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HEALTH ADVISORY FOR DIMETHYL METHYLPHOSPHONATE (DMMP)

Office of Water
U.S. Environmental Protection Agency
Washington, DC 20460

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DIMETHYL METHYLPHOSPHONATE (DMMP)

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PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environment Safety and Occupational Health (OASA(IL&E)), and the U.S. Environmental Protection Agency (EPA), Office of Water (OW), Office of Science and Technology for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. A Health Advisory provides health effects guidelines and analytical methods, and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, Ten-day, Longer-term and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water, nor are they legally enforceable standards. They are not issued as official regulations and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

This report is the product of the foregoing process. Available toxicological data, as provided by the Army and as found in open literature sources, on the munitions chemicals dimethyl methylphosphonate (DMMP) have been reviewed, and relevant findings are presented in this report in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted for critical internal and external review by the EPA.

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EXECUTIVE SUMMARY

Dimethyl methylphosphonate (DMMP) is a colorless liquid used both in commercial and military applications. Commercial uses include formulations of resins, latex, coatings, and flame retardants. The military uses DMMP to simulate nerve agents for testing chemical agent detection equipment and techniques.

No studies were found in the available literature on the toxicokinetics of DMMP. However, acute, subchronic, and chronic oral toxicity studies in mice and rats indicate that DMMP is absorbed via the gastrointestinal tract.

No information was available on occupational exposure to DMMP. Potential exposure may occur primarily under occupational settings at production sites where DMMP is used as a flame retardant and as a viscosity depressant in polyester and epoxy resins. Military field uses and effluents from commercial production facilities are potential sources that could contaminate water sources. Dimethyl methylphosphonate has been detected in groundwater at one military installation at levels that range from 6.5 μg/L to 1,300 μg/L.

In a repeated insult patch test with humans, aqueous solutions of DMMP produced mild skin irritation at a concentration of 10% (v/v) and moderate to severe irritation at the 20% (v/v) level.

Acute toxicity studies indicate that DMMP exhibits little toxicity when administered to mice and rats via the oral route; LD_{50} values were >6,810 mg/kg in mice and 10,190 mg/kg in rats. Animals exhibited ataxia, tremors, unsteady gait, muscular hypertonia and hypotonia, prostration, and reductions in spontaneous motility. DMMP was slightly toxic by the intravenous route; LD_{50} values were 912 and 1,050 mg/kg in mice and rats, respectively. DMMP elicited mild skin irritation and minimal ocular irritation in rabbits. DMMP was negative as a skin sensitizer in rabbits.

Oral administration of DMMP in tapwater at 0, 1, 10, 100, or 1,000 mg/kg/day for 3 days to male and female rats produced no adverse effects on mortality, clinical signs, body weight, or food consumption. Plasma cholinesterase levels were depressed only in rats treated with 1,000

mg/kg/day; approximate reductions of 30% in males and 40-50% in females were observed over a 4-hour period as compared with control values taken at the same time. No effects on plasma cholinesterase levels were observed in rats at the other dose levels. The No-Observed-Adverse-Effect Level (NOAEL) was 100 mg/kg/day based on plasma cholinesterase activity depression.

The profile of some kidney effects observed in the DMMP-treated male rats is consistent with that of alpha-2-microglobulin (alpha$_{2\mu}$globulin) nephrotoxicity. This protein is synthesized in the liver of the male rat and has not been detected in female rats. It has not been detected in any other species, including humans. Because this protein appears to occur only in the male rat, alpha$_{2\mu}$globulin nephrotoxicity, and subsequent effects, are not expected to occur in other species. Thus, some DMMP-induced renal noncarcinogenic and carcinogenic effects were not considered to be relevant to humans.

In a 15-day study with a limited number of mice and rats (five/sex/species), histologic changes were observed in the stomachs of male mice receiving ≥1,250 mg DMMP/kg/day by oral intubation and in females receiving ≥5,000 mg/kg/day. The Lowest-Observed-Adverse-Effect Level (LOAEL) was 1,250 mg/kg/day, based on the histologic changes in the stomach of male mice. No gross pathological changes were seen in male or female rats at levels up to 15,000 mg/kg/day; however, mortality was seen in both sexes dosed at ≥5,000 mg/kg/day. Neither a NOAEL nor LOAEL was established in rats because a target organ for toxicity was not identified and histopathology was not conducted.

In a 4-week dietary study with rats, DMMP caused an increase in the protein resorption droplets (hyaline droplets) in the proximal convoluted epithelium of the kidneys of males fed diets containing 2,000, 6,000, or 20,000 ppm (178, 535, or 1,790 mg/kg/day, respectively); the effect in males was more pronounced with increasing levels of DMMP and was not observed in females. The LOAEL for this study is 6,000 ppm (535 mg/kg/day) based on increased kidney weights in female rats; the NOAEL is 2,000 ppm (178 mg/kg/day).

In a subchronic toxicity study, male and female mice were administered DMMP, by gavage, at doses ranging from 250 to 8,000 mg/kg, 5 days/week for 13 weeks. Except for mortality in both sexes at higher (4,000 or 8,000 mg/kg) dose levels, no adverse effects on body weight, clinical signs, food consumption, gross necropsy, or histopathology were observed. The stomach lesions observed in the 15-day study were not seen in this study. No compound-related cause for the mortality at the two upper levels was reported. Therefore, neither a NOAEL nor LOAEL was established.

In a subchronic toxicity study, male and female rats were administered DMMP, by gavage, at doses ranging from 250 to 4,000 mg/kg, 5 days/week for 13 weeks. DMMP, at doses $\geq$2,000 mg/kg, caused decreased survival, body weight gain, and increased liver-to-body weight ratios in males and females. Histopathology revealed kidney changes (nephrosis and hyaline droplet generation) in males at all dose levels starting at 250 mg/kg; no histopathological changes were seen in the kidneys of females. The kidney lesions are considered to be associated with $alpha_{2\mu}$globulin found only in male rats and therefore not appropriate for determining a LOAEL. The minimal to mild hypospermatogenesis seen in the testes of treated rats was not dose dependent. Based on increased relative liver weights in males and females, the LOAEL is 2,000 mg/kg/day and the NOAEL is 1,000 mg/kg/day.

In a chronic toxicity study, DMMP was administered, by gavage, at doses of 0, 1,000, or 2,000 mg/kg, 5 days/week for 103 weeks, to male and female mice. Survival was decreased in males at both dose levels and in females at the high dose because of fighting in males and dosing errors in females. Mean body weights were decreased in both sexes in the high-dose group, and the incidence of hepatocytomegaly was increased in treated males at both levels compared with vehicle controls. No increases in nonneoplastic liver lesions were seen in female mice. This study was judged to be inadequate owing to poor survival; therefore, neither a NOAEL nor LOAEL was established.

DMMP was administered, by gavage, at doses of 0, 500, or 1,000 mg/kg to male and female rats, 5 days/week for 103 weeks. Survival and mean body weights were decreased in both sexes at the high dose. Treatment-related kidney changes observed in males at both levels included nephropathy and calcification of the renal papilla. No kidney lesions were seen in females.

Discounting the kidney lesions observed in males due to alpha$_{2\mu}$globulin, the LOAEL is 1,000 mg/kg/day and the NOAEL is 500 mg/kg/day based on decreased body weights in both sexes.

DMMP was found to be noncarcinogenic to female F344/N rats and male or female B6C3F$_1$ mice. However, on the basis of poor survival in both sexes (see discussion above), the study with mice was judged to be inadequate to evaluate the carcinogenic potential of DMMP.

DMMP was carcinogenic to male F344/N rats, as shown by an increased incidence of mononuclear cell leukemia, hyperplasia of the transitional epithelium, and transitional cell papilloma or carcinoma (combined) of the kidneys following oral administration at doses of 500 or 1,000 mg/kg, 5 days/week for 103 weeks. Increased incidences of tubular cell hyperplasia and adenocarcinomas were considered to be related to alpha$_{2u}$ globulin and not relevant to estimating human cancer risk. DMMP is classified in Group C: Possible Human Carcinogen.

DMMP exhibited mutagenicity by inducing forward mutations in mouse lymphoma cells in the absence of metabolic activation and sister chromatid exchanges in Chinese hamster ovary (CHO) cells with and without activation. It gave limited evidence of clastogenicity in CHO cells in the absence of metabolic activation. *In vivo*, DMMP induced sex-linked recessive lethal mutations in *Drosophila*. DMMP was nonmutagenic in bacterial mutagenicity tests in several strains of *Salmonella*, did not increase neoplastic transformations in BALB/c 3T3 cells, and did not induce reciprocal translocations in *Drosophila in vivo*. DMMP gave positive results in dominant-lethal assays in mice and rats.

DMMP was shown to be toxic to the male reproductive system. DMMP induced dose-dependent decreases in sperm motility, sperm counts, and the male fertility index in male rats following oral administration at doses ranging from 250 to 2,000 mg/kg, 5 days/week for 90 days. Histologic changes were observed in the testes, epididymis, and prostate glands. DMMP altered reproductive functions at all dose levels by causing a decrease in the number of live fetuses per litter and an increase in the percentage of resorptions. The LOAEL for reproductive effects was the lowest dose tested, 250 mg/kg/day.

In an inhalation study, exposure of male rats to DMMP vapors at concentrations of 25 or 250 ppm continuously for 90 days induced histologic changes in the testes and epididymis.

DMMP was not found to be teratogenic. No developmental effects were observed in mice following oral administration at 4,175 mg/kg/day on gestation days 6-13. In another study, when administered to rats during gestation days 6-15, DMMP induced minimal fetotoxicity (reduced fetal weight and delayed skeletal maturation) only at maternally toxic (1,000 or 2,000 mg/kg/day) doses. No maternal toxicity, embryotoxicity, or fetotoxicity was observed at the 100-mg/kg/day dose. Fetal anomalies were within the historical range and were not considered to be compound related.

No delayed neurotoxicity was seen in hens observed for 21 days following oral administration of 1,999 or 3,998 mg DMMP/kg initially and after 21 days. However, birds exhibited acute toxicity, including ataxia, curved position, apathy or sedation, and fluttered feathers. In another study, a single intraperitoneal injection of 50 mg DMMP/kg daily for 10 days produced no delayed neurotoxicity in hens.

Based on increased relative liver weights in rats administered DMMP via gavage, the Longer-term Health Advisory (HA) for exposure in a 10-kg child is 2 mg/L (2,000 μg/L). In the absence of adequate animal data to determine a One-day or Ten-day Health Advisory, the Longer-term HA for a 10-kg child, 2 mg/L (2,000 μg/L), is used as a conservative estimate of the One-day or Ten-day HA. The Longer-term HA for an adult is 6 mg/L (6,000 μg/L). The Lifetime HA of 0.1 mg/L (100 μg/L) is for a 70-kg adult, based on a Drinking Water Equivalent Level (DWEL) of 7 mg/L (7,000 μg/L). The DWEL is based on a Reference Dose (RfD) of 0.2 mg/kg/day. The RfD is based on a LOAEL of 179 mg/kg/day, where the effect was increased resorptions in female rats that were impregnated by males that had been gavaged with DMMP for 13 weeks.

DMMP is classified as Group C: possible human carcinogen. Its cancer potential was considered in the development of the lifetime HA. The estimated cancer risk associated with lifetime exposure to drinking water containing DMMP at the level of the DWEL (7 mg/L) is approximately 1×10^{-3} (the actual calculated value is 7×10^{-4}). Derived by the linearized multistage

extrapolation method, the slope factor that was derived from oral exposure studies (oral slope factor, q_1*) for DMMP is 5×10^{-3} $(mg/kg/day)^{-1}$ and the drinking water unit risk is 1×10^{-7} per $\mu g/L$. At concentrations of 700 $\mu g/L$, 70 $\mu g/L$, and 7 $\mu g/L$, excess cancer risks are estimated to be 10^{-4}, 10^{-5}, and 10^{-6}, respectively, based on equivocal evidence of carcinogenicity. For comparison, drinking water concentrations associated with a 10^{-6} cancer risk are 2 $\mu g/L$ by the one hit model and less than 1×10^{-3} $\mu g/L$ for the multihit, probit, logit, and Weibull models.

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Water (OW), provides information on the health effects, analytical methodology, and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State, and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. HAs are subject to change as new information becomes available.

HAs are developed for One-day, Ten-day, Longer-term (approximately 7 years, or 10% of an individual's lifetime), and Lifetime exposures based on data describing noncarcinogenic endpoints of toxicity. For those substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linear multistage model with 95% upper confidence limits. This provides a low-dose estimate of cancer risks to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated values. Excess cancer risk estimates may also be calculated using the one-hit, Weibull, logit, and probit models. Current understanding of the biological mechanisms involved in cancer does not suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

II. GENERAL INFORMATION

Dimethyl methylphosphonate (CAS No. 756-79-6), commonly known as DMMP, is a low-viscosity, colorless liquid that is miscible in a variety of industrial solvents and miscible to soluble in water (Aldrich, 1988; U.S. EPA, 1983). It contains high levels of phosphorus (25% by weight), and is therefore highly efficient, on a weight basis, as a flame retardant. The general chemical and physical properties of DMMP are presented in Table II-1.

DMMP is used for a variety of purposes in both commercial and military activities. Fyrol DMMP, an additive, high-phosphorus flame retardant, may be used in formulations where its good solvent power, low viscosity, and high phosphorus content are desired. Recommended applications include use in epoxy resins, acrylic latexes, unsaturated polyester, urethane coatings, urethane rigid foam, and vinyl copolymers (Stauffer, 1980, as cited in U.S. EPA, 1983).

