mg/kg/day in corn oil, gavage, 5 days/week	Observation, necropsy and histology	Decreased survival of males at both doses, females at high dose. Decreased body weight of males and females at 1000 mg/kg. Non-neoplastic lesions in male kidney	NOAEL: 500 mg/kg/day
	Mouse-B6C3F$_1$ (50 male; 50 female)	0, 1000, or 2000 mg/kg/day in corn oil, gavage, 5 days/week	Observation, necropsy and histology	Decreased survival at high dose (M/F), decreased body weight (M/F), hepatocytomegaly in males	Inadequate study

The potential for ocular irritation of DMMP was assessed in groups of 3 male and 3 female rabbits (Ciba-Geigy, 1976 cited by Rowland [*1*]). DMMP was instilled into the conjunctival sac of the left eye for 1

minute and was then washed off. Minimal ocular irritation consisting of a conjunctival and corneal reaction was observed.

The effects of DMMP on plasma cholinesterase in Sprague-Dawley rats were assessed in groups of 5 male and 5 female rats administered 1, 10, 100, or 1000 mg/kg DMMP in tapwater by gavage for 3 days. A control group of 10 male and 10 female rats were dosed with an equivalent volume of tapwater. Blood sample collection occurred at pretest, 0.5, 1, 2, and 4 hours following administration of the final dose. DMMP did not affect mortality, body weight, or food consumption and no signs of clinical toxicity were observed. Plasma cholinesterase levels were decreased by 30% in male and 40–50% in female rats treated with 1000 mg/kg/day DMMP compared to control rats.

A subacute (4-week) toxicity study of DMMP in the diet was performed in Sprague-Dawley rats (Ciba-Geigy, 1977 cited by Rowland [*1*]). Groups of 5 male and 5 female rats were administered DMMP in the diet for a 4 week period at concentrations of 0, 2000, or 6000 ppm (0, 178, or 535 mg/kg/day). Additional groups of 10 male and 10 female rats were administered diets containing 20,000 ppm (1790 mg/kg/day) for 4 weeks and, of these, 5 males and 5 females were switched to a control diet for a further 4 weeks after treatment ended to examine recovery. Daily observations were made, and body weight and food consumption were measured weekly. Ophthalmologic examinations, hematology, clinical chemistry, urinalysis, gross necropsy, and histopathology were performed during the course of the study and/or at termination. DMMP had no effects on survival, body weight, food consumption, hematology, clinical chemistry, urinalysis, or eye abnormalities at the concentrations tested. Absolute and relative kidney weight in males and females and liver weight in males were increased significantly at 20,000 ppm. Absolute and relative kidney weight in females was increased at 6000 ppm. Increased resorption of protein droplets in the proximal convoluted epithelium of the kidney was observed in male rats in the 2000, 6000, and 20,000 ppm groups. Rats in the recovery group showed no adverse effects after cessation of treatment.

2.3 Genetic toxicity

DMMP was found to be negative in the Salmonella/microsome preincubation assay using a standard NTP-approved protocol (Mortelmans et al., 1986 cited by HSDB [*2*]). Testing was conducted in as many as 5 *S. typhimurium* strains (TA1535, TA1537, TA97, TA98, and TA100) in the presence and absence of rat and hamster liver S-9 fraction. DMMP exposure levels were 0.1, 0.333, 1.0, 3.33, and 10 mg/plate. The highest dose tested, 10 mg/plate, was ineffective in any strain of *S. typhimurium*. In unpublished studies, DMMP at up to 50 mg/plate was not mutagenic in several *Salmonella* strains either in the presence or absence of metabolic activation by rat liver S-9 fraction (Ciba-Geigy, 1978 and Inveresk Research International, 1976, cited in HSDB [*2*]). Sex-linked recessive lethal testing in *Drosophila melanogaster* gave positive results (0.7% lethals) with feeding of 24,000 ppm DMMP [*11*]. In a panel of genotoxicity tests performed for the Air Force [*12*], DMMP was not mutagenic in the Ames test or at the HGPRT locus in Chinese hamster ovary (CHO) cells. Sister chromatid exchanges were not increased in CHO cells. Chromosome aberrations were increased in CHO cells at 250 and 1000 µg/mL but not at 125 or 500 µg/mL. Transformation was not induced in mouse BALB/c-3T3 cells at up to 100 µg/mL DMMP. A study in male mice was characterized by its authors as a dominant lethal study [*13*]; this study includes end points that are useful in an assessment of male reproductive toxicity and this paper will be discussed in section 4.2.2.

2.4 Carcinogenicity

In 2-year studies by the NTP [*5*], DMMP was administered in corn oil by gavage at doses of 0, 500, and 1000 mg/kg/day to groups of 50 Fischer-344 rats of each sex and at doses of 0, 1000, or 2000 mg/kg/day to groups of 50 $B6C3F_1$ mice of each sex. Treatments were administered 5 days/week for 103 weeks. DMMP was not carcinogenic in female rats in this study. There was some evidence of DMMP carcinogenicity in male rats evidenced by an increased incidence of tubular cell hyperplasia, renal tubular

cell adenocarcinoma, hyperplasia of the transitional cell epithelium, and transitional cell papilloma or carcinoma (combined) of the pelvic epithelium in the kidneys. Because these tumor types are rare in rats, the NTP considered them to be related to treatment. Renal tubular cell adenocarcinoma, however, is associated with α-2u–globulin, a protein occurring only in the male rat liver, and production of this tumor type in rats is not considered relevant to humans. A significantly increased incidence of mononuclear cell leukemia was observed in male rats at 1000 mg/kg. Decreased survival in both dose groups of male mice made the study inadequate for assessing the carcinogenicity of DMMP. No evidence of carcinogenicity was observed in female mice at 1000 mg/kg but decreased survival of females at 2000 mg/kg made the study inadequate for assessing carcinogenicity in mice at this dose. DMMP was classified as a Group C: Possible Human Carcinogen by the EPA [*1*].