DMMP is also a nerve gas "simulant" (i.e., structural analog of nerve agents but with much lower acute toxicity). DMMP is structurally similar to the nerve gas GB (isopropyl methylphosphonofluoride) and is used as a simulant in testing methods for trapping and destruction of nerve gas in air (Berkowitz et al., 1978). Nerve gas simulants are used in the military to test equipment such as chemical protective masks and chemical detection systems (Dunnick et al., 1984a). DMMP has been used by the U.S. Armed Forces in experimental situations (Dunnick et al., 1983), which have included releasing DMMP canisters at geographically isolated proving grounds and then monitoring the dispersion of the plume.

Other applications of DMMP, which revolve around its viscosity-depressant effect and its volatility, include use as a solvent, reaction medium, or catalyst; methylating reagent; preignition additive for gasoline; antifoam agent; plasticizer and stabilizer; textile conditioner and antistatic agent; additive in solvents and low-temperature hydraulic fluids; and chemical intermediate in the production of other flame retardants. DMMP is also used in heavy metal extraction and solvent separation (Kirk-Othmer, 1980; U.S. EPA, 1983).

Table II-1. General Chemical and Physical Properties of Dimethyl Methylphosphonate

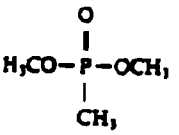
Property	Value	Reference
CAS Registry Number	756-79-6	Aldrich (1988)
Synonyms	Dimethyl methanephosphonate; DMMP; Fyrol DMMP; Methanephosphonic acid dimethyl ester; Phosphonic acid, methyl-, dimethyl ester	U.S. EPA (1983)
Molecular Formula	$CH_3P(O)(OCH_3)_2$	Aldrich (1988)
Molecular Weight	124.08	Aldrich (1988)
Chemical Structure		
Physical State	Colorless liquid	Albright and Wilson (1982)
Melting Point	<50°C	Stauffer (1980a)
Boiling Point	181°C	Aldrich (1988)
Density	1.145	Aldrich (1988)
Vapor Pressure	<0.1 torr @ 20°C 1.0 torr @ 30°C 1.1 torr @ 10°C 2.4 torr @ 25°C 5.4 torr @ 37.8°C 10.0 torr @ 62.5°C 20.0 torr @ 65°C	U.S. EPA (1983)
Solubility		
Water	Soluble	Albright and Wilson (1982)
	Soluble	U.S. EPA (1983)
Solvents	Miscible in alcohol, ether, benzene, acetone, carbon tetrachloride; insoluble in heavy mineral oil.	Albright and Wilson (1982)

Table II-1 (cont.)

Property	Value	Reference
Refractive Index	1.411	U.S. EPA (1983)
Log P octanol/water (estimated)	0.46	U.S. EPA (1983)
Log octanol/water partition coefficient (K_{ow}) (Fragment method)	-1.88	PMRMA (1990)

DMMP is produced by the molecular rearrangement of trimethylphosphite, in a reaction catalyzed by a halogenated organic compound (Kirk-Othmer, 1980; U.S. EPA, 1983). Mobil Chemical Company in Charleston, SC, is the only current U.S. manufacturer of DMMP listed in the available literature. Production estimates for 1977 were reported to be from 100,000 to 1 million pounds (U.S. EPA, 1983).

III. SOURCES OF EXPOSURE

No information concerning occupational or environmental DMMP exposure concentrations was found in the available literature. However, considering its low acute toxicity and irritancy, and its high vapor pressure, little warning of exposure to DMMP may be given; therefore, there is a potential for high exposure (U.S. EPA, 1983).

Potential water contamination could occur from military and commercial sources. When DMMP is used by the military as a nerve gas simulant, it may be released to the environment (U.S.EPA, 1983). During production, DMMP may be released to the environment in waste effluent. Quantitative data on military or commercial environmental releases is limited. In 1989, DMMP was detected in groundwater at the Rocky Mountain Arsenal (RMA), Colorado, at concentrations that ranged from 6.5 μg/L to 1,300 μg/L (PMRMA, 1991). In 1991, DMMP was detected in groundwater at two RMA sites at concentrations of 490 μg/L and 760 μg/L, respectively.

DMMP has high water solubility and volatility. It evaporates slowly from aqueous solutions and, therefore, should enter air and water in the environment (U.S. EPA, 1983).

IV. ENVIRONMENTAL FATE

Little information is available concerning the environmental fate of DMMP. The dialkyl alkylphosphonates (e.g., DMMP) are characterized as neutral esters that undergo few chemical reactions. The group is resistant to oxygen, oxidizing agents, and reducing agents (U.S. EPA, 1983). DMMP is, however, subject to hydrolysis and ultraviolet (uv) photolysis.

Christol et al. (1968) studied the hydrolysis of phosphonates by alkali in aqueous dioxane. The pK_a values at 20°C in water and water:dioxane (50:50) are reported to be 2.37 and 3.11, respectively. Mabey and Mill (1978) conducted a critical literature review of the hydrolysis of organic compounds in water under environmental conditions. Their objective was to use this data to estimate persistence in freshwater aquatic systems. The hydrolysis of pentavalent phosphorus compounds (e.g., DMMP) occurs by nucleophilic displacement at the phosphorus- or carbon-oxygen bond with the formation of leaving groups. Phosphorus-oxygen cleavage is more common with base hydrolysis; as pH is lowered, a decrease in the overall rate of hydrolysis is observed. Estimated environmental acid and base hydrolysis rate constants for DMMP were $\approx 1.36 \times 10^{-9}$ and 2.5×10^{-3} $M^{-1}s^{-1}$, respectively. The reported environmental half-life is estimated to be 88 years at 298°K and pH 7. Extrapolations from studies of DMMP hydrolysis to reaction temperatures of 80, 90, and 98°C indicate a half life of 13 years at 15°C (Berkowitz et al., 1978).

Laser-induced photodestruction of DMMP was studied by Radziemski (1981). Experiments were carried out with the liquid and gaseous forms of DMMP. Liquid DMMP irradiated (time not reported) at 266 nm had a carbon atom quantum yield of 1.0%, and gaseous DMMP irradiated at 193 nm for 30 minutes had a yield of 8.4%. Dissociation products were simple hydrocarbon gases, CO, CO_2, H_2, and H_2O.

The average half-life of DMMP on Cheat grass, in soil, and in water were estimated in environmental chambers (U.S. EPA, 1983). Corresponding values for the above media were a few hours, 12.4 days, and 1 to 30 weeks. The half-life values (not given) indicated a more rapid disappearance at higher temperatures. Various environmental fate processes may have been represented

(hydrolysis, photolysis, biodegradation), and the major fate mechanism may have been volatilization.

Based on the available data, DMMP would be expected to be stable in aqueous environments. Photolysis is not expected to be a major fate, and hydrolysis is extremely slow; therefore, DMMP is expected to be moderately persistent in the environment.

V. TOXICOKINETICS

No information was found in the available literature on the toxicokinetics (absorption, distribution, metabolism, and excretion) of DMMP in animals or humans. However, acute, subchronic, and chronic oral toxicity studies in mice and rats indicate that DMMP is absorbed via the gastrointestinal tract.

VI. HEALTH EFFECTS

A. HUMANS

In a repeated-insult patch test with humans, a series of nine 24-hour occluded induction patches of DMMP were applied to 50 subjects (25 males and 25 females). DMMP (0.05 mL) was tested initially as a 20% (v/v) aqueous solution; after the sixth application, the dose was reduced to the 10% (v/v) level for the remaining applications. The induction phase was followed 12 days later by a single challenge with DMMP to detect sensitization. At the 20% level, DMMP induced moderately severe skin irritation by the sixth application; at the 10% level, DMMP caused very slight to mild skin irritation. One subject showed a Grade 4 irritation after the challenge dose. The reaction did not persist beyond 24 hours; consequently, this was not considered to be a sensitizing response. DMMP was classified a moderate irritant at a concentration of 20% and a mild irritant at 10%. DMMP was classified as a non-skin-sensitizer (Ciba-Geigy, 1976a).

B. ANIMAL EXPERIMENTS

1. Short-term Exposure (<4-week studies)

a. Acute

The acute toxicity data are summarized in Table VI-1. A summary of short-term studies for DMMP is presented in Table VI-2. DMMP exhibits little toxicity when administered to rats and mice via the oral route; the acute oral LD_{50} values are 10,190 mg/kg in rats and >6,810 mg/kg in mice.

In an oral toxicity study, groups of five male and five female Tif:RAIf rats were given a single dose, by gavage, of undiluted DMMP at 1,000, 3,000, 4,500, 6,000, 8,000, 10,000, or 15,000 mg/kg, and the animals were observed for 15 days. No deaths were seen in rats dosed at 1,000 to 6,000 mg/kg. Mortality occurred in one female dosed at 8,000 mg/kg, in one male and four females dosed at 10,000 mg/kg, and in all animals dosed at 15,000 mg/kg. Rats at all dose levels exhibited ataxia, muscular hypotonia, prostration, hypoventilation, and reduced spontaneous motility. Pharmacotoxic signs lasted

Table VI-1. Acute LD_{50} Values for DMMP in Laboratory Animals

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg)	Reference
Mouse	$B6C3F_1$	M,F	Oral	Corn oil	>6,810	NTP (1987)
Mouse	Tif: MAGf	M,F	Intravenous	Distilled water	912	Ciba-Geigy (1977a)
Rat	Tif: RAIf	M,F	Oral	None	10,190 (9,140-11,360)[a]	Ciba-Geigy (1976b)
Rat	F344/N	M,F	Oral	Corn oil	>6,810	NTP (1987)
Rat	--[b]	M,F	Oral	--	>5,000	Stauffer (1983)
Rat	Tif: RAIf	M,F	Intravenous	Distilled water	1,050 (940-1,180)	Ciba-Geigy (1977a)
Rat	Tif: RAIf	M,F	Dermal	Undiluted	>4,600	Ciba-Geigy (1976c)
Rabbit	--	--	Dermal	--	>2,000	Stauffer (1983)

[a]The values in parenthesis are 95% confidence limits.
[b]Data not reported.

Table VI-2 Summary of Studies: Short-term Exposure of Animals to DMMP

Reference	Species	Dose (mg/kg/day)[a]	Route	Duration (days)[a]
Ciba-Geigy, 1976b	Rat	1,000 - 15,000	Oral	LD_{50}
Stauffer, 1983	Rat	5,000	Oral	LD_{50}
	Rabbit	2,000	Dermal	LD_{50}
NTP, 1987	Rat, Mouse	1,470 - 6,810	Oral	LD_{50}
Ciba-Geigy, 1977a	Rat	600 - 1,500	Intravenous	LD_{50}
	Mouse	100 - 930	Intravenous	LD_{50}
Ciba-Geigy, 1977b	Rat	1,355 ± 107 mg/m³ (m) 2,589 ± 176 mg/m³ (f)	Inhalation	1(14)[b]; LC_{50}
Ciba-Geigy, 1976c	Rat	4 mL/kg	Dermal	1(8)
Ciba-Geigy, 1978a	Rat	1, 10, 100, 1,000	Oral	3
Ciba-Geigy, 1976d	Rabbit	278 - 337	Dermal	1(3)
Ciba-Geigy, 1976e	Rabbit	0.5mL (1, 10, 20, 50% sol)	Dermal	1(3)
Ciba-Geigy, 1976f	Rabbit	0.1 g	Ocular	1(14)
Ciba-Geigy, 1976g	Guinea Pig	0.5 mL (10% sol)	Dermal	1(14)
NTP, 1987	Rat	0, 1, 250, 2,500, 5,000, 10,000, 15,000	Oral	15
	Mouse	0, 1, 250, 2,500, 5,000, 10,000, 15,000	Oral	15
Ciba-Geigy, 1977c	Rat	0, 178, 535	Diet	30

[a]Unless otherwise stated.
[b]n(n) = days dosed (total days).

for >6 hours and were not seen after 24 hours. Rats receiving the high dose exhibited cyanosis in addition to all the above signs and died within 24 hours (Ciba-Geigy, 1976b). The acute oral LD_{50} in male and female rats was reported to be >5,000 mg/kg and a dermal LD_{50} in rabbits was 2,000 mg/kg; study details (species, number of animals, effects, etc.) were not provided (Stauffer, 1983).

The National Toxicology Program (NTP) conducted single-dose toxicity studies in male and female $B6C3F_1$ mice and F344/N rats. Groups of five males and five females of each species were administered DMMP in corn oil, by gavage, at doses of 1,470, 2,150, 3,160, 4,640, or 6,810 mg/kg. Animals were observed for 14 days. Mortality occurred in two of five female mice at the highest dose; no other deaths were seen. Mice at the two highest dose levels exhibited transitory inactivity 1 to 4 hours postdosing. In rats, no treatment-related deaths occurred at doses up to 6,810 mg/kg. Clinical signs observed 1 to 4 hours postdosing in all but the lowest dose (1,470 mg/kg) included transitory inactivity, unsteady gait, and prostration (NTP, 1987).

Ciba-Geigy (1977a) studied the toxicity of DMMP following intravenous (iv) injection to Tif:MAGf mice and Tif:RAIf rats. Groups of five male and five female mice were given a single iv injection of DMMP diluted in distilled water at 100, 300, 600, 800, 900, 910, 920, or 930 mg/kg. DMMP was slightly toxic by this route; the LD_{50} in mice was 912 mg/kg. Mice at all dose levels exhibited ataxia, roughness of the hair coat, restlessness, humpback, tremors, ventricumbency, labored respiration, muscular hypertonia, exophthalmos, twitching muscles, salivation, lacrimation, convulsions, and reduced spontaneous motility.