2.5 Potentially sensitive subpopulations

No information was located on potentially sensitive subpopulations..

2.6 Summary of general toxicology and biological effects

2.6.1 Toxicokinetics

No human data were located on DMMP toxicokinetics. In rats exposed to DMMP by mouth, absorption was rapid. Most of the administered compound was eliminated unchanged in the urine with an elimination half-life of 3–6 hours [*9*]. The hydrolysis product methyl methylphosphonate was also identified in urine. Elimination of parent compound and metabolite was complete by 36 hours after oral administration, with possible retention of DMMP in the kidneys of male rats in comparison to female rats [*9*].

2.6.2 General toxicity

No human data on DMMP toxicity was identified. The acute oral LD_{50} in rats is 10,190 mg/kg, and in mice is >6810 mg/kg [*1*]. Subacute toxicity in rats includes inactivity and unsteady gait and in mice includes gastric lesions [*5*]. Subchronic and chronic dosing in rats produces non-neoplastic renal lesions in males [*5*]. Inhalation of DMMP aerosol for 4 hours at 1355 and 2589 mg/m^3 **[approximately equivalent to 22 and 432 mg/kg]** produced no mortality but produced clinical signs of toxicity and hemorrhage in the lungs and other **[unstated]** organs [*1*]. Application of an estimated 4580 mg/kg to the shaved back of rats for 24 hours did not produce evidence of toxicity [*1*].

2.6.3 Genetic toxicity

DMMP was not mutagenic in *S. typhimurium* with or without S-9 activation [*2*] or in CHO cells in culture. Chromosome aberrations were increased in CHO cells but not in a dose-dependent manner.

2.6.4 Carcinogenicity

DMMP was not carcinogenic in female rats at gavage doses of up to 1000 mg/kg/day 5 days/week for 2 years [*5*]. In male rats, there was an increased incidence of tubular cell hyperplasia, renal tubular cell adenocarcinoma, hyperplasia of the transitional cell epithelium, and transitional cell papilloma or carcinoma (combined) of the pelvic epithelium in the kidneys [*5*]. Because these tumor types are rare in rats, the NTP considered them to be related to treatment. Renal tubular cell adenocarcinoma, however, is associated with α-2u–globulin, a protein occurring only in the male rat liver, and production of this tumor type in rats is not considered relevant to humans. Carcinogenicity studies in male mice could not be interpreted due to excessive mortality. DMMP was not carcinogenic in female mice given 1000 mg/kg/day by gavage 5 days/week for 2 years. Excessive mortality at 2000 mg/kg/day precluded evaluation of carcinogenicity at this dose in female mice NTP.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human data

No information was located on human developmental effects of DMMP.

3.2 Experimental animal

DMMP was evaluated in the Chernoff-Kavlock protocol, which is a short-term in vivo screening assay (Hardin et al. [*14*]; supported by NIOSH). Pregnant CD-1 mice (n=50) were dosed by gavage from gestational day (GD) 6–13 (plug = GD 0) with DMMP **[purity not specified]** 4175 mg/kg/day (estimated from preliminary studies to represent the LD_{10}). Treatments were based on GD 6 weights. Dams were allowed to deliver their litters (considered postnatal day [PND] 1) and litter size, weight of the litter, and pup mortality was recorded. Neonatal growth and survival and maternal weight were assessed on PND 3. A control group of 50 mice given corn oil underwent the same evaluations. One of the 50 DMMP-treated dams died. There were 32 viable litters among 36 pregnant dams in the DMMP group, which was not different from 37 viable litters among 39 pregnant control dams. The maternal weight change between GD 6 and PND 3 did not differ between DMMP and control groups. Mean birth weight (±SD) of pups was significantly (P<0.05) decreased in the DMMP group (1.15 ± 0.1 g) compared to the control group (1.6 ± 0.2 g). Number of live pups per litter, survival, and pup weight gain were not affected by treatment with DMMP. The data in this study are also contained in a NIOSH report, *Screening of priority chemicals for potential reproductive hazard* [*15*].

Strengths/Weaknesses: The Chernoff-Kavlock protocol offers the advantage of providing a relatively rapid and simply assessment of the potential of a compound to disrupt development. The test is based on the observation that developmental toxicology studies typically show a reduction in litter size, weight, or viability at doses of a test compound that produce an increase in malformations. The disadvantage of this assay is the use of a single dose, preventing a quantitative assessment of developmental response, and the lack of a detailed anatomic evaluation of the offspring.

Utility (Adequacy) for the CERHR Evaluation Process: This study has limited utility in the evaluation, and would be suitable only as supplementary information if other information on developmental toxicity is available. By itself, this study has no utility in the evaluation process except to indicate that 4175 mg/kg/day is an effect level in mice.

Experiments conducted in 1978 by Ciba-Geigy are described in a review by Rowland et al. [*1*]. Female Sprague-Dawley rats in groups of 25 were administered 100, 1000, or 2000 mg/kg/day DMMP by gavage on GD 6–15 and killed on day 21. Dams in the 1000 mg/kg/day group showed "slightly" decreased food intake **[not otherwise specified]**, and dams in the 2000 mg/kg/day group showed decreased body weight gain and food consumption. No maternal or fetal toxicity was seen at 100 mg/kg/day. At higher doses, toxicity was limited to reduced fetal weight and delayed skeletal maturation. No increase in structural malformations was seen at the doses tested.

A second experiment conducted in 1978 by Ciba-Geigy (reported in Rowland et al. [*1*]) was similar except that female rats were administered 2000 mg/kg/day or 2500 mg/kg/day on gestation days 6–10. Maternal food consumption was decreased in both dose groups and maternal body weight was decreased in the 2500 mg/kg/day dose group. At these doses, fetal body weight was "slightly decreased" **[not otherwise specified]** and ossification was increased. No gross malformations, visceral, or skeletal anomalies occurred in the 2000 mg/kg/day dose group, and the few that were seen in the 2500 mg/kg/day dose group were considered to be within the normal range for this species [*1*].

Strengths/Weaknesses: These experiments are reported in a secondary source and little detail is provided. The information that was provided is consistent with a standard Segment II design. The report

may be of importance in an assessment of DMMP developmental effects and an attempt is being made to obtain it.

Utility (Adequacy) for CERHR Evaluation Process: The lack of detail prevents these studies, as reported in a review article, from being used in the evaluation process.

3.3 Utility of data

A Chernoff-Kavlock study design was reported in mice and demonstrates an effect of DMMP on development at the single dose used in this protocol. This study by itself is not adequate for an assessment of DMMP developmental toxicity. There is an unpublished 1978 report submitted to EPA that may include a developmental toxicity study of appropriate design for the evaluation process; however, unless and until that report can be reviewed, the data base on DMMP developmental toxicity is insufficient for the evaluation process.

3.4 Summary of developmental toxicity

Pregnant CD-1 mice were given DMMP by gavage from GD 6–13 at 0 or 4175 mg/kg/day [*14*]. Mean birth weight was decreased by DMMP treatment. There was no effect of treatment on number of live pups/litter, survival of pups to PND 3, or pup weight gain, or on maternal weight in the interval from GD 6 to PND 3. This Chernoff-Kavlock study design has been proposed as a screen for chemicals with developmental toxicity potential. The single dose was chosen based on preliminary experiments to represent the LD_{10}. This study permits the identification of 4175 mg/kg/day as an effect level for developmental toxicity in mice, but does not permit additional evaluation.

An unpublished study (reported in Rowland et al [*1*]) was not available in sufficient detail to permit use in the evaluation process.

There are insufficient data with which to evaluate developmental toxicity of DMMP in humans or experimental animals.

4.0 REPRODUCTIVE TOCICITY

4.1 Human data

No information was identified on possible human reproductive effects of DMMP.

4.2 Experimental animal toxicity

4.2.1 Female reproductive toxicity

No information was identified on female reproductive toxicity.

4.2.2 Male reproductive toxicity

Chapin et al. (1984 [*16*]; NIEHS) examined the testis and epididymis in Fischer 344 rats 3, 4, 5, 7, 9, and 12 weeks after gavage dosing with DMMP **[purity not specified]** 1750 mg/kg/day 5 days/week. An additional group of animals treated for 12 weeks with DMMP was examined following 14 weeks of recovery. Control animals were gavaged with the tap water vehicle on the same schedule. Seven DMMP-treated animals and two controls animals were evaluated at each time point. Body weight gain was described as statistically decreased in the DMMP group by the end of the study **[the decrease was estimated from a figure to be about 3%]**. Absolute testis weight was not affected by treatment. Absolute epididymal weight was described as decreased **[a data figure shows divergence of epididymal weight at 7 weeks of DMMP dosing with about a 20% reduction in epididymis weight in the DMMP group by the end of the dosing period**. **Relative organ weights were not provided but the figures do not suggest that body weight change would account for the decrease in epididymal weight].** Testis and epididymis were fixed in Karnofsky's fixative, embedded in plastic and sectioned at 3 μm, and stained with periodic acid Schiff's (PAS) stain for light microscopy. **[Results of electron microscopy were reported but electron microscopy is not mentioned in the methods]**. Testicular lesions were evident by light microscopy after 5 weeks of DMMP dosing; these lesions were luminal PAS-positive bodies that on electron microscopy were seen to contain a matrix characteristic of spermatids. There was also an increase in "anachronistic spermiation" (Step 19 spermatid in Stage IX or X epithelia) at week 5 and focal exfoliation of spermatids and spermatocytes at week 7. The effects on the testis became more severe as the duration of exposure increased. Effects of DMMP in epididymis included PAS- or hematoxylin-positive bodies at 5 weeks followed by an increase in size and number, accompanied by immature germ cells, early spermatids, and zygotene and pachytene spermatocytes. Sperm density decreased after 9 and 12 weeks of DMMP dosing. Tubules in stages VI through XIV appeared more sensitive to DMMP treatment at 7 or 9 weeks while after 12 weeks treatment, stages VI to IX contained the maximum number of lesions. Animals that were allowed to recover for 14 weeks after 12 weeks of DMMP treatment showed mostly normal tubular spermatogenesis. Some of the tubules (up to 20%) remained abnormal, but around 95% of germ cells in abnormal tubules were cytologically normal and sperm density was normal. The authors observed in the discussion section that the changes were consistent with androgen deficiency but that serum testosterone was not reduced by DMMP treatment **[data not shown]**. They observed that testicular weight was not predictive of the effects on spermatogenesis and postulated that the effects might be due to premature shedding of germ cells by the Sertoli cells.

Strengths/Weaknesses: The strength of this study is the careful evaluation of testis histology using appropriate fixation and embedding methods. The identification of the time course of the histologic changes in the testis may be used to develop mechanistic theories. The use of a single dose and the absence of fertility data, particularly for the evaluation of recovery, are important weaknesses of the study in the context of a quantitative assessment of risk.

Utility (Adequacy) for the CERHR Evaluation Process: This study is of limited utility in a quantitative assessment, and can be used only as supplemental information. The recovery data are useful in a qualitative description of possible reproductive effects but limited by the lack of fertility data.