In the study with rats, groups of five males and five females were given a single iv injection of 600, 800, 1,000, 1,200, or 1,500 mg/kg DMMP diluted in distilled water. DMMP was slightly toxic; the LD_{50} was 1,050 mg/kg. Mortality occurred in all but the lowest dose. Clinical signs of toxicity observed in rats at all dose levels were similar to those observed in mice (Ciba-Geigy, 1977a).

In an inhalation study, groups of 10 male and 10 female Tif:RAIf (SPF) rats were exposed, nose-only, to aerosol concentrations of 1,355 $\pm$ 107 or

2,589 ± 176 mg DMMP/m^3 for 4 hours and observed for 14 days. No mortality was seen at either concentration. Rats at both concentrations exhibited convulsions, exophthalmos, lateral or ventral position, and ruffled fur. These signs became more pronounced with increased concentration, but the rats recovered within 6 to 7 days. Necropsy revealed hemorrhages in the lungs and "congested organs" (Ciba-Geigy, 1977b).

In a dermal toxicity study, five male and five female Tif:RAIf (SPF) rats received a single 24-hour dermal application of undiluted DMMP (4.0 mL/kg) on their shaved backs. No deaths were seen during an 8-day observation period, and the animals exhibited neither local effects nor systemic toxicity (Ciba-Geigy, 1976c).

Ciba-Geigy (1978a) assessed the effects of DMMP on plasma cholinesterase activity in Sprague-Dawley rats. Groups of five males and five females were orally administered, by gavage, DMMP mixed in tapwater at doses of 1, 10, 100, or 1,000 mg/kg body weight (bw) once daily for 3 days. The dose volume was 10 mL/kg. A control group of 10 males and 10 females received only tapwater at the same rate. Blood samples for plasma cholinesterase measurements were taken pretest, and 0.5, 1, 2, and 4 hours after the third administration. All survivors were sacrificed after 3 days, but no necropsies were performed. No mortality occurred, and no differences were observed in daily body weight or food consumption. Rats showed no signs of clinical toxicity. Plasma cholinesterase levels were depressed only in rats treated with 1,000 mg/kg/day DMMP. Approximate reductions of 30% in males and 40 to 50% in females were observed over a 4-hour period as compared with control values taken at the same time. No effects on plasma cholinesterase levels were observed in rats at the other dose levels. In this study, the NOAEL of DMMP for plasma cholinesterase depression was 100 mg/kg/day.

b. Primary irritation and skin sensitization

DMMP (0.5 mL) elicited minimal irritation when applied to the intact skin and slight irritation when applied to the abraded skin of three male and three female Russian rabbits (weighing 1.7 to 2.0 kg) under 24-hour occlusive patch conditions. Assuming 100% purity of the test compound, the dose ranged

from approximately 278 mg/kg to 337 mg/kg. No irritation was noted 72 hours postapplication (Ciba-Geigy, 1976d).

In a primary skin irritation test, groups of two albino rabbits (sex not reported) received a dermal application of 0.05 mL of DMMP at concentrations of 1, 10, 20, or 50% in tapwater. Test sites were scored at 24 and 72 hours. DMMP was a nonirritant at 1, 10, or 20%. A slight irritation was noted at the 50% level (mean primary irritation score = 0.8/8.0) after 24 hours, which disappeared 72 hours postapplication (Ciba-Geigy, 1976e).

DMMP caused minimal ocular irritation when instilled (0.1 g) into the conjunctival sac of the left eye of three male and three female rabbits for 1 minute before being washed off. The irritation consisted of conjunctival and corneal reaction (Ciba-Geigy, 1976f).

In a skin sensitization test, DMMP was shown to be a non-skin-sensitizer. Ten albino guinea pigs were exposed to 0.5 mL of a 10% (w/v) aqueous solution of DMMP for 5 hours followed 2 weeks later with a challenge dose of DMMP (Ciba-Geigy, 1976g).

c. Subacute

The National Toxicology Program (NTP, 1987) conducted a subacute (15-day) oral toxicity study of DMMP in male and female $B6C3F_1$ mice and F344/N rats. Groups of five males and five females of each species were administered DMMP in corn oil, by gavage, at 0, 1,250, 2,500, 5,000, 10,000, or 15,000 mg/kg/day for 15 consecutive days. The 15,000-mg/kg dose for mice was administered neat, i.e., without vehicle. All animals were observed twice daily, and body weight measurements were taken on study days 0 and 15 for mice and on day 0 for rats. Necropsies were performed on all animals; the stomachs of all mice underwent histopathological examination.

Mortality occurred in 4/5 male and 5/5 female mice at the 10,000-mg/kg/day level and in all mice at the 15,000-mg/kg/day level. Mice that died at these levels exhibited inactivity, prostration, and shallow breathing before death. Histopathology revealed increased incidence of stomach (fore- or glandular section not specified) lesions (gastropathy, gastritis,

hyperkeratosis, or epithelial ulceration) in females in the three highest dose groups and in males (squamous atrophy, gastropathy, or gastritis) in all dose groups (Table VI-3). Treatment-related deaths were seen in 4/5 male rats and 4/5 female rats dosed at 5,000 mg/kg and in all rats in the two highest dose groups. Rats treated at ≥2,500 mg/kg/day were inactive after dosing, and animals treated at 5,000 or 10,000 mg/kg/day had unsteady gait. No treatment-related gross pathology was seen.

In this study, based on stomach lesions, the LOAEL was 1,250 mg/kg/day for male mice and 5,000 mg/kg/day for female mice. Since histopathology was not performed and no target organs were identified in the rats, a LOAEL or NOAEL cannot be established.

The toxic effects of subacute (4-week) dietary administration of DMMP were evaluated in Sprague-Dawley rats (Ciba-Geigy, 1977c). DMMP was included in the daily diets of five rats/sex/dose for a 1-month period at the following concentrations: 0, 2,000, or 6,000 ppm (0, 178, or 535 mg/kg/day, respectively). An additional group of 10 male and 10 female rats were fed diets containing 20,000 ppm (1,790 mg/kg/day) for the same period; 10 rats (5 males and 5 females) from this group were retained for 4 weeks on a control diet after cessation of treatment (recovery group). Animals were observed daily, and body weights and food consumption were measured weekly. Ophthalmological examinations were performed on all rats prior to initiation of treatment and on five males and five females from the 20,000-ppm group during study weeks 5 and 8. A battery of hematological and clinical chemistry tests and urinalyses was conducted on all rats at study termination. All animals underwent gross necropsy, and the adrenals, kidneys, heart, brain, liver, and gonads were weighed. All major organs underwent histopathological examination.

No deaths were seen during the study at any dietary level. Body weights and food consumption were within normal limits, and no signs of toxicity were observed. Hematology, clinical chemistry, urinalysis, and eye exams revealed no treatment-related effects. At the 20,000-ppm level, kidney weights in both sexes and liver weights in males were significantly ($p < 0.05$) increased on both an absolute and relative basis. At the 6,000-ppm level, kidney weights

Table VI-3. Number of Mice With Stomach Lesions After Oral Administration of DMMP for 15 Consecutive Days[a]

Lesion	Dose (mg/kg) 0	1,250	2,500	5,000	10,000	15,000
Males						
No. of animals examined	5	5	5	5	5	5
Insufficient tissue for evaluation	0	0	1	0	1	0
Autolysis	0	0	0	0	1	0
Epithelial necrosis	0	0	1	0	1	0
Submucosal necrosis	0	0	0	0	1	0
Squamous atrophy	0	0	0	0	1	3
Hyperplastic gastropathy	0	1	0	1	0	1
Hyperplastic gastritis, acute/chronic	0	0	1	0	2	0
Hyperkeratosis	0	0	0	0	0	0
Epithelial ulceration	0	0	0	0	0	0
Females						
No. of animals examined	5	5	5	5	5	5
Autolysis	0	0	0	0	0	0
Epithelial necrosis	0	0	0	0	0	0
Submucosal necrosis	0	0	0	0	0	0
Squamous atrophy	0	0	0	0	0	0
Hyperplastic gastropathy	0	0	0	2	0	1
Hyperplastic gastritis, acute/chronic	0	0	0	1	3	0
Hyperkeratosis	0	0	0	0	0	2
Epithelial ulceration	0	0	0	0	3	0

[a]Animals with more than one type of stomach lesion may have been counted more than once. Since individual data were not reported, the reviewers were unable to determine which animals were counted more than once.

SOURCE: NTP (1987).

in females were increased ($p < 0.05$) on both an absolute and relative basis. Histopathology showed a moderate increase in protein resorption droplets (hyaline droplets) in the proximal convoluted epithelium of the kidneys in male rats in the 2,000-ppm group. No other evidence of kidney damage was seen. Markedly and moderately increased protein resorption was also observed in the 6,000- and 20,000-ppm groups. No protein resorption droplets were seen in the kidneys of rats fed a control diet. No other treatment-related changes were seen in males or females. Rats in the recovery group showed no adverse effects following cessation of treatment. Because of the relationship between the presence of $alpha_{2\mu}$globulin in male rats, and the development of kidney lesions (Baetcke et al., 1991), the LOAEL is not based on male kidney effects. Because this protein appears to occur only in the male rat, $alpha_{2\mu}$globulin associated renal tubular cancers are not expected to be relevant to humans. The LOAEL for this study is 6,000 ppm (535 mg/kg/day) based on increased absolute and relative kidney weights in female rats. The NOAEL is 2,000 ppm (178 mg/kg/day).

2. Longer-term Exposure

a. 13-Week studies

A summary of longer-term studies for DMMP is presented in Table VI-4. NTP (1987) conducted 13-week oral toxicity studies in male and female $B6C3F_1$ mice and F344/N rats. DMMP in corn oil was administered, by gavage, to the animals 5 days/week for 13 weeks. All animals were observed daily, and body weights and food consumption were recorded weekly. Necropsies were performed on all animals, and histopathological examination of all major organs was conducted on all animals that died, on vehicle controls, on high-dose group rats, and on two high-dose group mice.

In the study with mice, groups of 10 males and 10 females received DMMP at 0, 250, 500, 1,000, 2,000, 4,000, or 8,000 mg/kg/day. Only seven males and six females were dosed at the 8,000-mg/kg level. Treatment-related deaths occurred in nine males and nine females dosed at the 4,000-mg/kg level and in seven males and five females dosed at the 8,000-mg/kg/day level. DMMP had no effect on body weight, food consumption, clinical signs, gross pathology, or histopathology. The stomach lesions observed in the 15-day study were not

Table VI-4 Summary of Studies: Longer-term Exposure of Animals to DMMP

Reference	Species	Dose (mg/Kg/day)[a]	Route	Duration (days)[a]
NTP, 1987	Rat	0, 250, 500, 1,000 2,000, 4,000, 8,000	Oral	90
	Rat	0, 250, 500, 1,000 2,000, 4,000, 8,000	Oral	90
	Mouse	0, 250, 500, 1,000 2,000, 4,000, 8,000	Oral	90
Dunnick, 1984a	Rat	0, 250, 500, 1,000, 2,000	Oral	90
Chapin, 1984	Rat	0, 1,750	Oral	12(14)[b]
Mattie, 1987	Rat	127, 1269 mg/m^3	Inhalation	90
Hardin, 1987	Mouse	4, 175	Oral	1 Generation 6-13 Gestation
Ciba-Geigy, 1978b	Rat	100, 1,000, 2,000	Oral	1 Generation 6-15 Gestation
	Rat	2,000	Oral	1 Generation 6-15 Gestation
	Rat	2,500	Oral	6-10 Gestation
Dunnick, 1984b	Mouse	0, 250, 500, 1,000, 2,000	Oral	90 (105)
Dunnick, 1988, NTP, 1987	Rat	0, 500, 1,000	Oral	103 Weeks
	Mouse	0, 1,000, 2,000	Oral	103 Weeks
Ciba-Geigy, 1978d	Chicken	1,999, 3,998	Oral	2(42)[c]
Hollinghaus, 1981	Chicken	50	Intraperitoneal	10

[a]Unless otherwise stated.
[b]n(n) = days dosed (total days).
[c]Doses were administered on days 1 and 21 and chickens were observed through 42 days.

seen in this study. Histopathology did not identify target tissues. No compound-related cause for the high mortality at the two upper levels was reported. Therefore, in the absence of any other data, a NOAEL or LOAEL cannot be determined.

In the study with rats, groups of 10 males and 10 females received DMMP at 0, 250, 500, 1,000, 2,000, 4,000, or 8,000 mg/kg/day. Treatment-related mortality occurred in rats dosed at ≥2,000 mg/kg/day; deaths observed at lower levels were due to technical error during the dosing regimen. The final body weights of rats in the 4,000-mg/kg group were more than 10% lower than those of the vehicle controls. Rats dosed at 8,000 mg/kg/day had rough hair coats, and decreased activity was seen in rats dosed at both 4,000 and 8,000 mg/kg/day. Histopathology revealed lesions in the kidneys (nephrosis), testes (atrophy and degeneration), and epididymis (degeneration) of treated males, and atrophy of the salivary glands in males and females (Table VI-5) (NTP, 1987).