Cho and Park (1994 [*17*]; supported by a university research fund), compared testicular histologic effects of DMMP and trimethylphosphate (TMP), a strong alkylating agent, using a design similar to that of Chapin et al [*16*]. Adult male Sprague-Dawley rats were treated by gavage with DMMP (97% purity) 1750 mg/kg/day 5 days/week for up to 12 weeks and compared to rats treated by gavage with one of five doses of TMP (400–1500 mg/kg/day, 5 days/week) for up to 5 weeks. There were 60 rats treated with DMMP, 20 rats/dose treated with TMP, and 5 rats treated in a similar manner with distilled water as a control (the diluent for DMMP and TMP was tap water). Five males in the DMMP group and 4 males in each TMP dose group were killed every week for testicular histology **[It does not appear possible that this design was followed inasmuch as 22.6% of the DMMP-treated rats and 75% of the TMP-treated rats died (within 5 days of treatment for the TMP-treated rats. Data for DMMP-treated animals are shown for 5, 7-9, and 12 weeks of treatment. The timing of evaluation of the control animals was not given]**. DMMP was described as causing an increase in the proportion of tubules at Stages IX and X **[statistical analysis not described; this observation is not readily apparent from the data figure in the paper]**. Testes abnormalities in rats after 5 weeks of DMMP included aggregates of multinucleated giant cells characterized by PAS-positive material. Abnormalities were predominantly associated with stages X, XI, and XII, and also occurred in stages XIII-VII. No aggregates were found in stages VIII and IX. After 7 weeks of dosing, the testis abnormalities decreased, but large vacuoles were observed in Sertoli cells. "Anachronistic spermiation" (step 19 spermatids in Stage XIII-XIV epithelium) was also seen. Treatment with TMP depleted spermatids after 4 weeks. At 1 week, tubules appeared to contain multinucleated giant cells composed of spermatids at stages III-V and X. TMP was more potent in producing histologic abnormalities than DMMP.

Strengths/Weaknesses: A weakness of this study is the lack of detail in describing data analysis. A more important weakness, however, is the high mortality rate in the treated animals.

Utility (Adequacy) for the CERHR Evaluation Process: This study is generally supportive of the findings of Chapin et al [*16*], but confidence in the results are decreased by inadequate details in the reporting of results and a high mortality rate in treated animals.

In a reproductive toxicity study by the NTP [*18*], DMMP (>99% purity, corn oil vehicle) was given to male Fischer 344 rats by gavage 5 days/week for 90 days at 0, 250, 500, 1000, and 2000 mg/kg/day. The rats were mated at day 84 to untreated female rats (2 females per male). There was no mortality and no clinical signs or neurotoxicity seen in any group. Body weight gain was decreased 10% in the 2000 mg/kg/day group relative to the control group. Relative weight of the testis and prostate were not different from control in any group. Relative epididymis weight was decreased in the 2000 mg/kg/day group and relative kidney weight was decreased in the 1000 and 2000 mg/kg/day groups. There was no difference in the plasma LH and FSH levels at the times of sacrifice. In the 2000 mg/kg/day group, 18 of 20 rats (90%) had testicular lesions characterized by lack of spermatogenesis and by degeneration, vacuolization, and necrosis of spermatogonial cells. Microscopic changes characterized by lymphocyte and plasma infiltration in the interstitium were also seen in the prostate of 1 of 20 (5%) rats in the 1000 mg/kg/day group and 4 of 20 (20%) rats in the 2000 mg/kg/day groups. Kidneys of treated rats showed varying pathologic changes that were observed most frequently in the 2000 mg/kg/day group. There was a dose-dependent decrease in sperm motility and sperm count that was significant ($P<0.01$) in the 2000 mg/kg dose group and consisted of headless sperm and sperm without a hook or with a blunt hook. In the mating trial, 2000 mg/kg/day group males were incapable of impregnating females. The effect was dose-dependent with the 250 mg/kg dose group not different from controls. A dose-dependent decrease in live pups was seen due to resorptions in all dose groups compared to controls. Selected dose-response curves are illustrated in Figure 3.

Across the dose range of this experiment, the effects on male reproduction included increased resorptions to complete sterility in the absence of clinical toxicity. Reproductive toxicity showed a continuum based

on dose with effects on resorptions, number of live fetuses, total number of pregnant females, and male fertility index at increasing doses (Table 3).

Strengths/Weaknesses: This study was well-designed and conducted, with a top dose of DMMP that produced an appropriate level of generalized toxicity in the animals. The presentation and analysis of data were clear and appropriate. A weakness is the use of formalin/Bouin's fixation and paraffin embedding for the testis and the use of manual rather than automated methods of sperm analysis (understandable given the time frame in which the study was performed). In spite of the possible increased sensitivity these modern methods might have accorded the study, the data that are presented permit reliable dose-response modeling of male reproductive effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is very useful for an evaluation of the reproductive toxicity of DMMP in male rats. The most sensitive end point is resorptions per litter in pregnancies sired by treated males. The LOAEL for this effect was 250 mg/kg/day, which was the lowest tested dose. This dose was also close to the calculated BMD_{10} of 259 mg/kg/day. The BMDL of 142 mg/kg/day is visually consistent with a NOAEL (Figure 3).