A second 13-week study was conducted with rats because of dosing errors in the first study (NTP, 1987; discussed above). Groups of 10 males and 10 females were given, by gavage, DMMP in corn oil at 0, 250, 500, 1,000, 2,000, or 4,000 mg/kg/day, 5 days/week for 13 weeks. Experimental design was identical to the first study. All rats at the 4,000-mg/kg/day level died during week 1, and six males and three females at the 2,000-mg/kg/day level died during the study. The final body weights of rats dosed at the 2,000-mg/kg/day level were 6% lower than those of the vehicle controls for males and 7% lower for females. The liver-to-body weight ratio was significantly ($p < 0.01$) increased in rats in this group. No clinical signs of toxicity were observed. Histopathology revealed an increased incidence of kidney nephrosis in treated males compared with vehicle control rats; however, the severity of the lesions was similar between the treated and vehicle control rats. The kidney lesions were not considered to be life threatening. Accumulation of hyaline droplets was observed in the convoluted tubules of the renal cortex in treated males at all dose levels. The minimal to mild hypospermatogenesis seen in the testes of treated males was not dose-dependent (Table VI-6) (NTP, 1987). Because of an association with $alpha_{2\mu}$globulin (Baetcke et al., 1991), kidney lesions that occurred in males at all dose levels were not considered for a LOAEL. The results of this study indicated a Frank-Effect-Level (FEL)

Table VI-5. Histopathological Changes in Rats After Oral Administration of DMMP for 13 Weeks (Study 1)

Site/lesion	Vehicle control	Dose (mg/kg) 250	500	1,000	2,000	4,000	8,000
Males							
Kidney nephrosis	0/10[a]	N[b]	N	N	0/10	0/10	4/10[c]
Testes							
Atrophy	0/10	1/10	2/10	2/10	3/9	10/10	2/9[c]
Degeneration	0/10	0/10	0/10	0/10	0/9	0/10	2/9[c]
Epididymis							
Degeneration	0/10	0/10	0/10	0/10	0/10	9/10	3/9[c]
Salivary gland							
Atrophy	0/10	N	N	N	0/10	0/10	6/10[c]
Females							
Salivary gland							
Atrophy	0/10	N	N	0/2	0/10	0/10	4/10

[a] No. of rats with changes/No. of rats examined.
[b] Not examined
[c] Nine of 10 rats dead by week 2; 10th rat dead by week 5.

SOURCE: NTP (1987).

Table VI-6. Histopathological Changes in Rats After Oral Administration of DMMP for 13 Weeks (Study 2)

Site/lesion	Vehicle control	Dose (mg/kg) 250	500	1,000	2,000	4,000
Males						
Kidney						
Nephrosis	2/10[a]	9/10	10/10	10/10	5/9	0/10[b]
Hyaline droplet degeneration	0/10	8/10	10/10	10/10	4/9	3/10
Hemorrhage	0/10	0/10	0/10	0/10	1/9	2/10
Testes						
Hypospermatogenesis	0/10	1/10	0/10	2/10	4/9	1/10
Giant cell degeneration	0/10	0/10	0/10	1/10	0/9	0/10
Salivary gland						
Acute inflammation	0/10	N[c]	N	0/10	0/10	2/10
Females						
Salivary gland						
Acute inflammation	0/10	N	N	N	1/10	1/10[b]

[a] No. of rats with changes/No. of rats examined.
[b] 100% mortality by week 1.
[c] Not examined.

SOURCE: NTP (1987).

of 2000 mg/kg/day based on mortality and increased liver-to-body weight ratios in both sexes. The NOAEL is 1000 mg/kg/day due to the absence of mortality and liver changes.

Dunnick et al. (1984a) observed histopathological changes in the testes, prostate glands, and kidneys of male Fischer-344 rats (20 rats/group) administered oral doses of DMMP in corn oil at 0, 250, 500, 1,000, or 2,000 mg/kg/day, 5 days/week for 90 days. This study is discussed in detail in section VI.B.3, Reproductive Effects. Rats in the high-dose group showed a significant ($p < 0.01$) decrease in total body weight gain (about 11%) relative to the control. The kidney-to-body weight ratios were significantly ($p < 0.01$) increased in rats in the 1,000- and 2,000-mg/kg/day groups. The kidney lesions observed at all dose levels included varying degrees of regeneration, hyaline droplet degeneration, cytoplasmic hyaline bodies, and cellular infiltrate, primarily lymphocytes, into the interstitium. Because of an association with $alpha_{2\mu}$globulin (Baetcke, 1991), male rat kidney lesions were not appropriate for determining a LOAEL. The LOAEL in this study is based on reproductive effects as discussed in section VI.B.3.

Dunnick et al. (1984b) studied the effect of DMMP on dominant lethal mutations in male $B6C3F_1$ mice (discussed in section VI.B.6, Genotoxicity). The mice were gavaged with DMMP in water at doses up to 2,000 mg/kg/day for 13 weeks. At the conclusion of the study, there was no mortality, nor any chemically related effects on body weight or relative weights for the testis, epidydimis, prostate, and kidney. Histological examination of selected organs, sperm concentration and morphology, and levels of plasma cholinesterase, follicle stimulating hormone (FSH), and luteinizing hormone (LH) did not demonstrate DMMP related effects.

In an inhalation study (discussed in section VI.B.3, Reproductive Effects), male Fischer-344 rats exposed continuously to DMMP vapors at 25 or 250 ppm for 90 days showed gross and histopathological testicular changes (Mattie et al., 1987).

b. Lifetime studies

DMMP in corn oil was administered by gavage at doses of 0, 1,000, or 2,000 mg/kg to groups of 50 male and 50 female $B6C3F_1$ mice and at doses of 0,

500, or 1,000 mg/kg to groups of 50 male and 50 female F334/N rats, 5 days/week for 103 weeks. All animals were observed twice daily, clinical signs were recorded weekly, and body weights were measured once each week during the first 13 weeks and once each month thereafter. Necropsy and histopathological examinations were performed on all animals (NTP, 1987; Dunnick et al., 1988).

In mice, the decrease in survival of the high-dose (2,000 mg/kg) group was partially attributed to fighting among males. There was an apparent overdose in both sexes on week 45 (dose mixture had a concentration 134% greater than the targeted amount) which caused the deaths of 17 and 22 high-dose males and females, respectively. However, when these deaths were censored from the analysis, survival of the treated groups was reduced in both sexes. At termination, 29/50 vehicle control males, 12/50 low-dose males, and 0/50 high-dose males were alive; 41/50, 30/50, and 2/50 females survived. Mean body weights of males in the high-dose group were 7-16% lower than those of the vehicle controls between study weeks 36 and 76; the females at this dose level showed 6-12% lower body weights between study weeks 88 and 103. No treatment-related clinical signs were observed. Histopathology revealed congestion of the lungs in the mice that died at weeks 45 and 75 but not in those surviving to the end of the studies. The lung congestion was associated with early deaths, dosing errors, and gavage accidents. The incidence of hepatocytomegaly was increased in treated males (17/50, low-dose group; 10/46, high-dose group) compared with vehicle control males (5/50). No increases in nonneoplastic liver lesions were seen in female mice (NTP, 1987; Dunnick et al. 1988).

In rats, survival was lower for males at both dose groups; final survival was 27/50, vehicle control; 17/50, low-dose group; and 4/50, high-dose group. The decrease in survival was partially due to treatment-induced renal toxicity. Survival of the low-dose females was comparable to that of the vehicle controls. However, the final survival of the high-dose females (23/50) was significantly ($p < 0.05$) lower than that of the vehicle controls (30/50). At the 1,000-mg/kg/day level, the mean body weights of males were 5-10% lower than those of the vehicle controls between weeks 28 and 76, and the mean body weights were 10-24% lower between weeks 80 and 104; females showed an 8-12% decrease after week 80. Mean body weights of both sexes of rats at the 500-mg/kg/day dose level were comparable with those of the vehicle

controls. Treatment-related nonneoplastic lesions observed in the kidneys of male rats are summarized in Table VI-7. Females showed no treatment-related nonneoplastic lesions (NTP, 1987; Dunnick et al., 1988).

In summary, the study with mice was considered to be inadequate owing to poor survival in males at both levels and in females at the high dose. Therefore, it is difficult to estimate a LOAEL or a NOAEL. In rats, DMMP induced mortality, body weight loss, and kidney lesions in males at both dose levels. Male kidney lesions are not considered to be relevant to humans because of the association with $alpha_{2\mu}$globulin (Baetcke et al., 1991) and were not considered as adequate endpoints for a LOAEL. Females at the high dose showed decreases in survival and mean body weights toward the end of the study. The LOAEL is 1,000 mg/kg/day based on significant body weight decreases in males and females; the NOAEL is 500 mg/kg/day.

3. Reproductive Effects

Dunnick et al. (1984a) assessed the reproductive toxicity of DMMP in groups of 20 male Fischer-344 rats following oral (gavage) administration of DMMP in corn oil at doses of 0, 250, 500, 1,000, or 2,000 mg/kg, 5 days/week for a total of 63 doses during a 90-day period. All female rats were sacrificed on day 100; their uteri were removed and examined for the number of live and dead pups and the number of resorptions. At day 84, each treated male was housed with two untreated female Fischer-344 (i.e., 40/group) rats for five days. The males were sacrificed on day 90, and the reproductive and genito-urinary systems underwent histopathological examination. Sperm samples were analyzed at sacrifice for sperm count, sperm motility, and sperm morphology. DMMP had no effect on survival, and no clinical signs or neurotoxicity were observed. Rats dosed at 2,000 mg/kg showed significantly ($p < 0.01$) decreased total body weight gain relative to the control group. A significant ($p < 0.01$) increase was observed in the kidney-to-body weight ratio in the two highest dose groups, and there was a decrease in the epididymis/body weight ratio in the high-dose group. No alterations were seen in the plasma levels of luteinizing hormone or follicle-stimulating hormone at any dose. Testicular lesions observed in 18 of 20 rats (90%) in the high-dose group were characterized by lack of spermatogenesis and by degeneration, vacuolization, and necrosis. Changes observed in the prostate of one rat dosed at 1,000 mg/kg/day and four rats dosed at 2,000 mg/kg/day included

Table VI-7. Incidences of Kidney Lesions Observed in Male F344N Rats After Oral Administration of DMMP for 103 Weeks

Lesion	Dose level (mg/kg)		
	0	500	1,000
Nephropathy	36/50[a] (72)[b]	43/50 (86)	40/49 (82)
Severity of nephropathy[c]	1.9	2.5	2.8
Calcification of renal papilla	12/50 (24)	41/50 (82)	36/49 (73)
Cortical tubular cell hyperplasia	0/50 (0)	8/50 (16)	9/49 (18)
Pelvic epithelial hyperplasia	0/50 (0)	23/50 (46)	21/49 (43)

[a] No. of rats with lesions/No. of rats examined.
[b] The numbers in parentheses are percentages.
[c] Mean severity of lesions graded from 1 to 4, minimal to marked.

SOURCE: NTP (1987); Dunnick et al. (1988).

multifocal infiltration of lymphocytes and plasma cells into the interstitium, and dilation of acini containing cellular debris.

DMMP was shown to be toxic to the male reproductive system. DMMP induced dose-dependent decreases in sperm motility and sperm counts; the decrease reached statistical significance ($p < 0.01$) at the high-dose level. Sperm head abnormalities characterized by headless sperm and sperm heads without a hook or with a blunt hook were significantly ($p < 0.01$) increased at the high-dose level (Table VI-8). DMMP altered reproductive functions at all dose levels, as shown in Table VI-9. Fertility was completely arrested at the high-dose level; rats failed to impregnate females at this level. A significant ($p < 0.01$), dose-dependent decrease was observed in the number of live fetuses per litter at doses $\geq$500 mg/kg. Treatment also caused significant ($p < 0.05$) dose-dependent increases in the percentage of resorptions. DMMP was shown to be a reproductive toxin. The LOAEL for reproductive effects was the lowest dose tested, 250 mg/kg/day (179 mg/kg/day when adjusted from a 5-day to a 7-day exposure) (Dunnick et al., 1984a).

Chapin et al. (1984) examined DMMP-induced development of reproductive lesions by light and electron microscopy. Groups of 49 male Fischer-344 rats were orally administered, by gavage, DMMP dissolved in tapwater at 1,750 mg/kg, 5 days/week for up to 12 weeks; a group of fourteen control rats received tapwater only. After 3, 4, 5, 7, 9, and 12 weeks of treatment, groups of seven treated and two control rats were sacrificed, and the testes and epididymis were examined. The remaining animals (seven treated and two controls) were allowed to recover for 14 weeks on a control diet, and the tissues were processed as above. Body weights of treated rats were lower than those of the vehicle control rats after week 5 of the treatment period. DMMP did not reduce testes weights but did impair epididymal weight gain. Changes in the testes and epididymis were first observed 5 weeks posttreatment. The approximate severity and the time course of the lesions seen in these tissues are presented in Table VI-10. DMMP also induced morphological alterations in Sertoli cells and elongating spermatids, as well as functional defects in spermatozoa. Animals left to recover for 14 weeks showed approximately 80% normal tubules; affected tubules varied in their degree of recovery, but all showed loss of normal epithelial organization, a characteristic of DMMP treatments.

Table VI-8. Analysis of Sperm From Male Fischer-344 Rats After Oral Administration of DMMP for 90 Days

Dose[a] (mg/kg)	% Motile sperm[b] (mean ± SEM)	Sperm count x 10^6 (mean ± SE per g caudal epididymal tissue)	Sperm-head abnormalities (mean ± SEM)
0	80.2 ± 2.7	541.4 ± 25.1	4.5 ± 0.3
250	80.5 ± 3.0	515.2 ± 38.9	5.6 ± 0.7
500	79.7 ± 3.6	459.2 ± 35.2	5.9 ± 0.5
1000	71.5 ± 3.1*	432.2 ± 38.5	6.9 ± 0.7
2000	35.8 ± 5.5**	219.6 ± 34.0**	41.7 ± 5.1**

[a]Twenty rats per group.
[b]Percent of sperm with any movement.
*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.01$).

SOURCE: Dunnick et al. (1984a).

Table VI-9. Changes in the Reproductive Function of Male Fischer-344 Rats After Oral Administration of DMMP for 90 Days

Dose (mg/kg)	% of males with sperm-positive females[a]	Male fertility index[b] (%)	No. of males impregnating 0, 1, or 2 females			Total No. (%) of pregnant females[c]	No. of live fetuses per litter (mean ± SEM)	% Resorptions[d]
			0	1	2			
0	75 (15/20)	70 (14/20)	6	8	6	20 (50)	7.6 ± 0.7	6.1 (10/163)
250	85 (17/20)	75 (15/20)	5	11	4	19 (47.5)	7.8 ± 0.4	14.9 (26/174)*
500	60 (12/20)	60 (12/20)	8	7	5	17 (42.5)	5.7 ± 0.6**	39.4 (63/160)[e],**
1000	50 (10/20)	40 (8/20)	12	5	3	11 (27.5)*	0.82 ± 0.5**	79 1 (34/43)**
2000	55 (11/20)	0**	20	0	0	0 (0.0)**	0.0 ± 0.0**	--

[a][(No. of males with 1 or 2 sperm-positive females)/(N=20)] x 100.
[b][(No. of males with 1 or 2 pregnant females)/(N=20)] x 100. This value also included pregnant females that did not have sperm-positive smears.
[c]Total of 40 females were exposed to 20 males (2 females per male) for each group.
[d][(No. of resorptions)/(total fetuses + total no. of resorptions)] x 100; statistical analysis based on % resorptions per litter.
[e]Includes 4 dead fetuses from 2 litters.

* Significantly different from control value ($p < 0.05$).
** Significantly different from control value ($p < 0.01$).

SOURCE: Dunnick et al. (1984a).

Table VI-10. Severity and Time Course of Lesions Observed in the Testes and Epididymis of Male Fischer-344 Rats After Oral Administration of DMMP for up to 12 Weeks

Effects	Weeks after treatment			
	5	7	9	12
Testes				
Focal exfoliation	+[a]	+	+ +[b]	+ +
PAS[d]-positive round bodies	+	+	+ +	+ +
Epididymis				
Regions 1-3				
PAS[d]-positive round bodies	+	+ +	+ +	+ + +[c]
Luminal spermatocytes	+	+ +	+ +	+ + +
Decreased sperm density			+	+
Regions 5-6				
Luminal spermatocytes	+	+	+ +	+ +
Number of affected animals	5/7	7/7	7/7	7/7

[a]Mild.
[b]Moderate.
[c]Severe.
[d]Periodic acid and Schiff's Stain

SOURCE: Chapin et al. (1984).

Mattie et al. (1987) exposed male Fischer-344 rats (number per group not reported) to DMMP vapors at concentrations of 25 or 250 ppm (127 and 1269 mg/m^3, respectively) continuously for 90 days. Light microscopy showed segmental to diffuse degeneration of the seminiferous epithelium; the degeneration observed also was seen in rats sacrificed 1 year later. Ultrastructurally, the testes of rats exposed to 250 ppm DMMP vapors showed an increased number of lipid droplets in cells lining the seminiferous tubules, increased numbers of lysosomes in Sertoli cells, dilation of mitochondria, presence of multinucleate giant cells, and a decreased number of spermatogenic cells.

4. Developmental Toxicity

In a short-term screening assay, 50 female CD-1 mice were given, by gavage, DMMP in corn oil at doses of 4,175 mg/kg/day on gestation days 6-13. The control group received corn oil only. Dams were allowed to litter. DMMP had no effect on the dams, did not reduce the number of live pups per litter, and did not affect pup survival or fetal body weight gain. However, pup birth weight was significantly ($p < 0.05$) lower in the treated (1.5 ± 0.1 g) group when compared with the vehicle control (1.6 ± 0.2 g) (Hardin et al., 1987).

Ciba-Geigy (1978b) evaluated the teratogenic potential of DMMP in female Sprague-Dawley rats following oral (gavage) administration. Groups of 25 rats were dosed with DMMP in 2% carboxymethylcellulose (CMC) at 100, 1,000, or 2,000 mg/kg/day on gestation days 6-15; dams were sacrificed on day 21. Maternal toxicity observed at the 2,000-mg/kg/day dose included decreases in body weight gain and food consumption. Dams at the 1,000-mg/kg/day level showed slightly decreased feed intake. No maternal toxicity was observed at the 100-mg/kg/day level. No embryotoxicity or fetotoxicity was seen at the low dose. Fetal toxicity seen at the higher levels included reduced fetal weight and delayed skeletal maturation (ossification) and were considered attributable to the maternal toxicity seen at these levels. DMMP did not alter the incidence of fetal anomalies.

In another experiment (Ciba-Geigy, 1978b), groups of 25 female Sprague-Dawley rats received oral intubation of DMMP in 2% CMC at 2,000 mg/kg/day on gestation days 6-15 and at 2,500 mg/kg/day on gestation days 6-10. Food

consumption was decreased at both levels, and maternal body weight was decreased at the 2,500-mg/kg/day level. Fetal body weight was slightly reduced and ossification was increased in both dose groups. No gross malformations and no visceral or skeletal anomalies were detected in the 2,000-mg/kg/day group. The few anomalies in the fetuses from the higher level were considered to be within the normal range of this strain of rats.

5. Carcinogenicity

DMMP was carcinogenic to male F344/N rats, inducing transitional cell papillomas or carcinomas (combined) of the pelvic epithelium in the kidneys. In addition, DMMP increased the incidence of mononuclear cell leukemia in male rats. Male rats also developed renal tubular cell adenocarcinomas; however, this effect is considered to be associated with $alpha_{2\mu}$globulin and not relevant to humans (Baetcke et al., 1991). DMMP was not carcinogenic to male and female $B6C3F_1$ mice, but the study was considered to be inadequate owing to decreased survival in both sexes (NTP, 1987).

In the NTP (1987) study (reviewed in detail in Section VI.B.2.b, Lifetime Studies), DMMP in corn oil was administered, by gavage, to $B6C3F_1$ mice at doses of 0, 1,000, or 2,000 mg/kg/day and to F344/N rats at doses of 0, 500, or 1,000 mg/kg/day in groups of 50 animals/sex/species, 5 days/week for 103 weeks. No treatment-related neoplastic lesions were observed in male or female mice. However, owing to poor survival, NTP considered the study to be inadequate to assess the carcinogenic potential of DMMP.

DMMP was not found to be carcinogenic following oral (gavage) administration to female F344/N rats. DMMP exhibited some evidence of carcinogenicity in male F344/N rats as shown by increased incidence of tubular cell hyperplasia, renal tubular cell adenocarcinomas, hyperplasia of the transitional cell epithelium, and transitional cell papillomas or carcinomas (combined) of the pelvic epithelium in the kidneys (Table VI-11). These tumors are rarely seen in this strain of rat and, therefore, are believed to be treatment related. The historical incidence of tubular cell adenomas or adenocarcinomas in NTP studies was 8/1,448 (0.6%), and the incidence of transitional cell papillomas was 1/1,448 (<0.01%). This is supported by the finding that the incidence of cortical tubular cell hyperplasia often

Table VI-11. Incidences of Preneoplastic and Neoplastic Lesions Observed in the Kidneys of Male F344/N Rats After Oral Administration of DMMP for 103 Weeks

Type	Dose level (mg/kg/day)		
	0	500	1,000
Cortical tubular cell hyperplasia	0/50(0)	8/50(16)	9/49(18)
Pelvic epithelial hyperplasia	0/50(0)	23/50(46)	21/49(43)
Tubular cell adenocarcinoma	0/50[a](0)[b]	2/50(4)	3/49(6)*
Transitional cell papilloma	0/50(0)	7/50(14)**	3/49(6)*
Transitional cell carcinoma	0/50(0)	1/50(2)	0/49(0)
Transitional cell papilloma or carcinoma	0/50(0)	8/50(16)**	3/49(6)*

[a]No. with tumors/No. examined.
[b]The numbers in parentheses are percentages.
*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.001$).

SOURCE: NTP (1987); Dunnick et al. (1988).

increases in association with the induction of tubular cell neoplasm, suggesting morphological progression from hyperplasia to adenomas to adenocarcinomas. Similarly, there is a morphologic spectrum from hyperplasia of the transitional epithelium to papilloma and carcinoma of the renal pelvis. No tubular cell or transitional cell neoplasms or other treatment-related neoplasms were seen in female rats. The incidence of mononuclear cell leukemia was significantly increased ($p < 0.001$) in high-dose male rats (vehicle control, 10/50; low dose, 11/50; high dose, 17/50), and the incidence exceeded the highest incidence seen in historical vehicle control groups (14/50) in the NTP studies (Table VI-12) (NTP, 1987).

The profile of some kidney effects observed in the DMMP-treated male rats is consistent with that attributed to $alpha_{2\mu}$globulin (Baetcke et al., 1991; NTP, 1987). This protein is synthesized in the liver of the male rat and has not been detected in female rats. It has not been detected in any other species, including humans. Because this protein appears to occur only in the male rat, $alpha_{2\mu}$globulin associated renal tubular cancers are not to be expected to occur in other species. Thus, such effects are not considered to be relevant to humans.

In summary, DMMP was carcinogenic to male rats but not to female rats. Based on the EPA Guidelines for Carcinogenic Risk Assessment, DMMP is classified in Group C: Possible Human Carcinogen.

6. Genotoxicity

Table VI-13 summarizes the results of the genetic toxicology assays with DMMP. DMMP was nonmutagenic in the Ames assay using several *Salmonella typhimurium* strains both in the presence and absence of metabolic activation (Ciba-Geigy, 1976h, 1978c; Sivak, 1983; Mortelmans et al., 1986).

DMMP induced forward mutations in a mouse lymphoma L5178Y/TK+/- assay in the absence of metabolic activation (NTP, 1987). DMMP did not induce mutations in the Chinese hamster ovary (CHO) cell HGPRT assay in the absence of activation (Sivak, 1983). Tice (1990) reported that DMMP induced forward mutations in this same assay in both the presence and absence of metabolic activation.

Table VI-12. Incidence of Mononuclear Cell Leukemia Observed in Male F344/N Rats After Oral Administration of DMMP for 103 Weeks

Site	Dose level (mg/kg/day)		
	0	500	1,000
Multiple organs	10/50[a](20)[b]	11/50 (22)	7/50 (34)[c]
Spleen	0/50 (0)	1/50 (2)	0/50 (0)
Total	10/50 (20)	12/50 (24)	17/50 (34)[c]

[a]No. with tumors/No. examined.

[b]The numbers in parentheses are percentages.

[c]Significantly different from control value ($p < 0.001$)

Table VI-13. Mutagenicity of DMMP in Various Test Systems

Assay	Test System	Dose or concentration range	Endpoint response (with S9/without S9)	Reference
1. Gene mutation				
Ames assay	*Salmonella typhimurium* TA1535, TA1538	10 µg to 10 mg/plate	-/-	Ciba-Geigy (1976h)
	S. typhimurium TA98, TA100, TA1535, TA1537	25 to 2,025 µg/0.1 mL	-/-	Ciba-Geigy (1978c)
	S. typhimurium TA98, TA100, TA1535, TA1537, TA 1538	0.16 to 200 µL/plate	-/-	Sivak (1983)
	S typhimurium TA98, TA100, TA1535, TA1537	0 to 10,000 µg/plate	-/-	Mortelmans et al. (1986)
Mouse lymphoma assay	L5178Y/TK$^{+/-}$ cells	0.25 to 5 µL/mL	[b]/+	NTP (1987)
		25 to 45 µL/mL	+/+	Tice (1990)
CHO/HGPRT assay	Chinese hamster ovary (CHO) cells	125 to 1,000 µg/mL	[b]/-	Sivak (1983)
2. Chromosomal aberration	CHO cells	2,000 to 5,000 µg/mL	-/-	NTP (1987)
		14,300 to 22,000 µg/mL	-/-	NTP (1987)
	CHO cells	125 to 1,000 µg/mL	[b]/+	Sivak (1983)
3 Sister chromatid exchange	CHO cells	125 to 1,000 µg/mL	[b]/-	Sivak (1983)
	CHO cells	160 to 5,000 µg/mL	-/+	NTP (1987)
		1,100 to 22,000 µg/mL	+/+	NTP (1987)
4. Sex-linked recessive lethal (SLRL) mutation and reciprocal translocation (RT)	*Drosophila melanogaster*	23,735 ppm (SLRL)	+[a]	NTP (1987)
		23,500 ppm (RT)	-[a]	NTP (1987)
5. *In vitro* neoplastic transformation	BALB/c 3T3 cells	0.8 to 100 µg/mL	[b]/-	Sivak (1983)

[a]Metabolic activation not applicable

[b]Either not tested with S9 metabolic activation, or activation not applicable for test.

Limited evidence of clastogenicity was seen in CHO cells; DMMP induced chromosomal aberrations at the highest concentration tested (1 mg/mL) without activation (Sivak, 1983), but did not induce aberrations when tested by NTP at concentrations up to 22 mg/mL either with or without activation (NTP, 1987).

Conflicting results were obtained in assays inducing sister chromatid exchanges (SCEs) in CHO cells. Sivak (1983) observed no significant increases in SCEs at doses up to 1 mg/mL. On the other hand, NTP (1987) reported positive results both in the absence (over a concentration range of 0.16-11 mg/mL) and presence (1.1-22 mg/mL) of metabolic activation.

In an *in vitro* cell transformation assay, DMMP did not induce neoplastic transformations in BALB/c 3T3 cells (Sivak, 1983).

In *in vivo* genotoxic studies, when DMMP was fed to *Drosophila* it induced significant increases in the frequency of sex-linked recessive lethal mutations but did not induce reciprocal translocations (NTP, 1987).