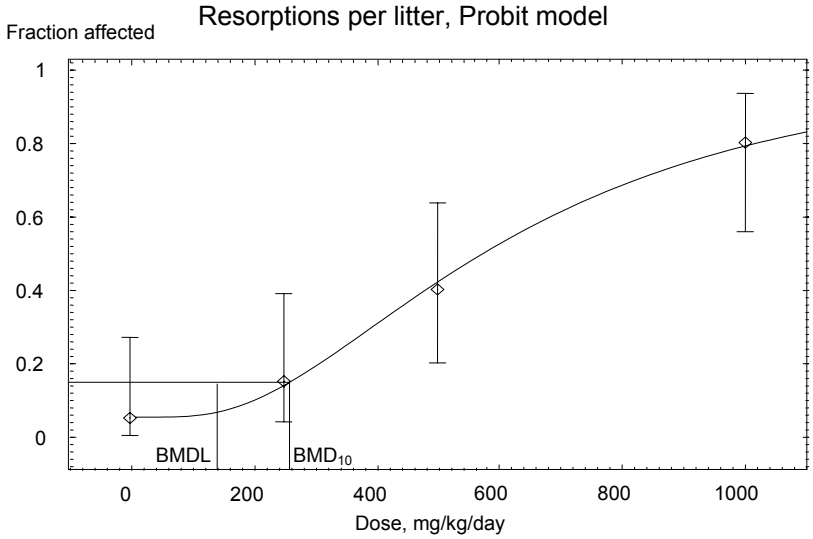
In an NTP study of similar design, Dunnick et al. [*13*] treated male $B6C3F_1$ mice 5 days/week by gavage with 0, 250, 500, 1000, or 2000 mg/kg/day aqueous DMMP (>99% purity). There were 20 males in each dose group. After 4, 8, and 12 weeks of dosing, males were cohabited with untreated CD-1 female mice for up to 4 days or until evidence of mating. Females were killed 16 days after mating and uterine contents evaluated for numbers of live and dead implants. Males were killed after 13 weeks of treatment and blood taken for measurement of luteinizing hormone and follicle stimulating hormone. Reproductive and selected other organs were weighed and evaluated by light microscopy. Epididymal sperm were counted and evaluated for morphology after staining with 1% eosin Y. An additional 20 males/dose were given 0, 1000, or 2000 mg/kg/day aqueous DMMP for 13 weeks followed by 15 weeks without treatment, after which mating trials were conducted.

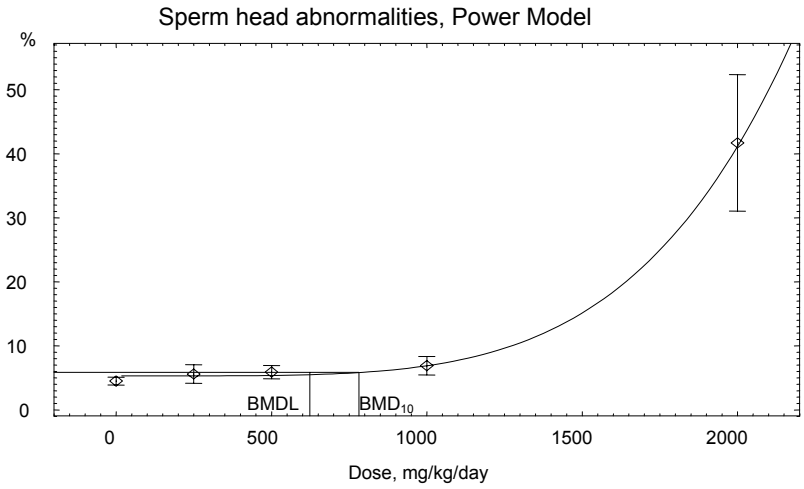
There were no treatment-related alterations in body weight or in relative weight of testis, epididymis, prostate, or kidney. There were no alterations in histopathology, sperm concentration, sperm morphology, or hormone levels. Dominant lethality, defined as the decrement in the average number of implants in the treated groups compared to the control group, was increased at 1000 and 2000 mg/kg/day after 4 and 12 weeks of treatment, and at 2000 mg/kg/day after 8 weeks of treatment. The litter means for dead and live implants are shown in Figure 4. The figure includes designation of the values that were different from control on pairwise comparison and benchmark doses calculated by CERHR using EPA benchmark dose software. The values for the benchmark doses are shown in Table 4. The authors concluded that DMMP effects in mice were similar to the effects in rats in their other study [*18*], but that mice were less sensitive than rats. Recovery appeared to be complete after 15 weeks without treatment.

Strengths/Weaknesses: This study is well designed, with similar strengths and weaknesses as the rat study by the same authors [*18*]. The use of pairwise comparisons appears to be less informative than the benchmark dose approach.

Utility (adequacy) for CERHR Evaluation Process: This study is very useful in the evaluation. In contrast to the author conclusion that mice are less sensitive than rats to DMMP, a comparison of the 12–13 week benchmark doses from the two studies [*13*],[*18*] suggests similar sensitivity. The observations at 4 weeks of treatment are consistent with somewhat lower effect levels in mice, but comparable data were not reported in the rat study.

Figure 3. Resorptions, motile sperm, and sperm head abnormalities after treatment of male Fischer 344 rats with DMMP for 13 weeks, graphed using the EPA benchmark dose software. BMD_{10} = benchmark dose corresponding to a 10% effect level; BMDL = lower bound of the 95% confidence interval around the 10% effect level. From data in Dunnick et al. [*18*] using EPA benchmark dose software.





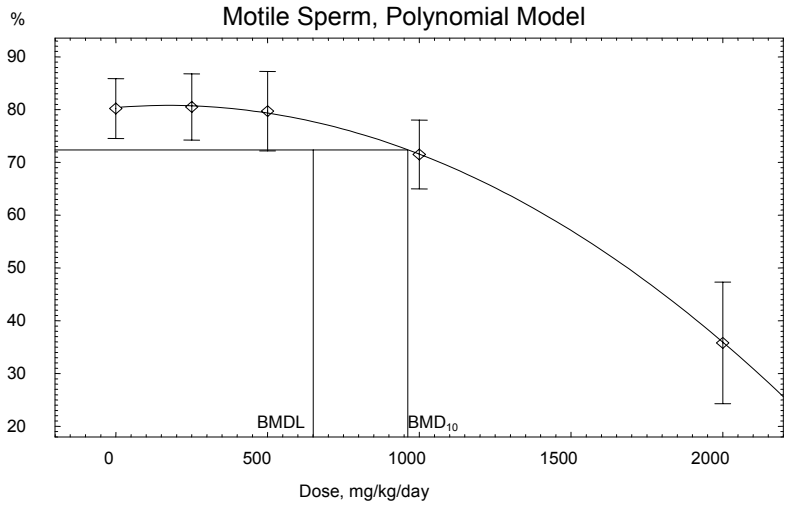


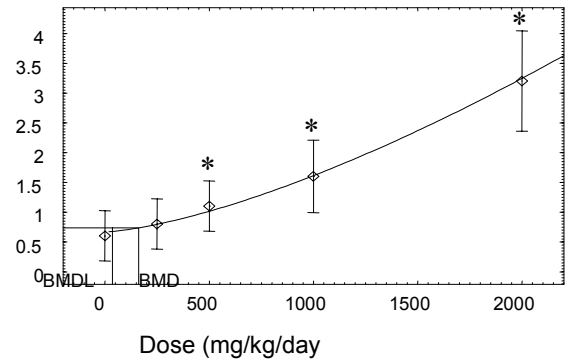
Table 3. Reproductive effects in male Fischer-344 rats after oral administration of DMMP for 90 days (data from Dunnick et al [*18*])

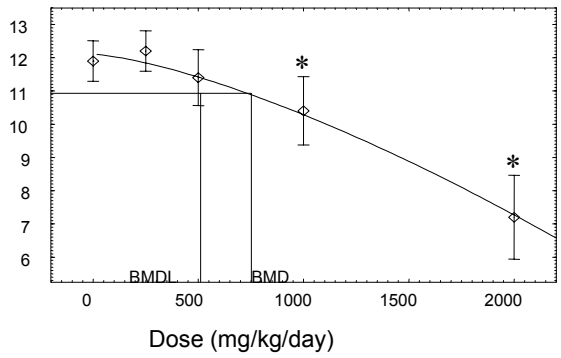
Dose (mg/kg/day)	Males with sperm positive females, %	Males impregnating at least 1 female, %	Number of pregnant females (n = 40)	Live fetuses per litter, n	Resorptions/ litter, %	Sperm count × 10^6/g caudal epididymal tissue)	Motile sperm, %	Sperm head abnormalities, %
0	75	70	20	7.6 ± 0.7	6.1	541.4 ± 25.1	80.2 ± 2.7	4.5 ± 0.3
250	85	75	19	7.8 ± 0.4	14.9*	515.2 ± 38.9	80.5 ± 3.0	5.5 ± 0.7
500	60	60	17	5.7 ± 0.6**	39.4**	459.2 ± 35.2	79.7 ± 3.6	5.9 ± 0.5
1000	50	40	11	0.82 ± 0.5**	79.1**	432.2 ± 38.5	71.5 ± 3.1 *	6.9 ± 0.7
2000	55	0	0	0	na	219.6 ± 34.0 **	35.8 ± 5.5 **	41.7 ± 5.1 **
BMD_{10}	723[a]	877[a]	865[a]	330[a,b]	259[a]	539[c]	962[c]	782[d]
BMDL	402	381	252	164	142	260	651	624

Data are mean ± SEM, n = 20/dose group, except for number of pregnant females (n=40, 2 females per male). na = not applicable. *P < 0.05, **0.01 on pairwise comparison to control. Benchmark doses calculated using EPA benchmark dose software. BMD_{10} = benchmark dose corresponding to a 10% effect level; BMDL = lower bound of the 95% confidence interval around the 10% effect level. Benchmark doses are expressed in mg/kg/day. Models selected based on best data fit. [a]Probit model; [b]Calculated without top dose; [c]Polynomial model; [d]Power model.

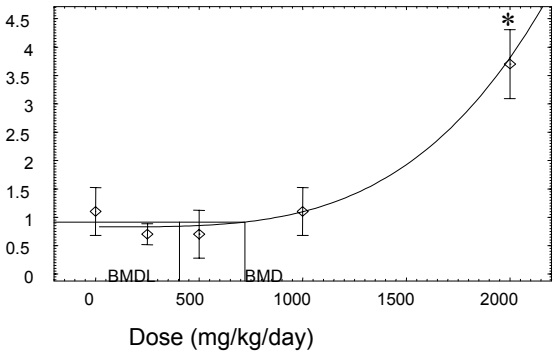
Figure 4. Dose–response curves for resorptions/female (left) and live implants/female (right) after treatment of male mice with DMMP. Drawn from Dunnick et al. [*13*] using EPA benchmark dose software

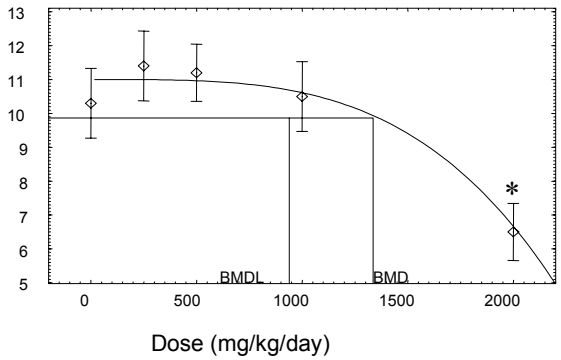
Mating after 4 weeks of treatment



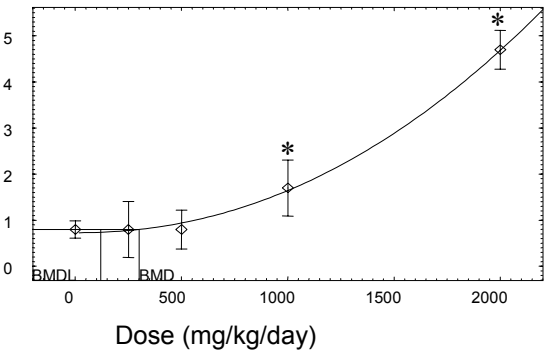


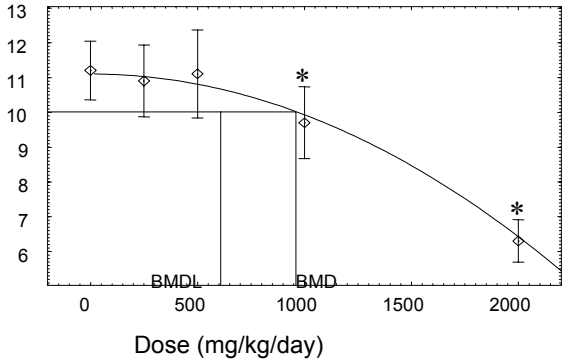
Mating after 8 weeks of treatment





Mating after 12 weeks of treatment





*Statistically different from control group by pairwise comparison. BMD = benchmark dose corresponding to a 10% response compared to control. BMDL = lower bound of the 95% confidence interval around the 10% response. Data graphed are litter means ± SD, n = 20 sires/group.