DMMP was shown to induce dominant lethal mutations in male $B6C3F_1$ mice when orally administered at 0, 250, 500, 1,000, or 2,000 mg/kg/day, 5 days/week for 13 weeks. The chemical was administered in water by gavage to treatment groups consisting of 40, 20, 20, 40, and 40 animals, respectively. After 4, 8, and 12 weeks of treatment, mice were mated with untreated CD-1 mice. Half the males dosed at 0, 1,000, or 2,000 mg/kg were mated after a 15-week recovery period on a control diet. DMMP at the two highest dose levels (1,000 or 2,000 mg/kg) caused dominant lethal mutations (early resorptions). The dominant lethal index was significantly ($p < 0.001$) increased in the 4, 8, and 12 week mating trials 1, 2, and 3 (40, 37, and 44%, respectively) at the 2,000-mg/kg/day level; and in the 4 and 12 week mating trials 1 and 3 (12 and 13%, respectively) at the 1,000-mg/kg/day level. Groups of mice mated after a 15-week recovery period had a resorption rate comparable to that of the vehicle control group (Dunnick et al., 1984b).

7. Neurotoxicity

Groups of 20 White leghorn hens were administered, by gavage, 1,999 (1/2 LD_{50}) or 3,998 mg DMMP/kg (LD_{50}) as a 60% dilution in 2% CMC twice, initially

and after 21 days. The animals were observed throughout a 42 day period. Acute toxicity observed included ataxia, curved position, and fluttered feathers in birds at the low dose, and ataxia, apathy or sedation, curved position, and fluttered feathers in birds at the high dose. The surviving birds partially recovered from the symptoms. Histopathology of the spinal cord and peripheral nerve of dosed hens showed no evidence of delayed neurotoxicity. It was concluded that DMMP did not cause delayed neurotoxicity (Ciba-Geigy, 1978d).

Hollinghaus et al. (1981) observed no delayed neurotoxicity in groups of three adult White leghorn hens following daily intraperitoneal injection of DMMP in corn oil at doses of 50 mg/kg for 10 days.

In a 3-day oral intubation study with rats, DMMP decreased plasma cholinesterase levels (30% in males and 40-50% in females) at a dose of 1,000 mg/kg/day over a 4-hour period when compared with control values taken during the same time period; no decreases in plasma cholinesterase levels were seen in rats dosed at 1, 10, or 100 mg/kg/day (Ciba-Geigy, 1978a). Mice dosed for 90 days at 250, 500, 1,000 or 2,000 mg/kg/day also did not have increased plasma cholinesterase levels (Dunnick et al., 1984b) (See detailed discussion of rat intubation study on page VI-5 and mouse study on page VI-28).

VII. HEALTH ADVISORY DEVELOPMENT

A. SUMMARY OF HEALTH EFFECTS DATA

Data on the systemic toxicity of DMMP in humans were not found in the available literature; patch testing with aqueous solutions of DMMP produced mild to moderate skin irritation in human subjects. DMMP was not a skin sensitizer (Ciba-Geigy, 1976a).

Acute toxicity studies indicate oral LD_{50} values of >6,810 mg/kg in both mice and rats (NTP, 1987). Ciba-Geigy (1976b) reported an oral LD_{50} of 10,190 mg/kg in rats. Animals exhibited ataxia, ventricumbency, unsteady gait, muscular hypertonia and hypotonia, tremors, prostration, and reduction in spontaneous motility. DMMP was slightly toxic by the intravenous route; the LD_{50} was 912 mg/kg in mice and 1,050 mg/kg in rats (Ciba-Geigy, 1977a). The acute dermal LD_{50} of DMMP was reported to be >4,600 mg/kg in rats (Ciba-Geigy, 1976c) and >2,000 mg/kg in rabbits (Stauffer, 1983). DMMP (0.5 mL) elicited mild skin irritation in rabbits (Ciba-Geigy, 1976d) and produced mild ocular irritation when instilled (0.1 g) into the eyes of rabbits (Ciba-Geigy, 1976f). DMMP was negative as a sensitizer in guinea pigs (Ciba-Geigy, 1976g).

In a 3-day oral intubation study with rats, DMMP decreased plasma cholinesterase levels (30% in males and 40-50% in females) at a dose of 1,000 mg/kg/day over a 4-hour period when compared with control values taken during the same time period; no decreases in plasma cholinesterase levels were seen in rats dosed at 1, 10, or 100 mg/kg/day (Ciba-Geigy, 1978a). Mice dosed for 90 days at 250, 500, 1,000 or 3,000 mg/kg/day also did not have increased plasma cholinesterase levels (Dunnick et al., 1984b).

In a 15-day study with mice, histopathological changes were observed in the stomachs of males receiving $\geq$1,250 mg/kg/day and in females receiving $\geq$5,000 mg/kg/day; no stomach lesions were seen in females dosed at $\leq$2,500 mg/kg/day (NTP,1987).

In a 15-day study with rats, no gross pathological changes were observed at levels up to 15,000 mg DMMP/kg/day; however, mortality was seen in both

sexes dosed at ≥5,000 mg/kg/day. No histopathological examination was conducted (NTP, 1987).

The profile of some kidney effects observed in the DMMP-treated male rats is consistent with that of $alpha_{2\mu}$globulin nephrotoxicity (Baetcke, 1991). This protein is synthesized in the liver of the male rat and has not been detected in female rats. It has not been detected in any other species, including humans. Because this protein appears to occur only in the male rat, $alpha_{2\mu}$globulin nephrotoxicity, and subsequent effects, are not expected to occur in other species. Thus, some DMMP-induced renal noncarcinogenic and carcinogenic effects were not considered to be relevant to humans. Subsequently, such endpoints are not used to develop advisory values to protect human health.

In a 4-week dietary study with rats, DMMP caused an increase in the protein resorption droplets (hyaline droplets) in the proximal convoluted epithelium of the kidneys of males fed diets containing 2,000, 6,000, or 20,000 ppm (178, 535, or 1,790 mg/kg/day); the effect was more pronounced with increasing levels of DMMP. No kidney changes were seen in females (Ciba-Geigy, 1977c).

In a 13-week study with male and female mice, oral administration of DMMP in corn oil at levels between 250 and 8,000 mg/kg/day produced no adverse effects on body weight gain, clinical signs, food consumption, or gross or histopathology. Mortality was seen in both sexes at the higher (4,000 or 8,000 mg/kg) dose levels (NTP, 1987).

In a 13-week study with rats, oral administration of DMMP in corn oil at 2,000 mg/kg/day caused decreased survival and body weight gain and increased liver-to-body weight ratio in males and females. Histopathology revealed kidney changes in males at all dose levels starting at 250 mg/kg/day; no kidney changes were seen in females at levels up to 2,000 mg/kg (NTP, 1987).

In a chronic study, male and female $B6C3F_1$ mice received oral intubation of 1,000 or 2,000 mg DMMP/kg, 5 days/week for 103 weeks. Mean body weights were decreased in both sexes in the high-dose group, and the incidence of hepatocytomegaly was increased in males. Females showed no treatment-related

nonneoplastic lesions. However, this study was considered to be inadequate owing to decreased survival in both sexes, which was attributed to fighting among males and dosing errors in females (NTP, 1987).

Oral administration of DMMP at 500 or 1,000 mg/kg, 5/days/week for 103 weeks, to male and female F344/N rats caused an increase in kidney lesions in males at both levels. No kidney lesions were seen in females at either level. Both males and females at the high dose showed lower survival and decreased body weight gain (NTP, 1987).

DMMP was not carcinogenic in female F344/N rats or male or female $B6C3F_1$ mice. However, the study with mice was judged to be inadequate to evaluate the carcinogenic potential of DMMP owing to poor survival in both sexes (NTP, 1987).

DMMP was carcinogenic in male F344/N rats as shown by increased incidences of mononuclear cell leukemia, hyperplasia of the transitional epithelium, and transitional cell papilloma or carcinoma (combined) of the kidneys. The males also developed renal tubular cell hyperplasia and adenocarcinoma which is considered to be in response to the presence of $alpha_{2\mu}$globulin, a protein found only in male rats (Baetcke, 1991). DMMP is classified in Group C: Possible Human Carcinogen.

DMMP was nonmutagenic in bacteria (Ciba-Geigy, 1976h, 1978c; Sivak, 1983; Mortelmans et al., 1983). It induced forward mutations in mouse lymphoma cells (NTP, 1987) but did not induce mutations in CHO cells in the CHO/HGPRT assay (Sivak, 1983). DMMP showed limited evidence for clastogenicity in CHO cells. In one study, it induced chromosomal aberrations at a concentration of 1 mg/mL (Sivak, 1983), but it did not do so at concentrations up to 22 mg/mL when tested by NTP (1987). Conflicting results were obtained in the sister chromatid exchange assays; Sivak (1983) observed no increases in SCEs, whereas NTP (1987) reported positive results. DMMP did not induce neoplastic transformations in the BALB/c 3T3 cell assay (Sivak, 1983). *In vivo*, DMMP increased the frequency of sex-linked recessive lethal mutations but did not induce reciprocal translocations in *Drosophila* (NTP, 1987). DMMP gave positive results in the dominant-lethal assays in mice and rats (Dunnick et al., 1984a,b).

DMMP was shown to be a reproductive toxin following oral administration to male rats. It caused decreases in sperm count, sperm motility, and male fertility index, and also induced histopathological changes in the testes, epididymis, and prostate glands. DMMP altered reproductive functions in male Fischer-344 rats at all levels (250 to 2,000 mg/kg, 5 days/week for 13 weeks) (Dunnick et al., 1984a). Inhalation exposure of rats to DMMP vapors (25 or 250 ppm) induced lesions in the testes and epididymis (Mattie et al., 1987).

DMMP was not found to be teratogenic; no developmental effects were observed following oral administration of 4,175 mg/kg/day during gestation days 6-13 (Hardin et al., 1984). DMMP at maternally toxic doses (1,000 or 2,000 mg/kg/day on gestation days 6-15) induced fetal toxicity that included reduced fetal weight and delayed skeletal maturation; no fetal anomalies were seen. In another study, DMMP at 2,000 or 2,500 mg/kg/day, administered on gestation days 6-13, reduced fetal weight but did not induce gross malformations or visceral or skeletal anomalies (Ciba-Geigy, 1978b).

DMMP did not induce delayed neurotoxicity in White leghorn hens following oral administration at 1,999 or 3,998 mg/kg (Ciba-Geigy, 1978d) or intraperitoneal injection at 50 mg/kg/day for 10 days (Hollinghaus et al., 1981).

B. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years), and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic endpoint of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(\text{NOAEL or LOAEL})\ (bw)}{(UF)\ (____\ L/day)} = _____\ mg/L\ \ (____\ \mu g/L)$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (in mg/kg bw/day).

bw = assumed body weight of a child (10 kg) or adult (70 kg).

UF = uncertainty factor (10, 100, or 1,000), in accordance with NAS/EPA guidelines.

L/day = assumed daily water consumption of a child (1 L/day) or adult (2 L/day).

1. One-day Health Advisory

The 3-day gavage study in rats (Ciba-Geigy, 1978a) was considered for derivation of a One-day HA. Plasma cholinesterase levels in Sprague-Dawley rats, 5/sex/dose, were measured at pretest, and 0.5, 1, 2, and 4 hours after the third administration of DMMP in tapwater at doses of 1, 10, 100, or 1,000 mg/kg/day. Plasma cholinesterase levels were depressed at the 1,000-mg/kg/day level only. In males, there was an approximate reduction of 30% over the 4-hour period when compared with vehicle control values taken at the same time period, and in females there was a 40-50% reduction. No effect on plasma cholinesterase levels was observed in the other groups. Based on the decreases in plasma cholinesterase levels in both sexes at the high dose, the NOAEL was 100 mg/kg/day. Since only plasma cholinesterase (butyl cholinesterase) but not brain or erythrocyte cholinesterase (acetyl cholinesterase) was measured, data gaps exist for acetylcholinesterase inhibition. The study is limited further by the small number of animals used per sex and treatment group, and the lack of a dose-response curve. Therefore, the Ciba-Geigy (1978a) study is limited in its use to derive a One-day HA. However, cholinesterase depression was considered in the assessment of effects from a one-day exposure to DMMP; the recommended One-day HA is protective of cholinesterase effects.

Since these data are judged unsuitable for derivation of a One-day HA, it is recommended that the Longer-term HA for a 10-kg child (2 mg/L) be used as a conservative estimate for the One-day HA value.

2. Ten-day Health Advisory

The 15-day gavage study with mice (NTP, 1987) was considered and rejected for the derivation of a Ten-day HA. This study was conducted on a limited number of animals (five/sex/dose), and histopathology revealed lesions (gastropathy, gastritis, squamous atrophy, hyperkeratosis, or epithelial

ulcerations) in the stomach (fore·or glandular not specified) of both male and female mice; the males were more sensitive than the females. Based on stomach lesions, the LOAEL was 1,250 mg/kg/day for males and 5,000 mg/kg/day for females. However, stomach lesions seen in this study were not observed in mice following oral administration of DMMP at comparable dose levels for 13 weeks (doses: 250 - 8,000 mg/kg/day) or 103 weeks (doses: 1,000 and 2,000 mg/kg/day). Therefore, it is difficult to attribute these effects to DMMP or to the method of administration (oral intubation), and the NTP report did not discuss the significance of the presence (in the 15-day study) or the absence (13- or 103-week studies) of the stomach lesions.

The 15-day study with rats (NTP, 1987) was judged unacceptable for use in derivation of a Ten-day HA, since no clear treatment-related pattern of toxicity was observed except for mortality. No histopathological examination was conducted, and no target organ was identified. Therefore, a LOAEL or NOAEL was not established.