Table 4. Benchmark doses for pregnancy outcome after treatment of sire with DMMP. Calculated from Dunnick et al. [*13*] using EPA benchmark dose software.

Weeks of treatment prior to mating	Increased resorptions per female		Decreased live implants per female	
	BMD_{10}	BMDL	BMD_{10}	BMDL
4	162	37	752	511
8	721	404	1337	939
12	300	120	960	608

BMD_{10} = Benchmark dose corresponding to a 10% effect level over control. BMDL = lower bound of the 95% confidence interval around the 10% effect level. Benchmark doses are expressed as mg/kg/day.

A study by Ginsberg et al. [*19*] examined the effects on male mouse germ cells of several chemicals including DMMP **[purity not stated]** following inhalation or i.p. injection. Using histochemical assays of individual sperm following treatment, levels of sperm enzymes such as acrosin, hyaluronidase, succinic dehydrogenase (SDH), and α-glycerolphosphate dehydrogenase (αGDH) were measured in conjunction with semiquantitative sperm count and motility evaluations. ICR mice (8–10 weeks old, 5–10 per group) were treated by injection once with 500, 2500, or 5000 mg/kg DMMP or by inhalation for 5 days, 24 hours/day to 25 or 250 ppm DMMP **[DMMP dose was not otherwise quantified or estimated; assuming a respiratory rate in male mice of 1.6 m^3/kg/day [*10*], these DMMP doses are approximately 198 and 1985 mg/kg/day]**. Injection of DMMP was reported to cause significantly decreased sperm number and motility at 6 weeks following exposure, accompanied by increase in sperm without αGDH activity. After inhalation, DMMP caused a significant increase in the frequency of sperm without SDH activity after 6 weeks of treatment, but not after 2 weeks, but sperm number and motility were not affected.

Strengths/Weaknesses: Weaknesses include lack of information on general toxicity of the treatment to the males including lack of information on body weight changes, feed consumption, and clinical condition. The semi-quantitative nature of the evaluation of sperm count and motility did not produce reliable information. Details of the statistical analysis were not provided, including how many males were used at each evaluation point and whether the analysis was performed with the male as the experimental unit. The enzyme findings may have been random and their meaning in the context of sperm function was not made clear.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

A study by Mattie et al. [*20*], published in abstract, investigated the toxicity of DMMP in male Fischer 344 rats exposed to 25 or 250 ppm DMMP by inhalation for 90 days. Histologic examination of the testes by light microscopy revealed degeneration in the seminiferous tubules immediately post-exposure and for up to one year after exposure. The number of lipid droplets in cells lining the seminiferous tubules and the number of lysosomes in Sertoli cells were increased and there was dilatation of mitochondria, multinucleate giant cells, and decreased spermatogenic cells.

Strengths/Weaknesses: There is inadequate detail in the abstract for an evaluation the methods or the findings.

Utility (Adequacy) for CERHR Evaluation Process: This abstract is not adequate for the evaluation process.

4.3 Utility of data

There are no data with which to evaluate human reproductive effects of DMMP or female reproductive effects in experimental animals. There is an adequate male reproductive toxicity study [*18*] in rats using 5 doses of DMMP plus a control and reported in adequate detail to permit an evaluation. A rat histopathology study [*16*] provides supplemental information. A study in mice using 4 doses of DMMP plus a control was presented as a dominant lethal study but contains useful data on male reproductive effects [*13*]. The data base for male reproductive toxicity in experimental animals is sufficient for an evaluation.

4.4 Summary of reproductive toxicity

4.4.1 Human

No information was found on human reproductive effects of DMMP.

4.4.2 Experimental animal

4.4.2.1 Female reproductive toxicity

No information was found on female reproductive effects of DMMP.

4.4.2.2 Male reproductive toxicity

In a study on testis histopathologic effects of DMMP, Fisher 344 rats were treated with 1750 mg/kg/day 5 days/week by gavage [*16*]. Animals were killed after 3, 4, 5, 7, 9, and 12 weeks of treatment and after 12 weeks of treatment plus 14 weeks of recovery. Seven animals were studied at each time point. Lesions identified on light microscopy of the testis appeared after five weeks of treatment and consisted of PAS-positive bodies in the tubule lumen that were believed by electron microscopy characteristics to be exfoliated spermatids. There was "anachronistic spermiation," referring to the presence of Step 19 spermatids in Stage IX or X epithelia. Histopathologic effects became more severe with increasing time of exposure. Epididymal sperm density was decreased at 9 and 12 weeks of exposure. After 12 weeks of exposure plus 14 weeks of recovery, at least 80% of tubules were normal and most of the cells in the abnormal tubules were cytologically normal. Epididymal sperm density was normal after the recovery period as well. The authors believed the effects of DMMP were consistent with premature Sertoli cell shedding of germ cells. A study by different authors using a similar design was noted [*17*], but was not considered reliable due to inadequate detail and high mortality among treated animals.