The 4-week dietary study (Ciba-Geigy, 1977c) was considered and rejected for derivation of a Ten-day HA. This study was conducted with a limited number (five/sex/dose) of animals. Histopathology revealed an increase in protein resorption droplets in the proximal convoluted epithelium of the kidneys in male rats. No histological changes were seen in female kidneys (however, kidney weights were increased significantly in the high-dose group). Similar findings (accumulation of hyaline droplets in the convoluted tubules of the renal cortex) were seen in the kidneys of male rats in a 13-week oral intubation study (NTP, 1987). The 4-week study was judged unsuitable because of the association between male rat kidney lesions and $alpha_{2\mu}$globulin (Baetcke et al., 1991) and the small number of animals tested.

Since these data were not judged suitable for derivation of a Ten-day HA, it is recommended that the Longer-term HA for a 10-kg child (2 mg/L) be used as a conservative estimate for the Ten-day HA value.

3. Longer-term Health Advisory

The 90-day reproductive toxicity study by Dunnick et al. (1984a), the basis for the Lifetime HA, is used to derive the Longer-term HA. The LOAEL for the study is 250 mg/kg/day (adjusted to 179 mg/kg/day) based upon DMMP-related resorptions in the female rats impregnated by treated males.

Using the adjusted study dose LOAEL of 179 mg/kg/day, the Longer-term HA for a 10-kg child is calculated as follows:

$$\text{Longer-term HA (child)} = \frac{(179\ \text{mg/kg/day})\ (10\ \text{kg})}{(1000)\ (1\ \text{L/day})} = 1.79\ \text{mg/L (rounded off to 2 mg/L or 2,000 } \mu\text{g/L)}$$

where:

179 mg/kg/day = LOAEL, based on increased resorptions in female rats that were impregnated by DMMP treated male rats.

10 kg = assumed weight of a child.

1000 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines in which a NOAEL from an animal study is employed. This UF includes a factor of 10 for interspecies variability, a factor of 10 for intraspecies variability, and a factor of 10 for use of a LOAEL in the absence of a NOAEL.

1 L/day = assumed daily water consumption of a 10-kg child.

The Longer-term HA for a 70-kg adult is calculated as follows:

$$\text{Longer-term HA (adult)} = \frac{(179)\ \text{mg/kg/day})\ (70\ \text{kg})}{(1000)\ (2\ \text{L/day})} = 6.27\ \text{mg/L (rounded off to 6 mg/L or 6,000 } \mu\text{g/L)}$$

where:

179 mg/kg/day = LOAEL, based on increased resorptions in female rats that were impregnated by DMMP treated male rats.

70 kg = assumed weight of an adult.

1000 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines in which a NOAEL from an animal study is employed. This UF includes a factor of 10 for interspecies variability, a factor of 10 for intraspecies variability, and a factor of 10 for use of a LOAEL in the absence of a NOAEL.

2 L/day = assumed daily water consumption of a 70-kg adult.

4. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), is identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL IS a medium-specific (i.e., Drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA is determined in step 3 by factoring in other sources of exposure, the relative source contribution (RSC). The RSC from drinking water may be based on actual exposure data or, if data are not available, a value of 20% is assumed. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then action should be exercised in assessing the risks associated with lifetime exposure to this chemical.

The 103-week chronic studies in $B6C3F_1$ mice and F344/N rats (NTP, 1987; Dunnick et al., 1988) were available for use in deriving the Lifetime HA. However, the study with mice was judged to be unacceptable for use in deriving the Lifetime HA because it was considered inadequate owing to poor survival in both sexes. The decreased survival was not treatment-related and was due to fighting in males and dosing errors in females.

In the study with rats, DMMP was orally administered at doses of 500 or 1,000 mg/kg, 5 days/week for 103 weeks. In males, an increased incidence of kidney lesions was noted at both dose levels. No kidney lesions were seen in females at either level. However, decreased survival and body weight gains were seen in females at the high-dose level. At the 1000 mg/kg/day level,

body weights were decreased significantly in males and females. Thus, a LOAEL of 1000 mg/kg/day and a NOAEL of 500 mg/kg/day was determined based on body weight.

No data on pharmacokinetics in either humans or animals were available. Therefore, it cannot be determined which species most closely resembles humans in absorption, metabolism, and excretion of DMMP.

Groups of 20 male Fischer 344 rats were administered dimethyl methylphosphonate (DMMP) in corn oil by gavage at doses of 0, 250, 500, 1000, or 2000 mg/kg/day, 5 days/week during a 90-day period (Dunnick et al., 1984a). At day 84, treated rats were mated with untreated female Fischer 344 rats. Reproductive functions were altered at all dose levels. In the 2000-mg/kg/day dose group, fertility was completely arrested; male rats completely failed to impregnate females. At 500-, 1000-, and 2000-mg/kg/day levels, there were significant ($p < 0.01$), dose-dependent decreases in the number of live fetuses per litter. Animals dosed at 250 mg/kg/day and above had significant ($p < 0.05$ or 0.01) dose-dependent increases in the percentage of resorptions. The LOAEL for this study is 250 mg/kg/day (179 mg/kg/day when adjusted from a 5-day to a 7-day exposure) based upon the increases in resorption. A NOAEL was not identified because adverse effects occurred at all dose levels.

The actual study dose of 179 mg/kg/day will be used as the LOAEL for the calculation of the DWEL. The DWEL is derived as follows:

Step 1: Determination of the Reference Dose (RfD)

$$RfD = \frac{(179\ mg/kg/day)}{(1{,}000)} = 0.179\ mg/kg/day$$

(rounded to 0.2 mg/kg/day or 200 μg/kg/day)

where:

179 mg/kg/day = LOAEL, based on adverse reproductive effects.

1,000 = uncertainty factor (UF), chosen in accordance with NAS/EPA guidelines using a LOAEL from an animal study. This UF includes a factor of 10 for interspecies variability, a factor of 10 for intraspecies

variability, and a factor of 10 for use of a LOAEL in the absence of a NOAEL. An additional factor for use of a less-than-lifetime study was not applied because adequate chronic and lifetime studies are available and were reviewed. A single well conducted chronic study (NTP, 1987) resulted in a NOAEL double that of the LOAEL for reproductive effects. The reproductive toxicity study used as a basis for the RfD demonstrated the most sensitive toxic endpoint.

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.2\ \text{mg/kg/day})\ (70\ \text{kg})}{(2\ \text{L/day})} = 7.0\ \text{mg/L or } 7{,}000\ \mu\text{g/L}$$

where:

0.2 mg/kg/day = RfD.

70 kg = assumed body weight of an adult.

2 L/day = assumed daily water consumption of an adult.

Step 3. Determination of Lifetime Health Advisory

$$\text{Lifetime HA} = \frac{(7.0\ \text{mg/L})\ \ (0.2)}{10} = 0.14\ \text{mg/L}$$

(rounded off to 0.1 mg/L or 100 μg/L)

where:

7 mg/L = Drinking Water Equivalent Level (DWEL).

0.2 = assumed Relative Source Contribution (RSC) (20%) if actual data are not available; assumed contribution from drinking water.

10 = OW policy, additional factor of 10 to account for possible (equivocal) evidence of human carcinogenicity for Group C chemicals.

C. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The carcinogenic potential of DMMP has been evaluated for male and female $B6C3F_1$ mice and F344/N rats. DMMP was not carcinogenic in female rats or in male or female mice. However, it was carcinogenic in male rats as shown by increased incidences of tubular cell hyperplasia, tubular cell adenocarcinomas, hyperplasia of the transitional cell epithelium, and

transitional cell papillomas or carcinomas (combined) of the kidney. In addition, an increased incidence of mononuclear cell leukemia was seen in male rats (NTP, 1985).

DMMP administered by gavage, to F344/N rats at 0, 500, or 1,000 mg/kg/day in groups of 50 animals/sex/species, 5 days/week for 103 weeks (NTP, 1987), had an increased incidence of mononuclear cell leukemia in high-dose male rats (vehicle control, 10/50 [20%]; low dose, 11/50 [22%]; high dose, 17/50 [34%]) which exceeded the incidence of that seen in historical vehicle controls (6-28%). The male rats also exhibited an increased incidence of transitional cell papillomas and carcinomas (combined) (8/50 low dose, 3/49 high dose) of the pelvic epithelium in the kidneys. Other kidney cancers that developed in the male rat, i.e., renal tubular carcinomas, were not considered to be relevant to humans, because of an association with $alpha_{2\mu}$globulin (Baetke et al., 1991).

1. Dose-Response Data (Carcinogenicity, Oral Exposure)

Individual and combined organ cancer incidence from the NTP (1987) study are shown in Table VII-1.

Tumor Type:	Mononuclear Cell Leukemia, Kidney Transitional Cell Papillomas and Carcinoma
Test Animals:	Rat, F344/N, Male
Route:	Oral, Gavage
Reference:	NTP, 1987

Dose		
Administered (mg/kg/day)	Human Equivalent* (mg/kg/day)	Tumor Incidence**
0	0	10/43
357	67	18/41
714	130	20/43

* Administered Dose ÷ (70 kg/rat weight) $^{0.33}$; Rat weights were averaged from study day 44 to 105 and were 0.476 kg, 0.465 kg, and 0.420 kg for the control, low- and high-dose groups, respectively.

** Animals that died before 60 days were excluded from the risk estimate.

Table VII-1. Summary of Data Used to Quantitate the Carcinogenic Potential of DMMP

	Dose Groups (Adjusted Dose)[a] (mg/kg/day)		
	0	500 (357)	1000 (714)
Number of Animals	50(43)[b]	50 (41)	50 (43)[c]
Mononuclear Cell Leukemia (MCL):			
- Multiple Organs	10	10	17
- Spleen	0	1	0
- Combined Multiple Organs and Kidney	10	11	17*,**
Kidney Transitional Cell:			
- Papilloma	0	7*,**,***	3*
- Carcinoma	0	1	0
- Combined Papilloma and Carcinoma	0	8	3
Combined MCL and Kidney[d]	10	18*,**,***	20*

[a]Actual dose adjusted from a 5-day/week exposure to a 7-day/week exposure, i.e., Dose x 5/7.

[b]The number in parenthesis is the number of animals that were included in the risk estimate after excluding those that died before the first case of cancer.

[c]Only 49 animals were examined for kidney effects.

[d]Number of tumor bearing animals. One animal in the 500 mg/kg/day group had both MCL and kidney cell cancer, it was counted only once in the combined totals.

*Significant by Life Table Tests ($p < 0.05$ or $p < 0.001$) (NTP, 1987).
**Significant by Incidental Tumor Test ($p < 0.05$ or $p < 0.001$) (NTP, 1987).
***Significant by Fisher Exact Test ($p < 0.05$) (NTP, 1987).

Source: NTP, 1987

The administered doses are time-weighted averages adjusted for frequency of exposure (5/7 days). Human equivalent doses were adjusted for the actual weights of the animals averaged from study day 45 to termination (0.476 kg, controls; 0.465 kg, low-dose group; 0.420 kg, high-dose group). The incidence of mononuclear cell leukemia in the vehicle control group was used in modeling. Vehicle controls did not develop transitional cell papilloma and carcinoma.

2. Summary of Risk Estimates

Oral Slope Factor $(mg/kg/day)^{-1}$ -- 5E-3

Drinking Water Unit Risk (per $\mu g/L$) -- 1E-7

Extrapolation Method -- Linearized Multistage Procedure, Extra Risk

3. Drinking Water Concentrations at Specified Risk Levels

Risk Level	Concentration
E-4 (1 in 10,000)	7E + 2 $\mu g/L$
E-5 (1 in 100,000)	7E + 1 $\mu g/L$
E-6 (1 in 1,000,000)	7E + 0 $\mu g/L$

The multistage model was used for high- to low-dose extrapolation (Crump and Watson, 1979; Howe and Crump, 1982). GLOBAL86 was used to fit the data in the experimental dose range and to obtain upper 95% confidence limits on risk. The multistage model conforms to a biological model of tumor initiation and promotion (Crump et al., 1977) and provided an adequate fit to the dose-response data for DMMP. The relationship of the concentration ($\mu g/L$) of a chemical in drinking water to cancer risk is expressed as follows:

$$\frac{35,000}{q_1^*} \times R = C$$

where:

35,000 = conversion factor for mg to μg and exposure assumption that a 70-kg adult consumes 2 L of water/day.

q1* = $(mg/kg/day)^{-1}$, human slope factor.

$R = 10^{-4}, 10^{-5}, 10^{-6}$, etc.

C = concentration of chemical in $\mu g/L$.

For comparison purposes, drinking water concentrations associated with an excess cancer risk of 10^{-6} are 2 $\mu g/L$ for the one-hit model, and significantly less than 0.0001 $\mu g/L$ for the multihit, probit, logit, and Weibull models, respectively. The parameter estimates for these models were computed with RISK81 (Kovar and Krewski, 1981) and MULTI80 (Rai and VanRyzin, 1980; Lavenhar, 1986).

4. Discussion of Confidence (Carcinogenicity Oral Exposure)

There are two carcinogenicity assays with DMMP, both conducted with rodents, rats and mice (NTP, 1987). Positive evidence in both studies are limited; male rat kidney effects are complicated by an association with $alpha_{2\mu}$globulin, and results in mice are inconclusive due to decreased survival. There is an increased incidence of mononuclear cell leukemia in only the high-dose group of male rats. Transitional cell papilloma and carcinoma in the renal pelvis also occur in male rats of both dose groups; the appearance of this additional tumor type increases the confidence in the Group C (possible human carcinogen) classification. Female rats did not develop cancer from DMMP exposure.

VIII. OTHER CRITERIA, GUIDANCE, AND STANDARDS

No information concerning other criteria, guidance, and standards for DMMP were found in the available literature.