In a reproductive toxicity study from NTP [*18*] used DMMP was given by gavage at 0, 250, 500, 1000, and 2000 mg/kg.day 5 days/week to male Fischer 344 rats (n=20/dose group). Males were cohabited with untreated females (2 females per male) beginning on day 84 of treatment. There was no mortality among males, and there were no clinical signs of toxicity. Males in the top dose group had a 10% decrease in body weight gain. Males in the top dose group failed to impregnate any females and had decrements in sperm count, motility, and percent normal forms. The percent resorptions per litter was increased in a dose-dependent manner at all DMMP doses, and was the most sensitive indicator of reproductive toxicity. Based on the resorption data, the LOAEL for male reproductive toxicity was 250 mg/kg/day. Benchmark dose calculations showed the BMD_{10} to be 259 mg/kg/day for the resorption data and the BMDL to be 142 mg/kg/day. Visual inspection of the dose–response curve suggested that the BMDL was probably close to the NOAEL. Effect levels and benchmark doses for other end points in this study are illustrated in Table 3.

A second study from NTP [*13*] using male $B6C3F_1$ mice involved gavage treatment 5 days/week with DMMP 0, 250, 500, 1000, or 2000 mg/kg/day. Mating trials were conduced after 4, 8, and 12 weeks of treatment and, in a separate group of animals, after 13 weeks of treatment plus 15 weeks of recovery (0,

1000, and 2000 mg/kg/day dose groups). There were 20 males in each dose group. Pairwise comparison with control showed a decrease in live fetuses per litter at 1000 and 2000 mg/kg/day for two of the on-treatment mating trials. The most sensitive end point was an increase in resorptions/litter after 4 weeks of treatment at doses of 500 mg/kg/day and above. The NOAEL in this study was 250 mg/kg/day. The authors concluded that male mice were less sensitive to DMMP toxicity than male rats based on these effect levels; however, a comparison of benchmarks dose calculations (Table 3Table 4) does not support this conclusion. Benchmark dose modeling of the resorption data after 4 weeks of treatment in mice suggests that the NOAEL may be closer to 37 mg/kg/day than 250 mg/kg/day.

Other reports [*19, 20*] were not adequate for an evaluation of male reproductive toxicity.

The data are sufficient to show that DMMP is a male reproductive toxicant in rats at oral gavage doses of 250 mg/kg/day manifest as an increase in resorptions in pregnancies sired by treated animals. Other end points of male reproductive toxicity become apparent at higher exposure levels. Data are sufficient to shown that DMMP is a male reproductive toxicant in mice at gavage doses of 500 mg/kg/day manifest as an increase in resorptions in pregnancies sired by treated animals. There are insufficient data with which to evaluate female reproductive toxicity in experimental animals or human reproductive toxicity in males or females. The data from male rats and mice are assumed relevant to humans.

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Summary and conclusions of reproductive and developmental hazards

There are no data with which to evaluate human developmental or reproductive effects of DMMP. There is a rat developmental toxicity study (cited by Rowland et al [*1*]); however, the report of this study is not available at present with an adequate level of detail for an evaluation. There are no data with which to evaluate female reproductive toxicity in any species. In male rats, DMMP produces reproductive toxicity at oral gavage doses of 250 mg/kg/day, manifest as an increase in resorptions in pregnancies sired by treated animals. Other end points of male reproductive toxicity become apparent at higher exposure levels. Based on mathematical modeling of the dose–response curve for male reproductive toxicity, the NOAEL may be approximately 142 mg/kg/day. DMMP is a male reproductive toxicant in mice at oral gavage doses of 500 mg/kg/day, manifest as an increase in resorptions in pregnancies sired by treated animals, with a NOAEL of 250 mg/kg/day based on pairwise comparison with the control group. Benchmark dose modeling suggests that the NOAEL may be as much as an order of magnitude lower.

5.2 Summary of human exposure

DMMP is used as a flame retardant, solvent, hydraulic fluid, antifoam agent, plasticizer and stabilizer, textile conditioner and antistatic agent, a preignition additive for gasoline, and an additive for solvents and low-temperature hydraulic fluids. DMMP is also used in the military to simulate nerve gas during testing of protective equipment. DMMP is also listed as a chemical weapons precursor. Current DMMP production volume is not known but in 1977, as much as 910,000 kg may have been produced in the U.S. Human exposure has not been measured. The most likely scenarios for human exposure involve production processes and military applications involving DMMP. The highest reported environmental concentration of DMMP was 1300 µg/L in groundwater at Rocky Mountain Arsenal, Colorado, although typical concentrations identified ground water have been 1 to 2 orders of magnitude lower. Assuming the use for drinking of water (2 L/day) with the highest reported level of contamination, DMMP would be ingested at a level of 2.6 mg/day (37 µg/kg/day for a 70-kg individual).

5.3 Overall conclusions

There are insufficient data with which to evaluate possible developmental or female reproductive toxicity of DMMP. Data from male rats and mice are sufficient to identify male reproductive toxicity of DMMP. In rats, the LOAEL for male reproductive toxicity is 250 mg/kg/day (oral gavage) and the BMD_{10} is 259 mg/kg/day. The BMDL of 142 mg/kg/day appears to be close to the NOAEL based on visual inspection of the dose–response curve. This BMDL is 3½ orders of magnitude higher than the worst-case scenario exposure to drinking water from a contaminated site at a military installation. In male mice, the NOAEL for reproductive toxicity is 250 mg/kg/day based on pairwise comparison with the control group. Benchmark dose modeling gives a BMDL of 37 mg/kg/day, which can be taken as a more conservative estimate of the NOAEL. This value is 3 orders of magnitude higher than the worst-case scenario exposure to drinking water from a contaminated site at a military installation. There is minimal concern for male reproductive toxicity in humans based on the assumption that human exposures are not above the worst-case scenario estimate.

5.4 Critical data needs

Data are needed on developmental toxicity and female reproductive toxicity in experimental animals. Human exposure estimates from measurements in drinking water and from monitoring of occupational sites would improve confidence in the conclusions about male reproductive toxicity concern and would contribute to evaluating concerns for developmental and female reproductive toxicity. Toxicokinetic data from exposed humans are necessary to evaluate whether there are important differences between humans and rats (or other species used in toxicity testing).

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