IX. ANALYTICAL METHODS

Traditional methods used in the analysis of organophosphorus compounds involved mass spectrometry (MS) analysis using electron impact ionization and chemical ionization (CI). Electron impact ionization generally leads to extensive fragmentation, while detection limits (2.0×10^{-11}g, DMMP) are estimated based on CI studies utilizing methane. Since neither method exhibits the necessary selectivity for organophosphorus compounds, they are usually used together with gas chromatography (GC) (Groenewold and Todd, 1985). Van der Veken and Herman (1983) used GC with an infrared detector to determine the existence of possible conformers of DMMP. DMMP was found to exist in the gas phase as a single conformer. Aurian-Blajemi and Boucher (1989) used fourier transform infrared (FTIR) spectroscopy to study the gas-solid interactions of DMMP with metal oxides. This method is a variation of traditional infrared GC analysis. However, GC/MS analysis does not allow for continuous analysis, and it is difficult to impossible when unknown inseparable interferents are present. Additional analytical methods for DMMP include nuclear magnetic resonance (NMR) and plasma chromatography (Berkowitz et al., 1978).

In an attempt to develop a mass spectrometry ionization method that is selective for organophosphorus compounds and would have the sensitivity of CI, Groenewold and Todd (1985) used molecular secondary ion mass spectrometry (SIMS) for the detection of gaseous organophosphorus compounds. The best matrix for the detection of DMMP was polyphosphoric acid, and the limit of detection was estimated to be 1.6×10^{-11} μg.

X. TREATMENT TECHNOLOGIES

There were no specific treatment technologies for removing DMMP from water reported in the literature. The following describe studies that resulted in the destruction or removal of DMMP in laboratory settings; the methods may not be appropriate for removal of the contaminant from drinking water sources.

Radziemski (1981) studied the laser-induced photodestruction of DMMP in vapor and liquid phases. Photodissociation was observed at all wavelengths, but was most efficient with argon-fluoride (ArF) in the vapor phase in the presence of oxygen. DMMP irradiated at 266 nm had a carbon atom quantum yield of 1.0%, while gaseous DMMP irradiated at 193 nm had a yield of 8.4%. Decomposition products included hydrocarbon gases, CO, CO_2, H_2, and H_2O.

Fraser et al. (1985) studied the decomposition mechanisms and products of DMMP in an alternating current discharge. DMMP vapor was placed in a helium stream in an atmospheric pressure alternating current discharge. Efficiency has been determined to be 50-100% and is dependent upon the input concentration and flow rate. Primary discharge products were trimethyl phosphite and methanol.

As part of a program to investigate catalytic methods for the removal of toxic chemicals from air, Graven et al. (1966) studied the catalytic conversion of DMMP vapor over two types of platinum-alumina catalysts. Values for temperatures, residue times, and catalyst particle sizes were 300-500°C, 0.15-2.7 seconds, and 0.31-2.4 mm, respectively. Product analyses indicated that the initial reaction was hydrolysis to yield methanol and a phosphorus acid.

Aurian-Blajemi and Boucher (1989) studied the gas-solid interactions of DMMP with metal oxide surfaces at various temperatures (25-102°C) for various periods (0.5 hours to several days). In an activated process, DMMP adsorbed to the oxide surface. The adsorption was virtually irreversible; therefore, no significant catalysis was observed. The authors concluded that the metal oxides were good for the concentration stage of decontamination.

Even though DMMP strongly adsorbs to activated carbon (Berkowitz et al., 1978), the use of granular activated carbon (GAC) treatment, a common technology for removing organic contaminants from water, was not found in the literature.

XI. CONCLUSIONS

Based on the adverse liver effects in rats following oral (gavage) administration of DMMP for 13 weeks, the One-day, Ten-day, and Longer-term HA for exposure in a 10-kg child is 2 mg/L (2,000 μg/L); the Longer-term HA for exposure in a 70-kg adult is 6 mg/L (6,000 μg/L). A Lifetime HA of 0.1 mg/L (100 μg/L) for a 70-kg adult is based on a Drinking Water Equivalent Level (DWEL) of 7 mg/L (7,000 μg/L). The DWEL is based on a Reference Dose (RfD) of 0.2 mg/kg/day. The RfD is based on a LOAEL of 179 mg/kg/day, where the effect was reproductive toxicity in rats orally administered DMMP for 90 days. Based on the study by NTP (1987), DMMP is classified as Group C: Possible Human Carcinogen. The classification of DMMP in EPA Group C is based on limited (equivocal) evidence of carcinogenicity in animals and the absence of human data.

A comparison report "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for DMMP" (see Appendix) summarizes the scope of existing data reviewed for this HA. This comparison report delineates the areas where additional data and/or a clarification of existing data would be appropriate for a sound HA.

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APPENDIX

Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for DMMP

DATA BASE DEVELOPMENT

A. OBJECTIVES

The objective of this document is to provide an evaluation of data deficiencies and/or problem areas encountered in the review process for DMMP and to make recommendations, as appropriate, for additional data base development. This document is presented as an independent analysis of the current status of DMMP toxicology, as related to its possible presence in drinking water, and it includes a summary of the background information used in development of the Health Advisory (HA). For greater detail on the toxicology of DMMP, the Health Advisory on DMMP should be consulted.

B. BACKGROUND

Dimethyl methylphosphonate (DMMP) is a colorless liquid used in military munitions formulations.

No studies were found in the available literature on the pharmacokinetics of DMMP. However, acute, subchronic, and chronic oral toxicity studies in mice and rats indicate that DMMP is absorbed via the gastrointestinal tract.

No information was available on human occupational exposure to DMMP. Exposure may occur primarily under occupational settings at production sites where DMMP is used as a flame retardant and as a viscosity depressant in polyester and epoxy resins. Dimethyl methylphosphonate has been detected in groundwater at one military installation at levels that range from 6.5 μg/L to 1,300 μg/L (PMRMA, 1991).

In a repeated-insult patch test with humans, aqueous solutions of DMMP produced mild skin irritation at a concentration of 10% (v/v) and moderate to severe irritation at the 20% (v/v) level (Ciba-Geigy, 1976a).

Acute toxicity studies indicate that DMMP is practically nontoxic when administered to mice and rats via the oral route with LD_{50} values of >6,810 mg/kg in mice (NTP, 1987) and 10,190 mg/kg in rats (Ciba-Geigy, 1976b). Animals exhibited ataxia, tremors, ventricumbency, unsteady gait, muscular

hypertonia and hypotonia, prostration, and reductions in spontaneous motility. DMMP was slightly toxic by the intravenous route with LD_{50} values of 912 and 1,050 mg/kg in mice and rats, respectively (Ciba-Geigy, 1977a). DMMP elicited mild skin irritation and minimal ocular irritation in rabbits. DMMP was not a skin sensitizer in rabbits (Ciba-Geigy, 1976c,d,e,f,g).

Oral administration of DMMP in tapwater at 0, 1, 10, 100, or 1,000 mg/kg/day for 3 days to male and female rats produced no adverse effects on mortality, clinical signs, body weight, or food consumption. Plasma cholinesterase levels were depressed only in rats treated with 1,000 mg/kg/day; approximate reductions of 30% in males and 40-50% in females were observed over a 4-hour period as compared with control values taken at the same time. No effects on plasma cholinesterase levels were observed in rats at the other dose levels. The No-Observed-Adverse-Effect Level (NOAEL) was 100 mg/kg/day for plasma cholinesterase (Ciba-Geigy, 1978a). By comparison, mice dosed by gavage with DMMP in water at doses up to 2,000 mg/kg/day for 90 days did not exhibit depressed plasma cholinesterase levels (Dunnick et al., 1984b).

In a 15-day study with a limited number of mice and rats (five/sex/species), histologic changes were observed in the stomachs of male mice orally administered $\geq$1,250 mg DMMP/kg/day and in females orally administered $\geq$5,000 mg DMMP/kg/day. The Lowest-Observed-Adverse-Effect Level (LOAEL) was 1,250 mg/kg/day, based on the stomach changes in males. No gross pathological changes were seen in male or female rats at levels up to 15,000 mg/kg/day; however, mortality was seen in both sexes dosed at $\geq$5,000 mg/kg/day. Neither a NOAEL nor LOAEL was established in rats because a target organ for toxicity was not identified and histopathology was not conducted (NTP, 1987).

In a 4-week dietary study with rats, DMMP caused an increase in the protein resorption droplets in the proximal convoluted epithelium of the kidneys of males fed diets containing 2,000, 6,000, or 20,000 ppm (178, 535, or 1,790 mg/kg/day, respectively); the effect was more pronounced with increasing levels of DMMP. No kidney changes were observed in females (Ciba-Geigy, 1977C).

In a subchronic toxicity study, male and female mice were administered DMMP, by gavage, at doses ranging from 250 to 8,000 mg/kg/day, 5 days/week for 13

weeks. Except for mortality in both sexes at higher (4,000 or 8,000 mg/kg/day) dose levels, no adverse effects were observed on body weight, clinical signs, food consumption, gross necropsy, or histopathlogy. The stomach lesions observed in the 15-day study were not seen in this study. No compound-related cause for the mortality at the two upper levels was reported. Therefore, neither a NOAEL nor LOAEL was established (NTP, 1987).

In a subchronic toxicity study, male and female rats were administered DMMP, by gavage, at doses ranging from 250 to 4,000 mg/kg, 5 days/week for 13 weeks. DMMP, at doses ≥2,000 mg/kg/day, caused decreased survival, body weight gain, and increased liver-to-body weight ratios in males and females. Histopathology revealed kidney changes (nephrosis and hyaline droplet generation) in males at all dose levels starting at 250 mg/kg; no kidney changes were seen in females. The minimal to mild hypospermatogenesis seen in the testes of treated rats was not dose-dependent. Based on the increased relative liver weights in males and females, the LOAEL is 2,000 mg/kg/day and the NOAEL is 1,000 mg/kg/day (NTP, 1987).

In a chronic study, DMMP was administered, by gavage, to male and female mice at doses of 0, 1,000, or 2,000 mg/kg, 5 days/week for 103 weeks. Survival was decreased in males at both dose levels and in females at the high dose. The decreased survival was not treatment-related; it was due to fighting in males and dosing errors in females. Mean body weights were decreased in both sexes in the high-dose group, and the incidence of hepatocytomegaly was increased in treated males at both levels compared with vehicle controls. No increases in nonneoplastic liver lesions were seen in female mice. This study was judged to be inadequate owing to poor survival; therefore, neither a NOAEL nor LOAEL was established (NTP, 1987; Dunnick et al., 1988).

DMMP was administered, by gavage, at doses of 0, 500, or 1,000 mg/kg to male and female rats, 5 days/week for 103 weeks. Survival and mean body weights were decreased in both sexes at the high dose. Treatment-related kidney changes observed in males at both levels included nephropathy and calcification of the renal papilla. No kidney lesions were seen in females. Based on the significant body weight decreases, the LOAEL is 1,000 mg/kg/day (NTP, 1987; Dunnick et al., 1988) and the NOAEL is 500 mg/kg/day.

DMMP was found to be noncarcinogenic to female F344/N rats and male or female $B6C3F_1$ mice. However, the study with mice was judged to be inadequate to evaluate the carcinogenic potential of DMMP owing to poor survival in both sexes (see discussion above) (NTP, 1987).

Following oral administration at doses of 500 or 1,000 mg/kg, 5 days/week for 103 weeks, DMMP was carcinogenic to male F344/N rats, inducing transitional cell papillomas or carcinomas (combined) of the pelvic epithelium in the kidneys. In addition, DMMP increased the incidence of mononuclear cell leukemia in male rats. Male rats also developed renal tubular cell adenocarcinomas; however, this effect is considered to be associated with $alpha_{2\mu}$globulin and not relevant to humans (Baetcke et al., 1991). DMMP was not carcinogenic to male and female $B6C3F_1$ mice, but the study was considered to be inadequate owing to decreased survival in both sexes (NTP, 1987). DMMP is classified in Group C: Possible Human Carcinogen (NTP, 1987).

DMMP was found to be mutagenic by inducing forward mutations in mouse lymphoma cells in the absence of metabolic activation and sister chromatid exchanges in Chinese hamster ovary (CHO) cells with and without activation (NTP, 1987). It gave limited evidence of clastogenicity in CHO cells in the absence of metabolic activation (Sivak, 1983; NTP, 1987). Tice (1990) demonstrated that DMMP induced forward mutations in the CHO cell HGPRT assay with and without metabolic activation. In vivo, DMMP induced sex-linked recessive lethal mutations in Drosophila (NTP, 1987). DMMP was nonmutagenic in bacterial mutagenicity tests in several strains of Salmonella (Ciba-Geigy, 1976h, 1978c; Sivak, 1983; Mortelmans et al., 1986), did not increase neoplastic transformations in BALB/c 3T3 cells (Sivak, 1983), and did not induce reciprocal translocations in Drosophila in vivo (NTP, 1987). DMMP gave positive results in dominant-lethal assays in mice and rats (Dunnick et al., 1984a,b).

DMMP was shown to be toxic to the male reproductive system. In male rats following oral administration at doses ranging from 250 to 2,000 mg/kg, 5 days/week for 90 days, DMMP induced dose-dependent decreases in sperm motility, sperm counts, and male fertility index. Histologic changes were observed in the testes, epididymis, and prostate glands. DMMP altered reproductive functions at all dose levels by causing a decrease in the number

of live fetuses per litter, and an increase in the percentage of resorptions. The LOAEL for reproductive effects was the lowest dose tested, 250 mg/kg/day (Dunnick et al., 1984a). In an inhalation study, exposure of male rats to DMMP vapors at concentrations of 25 or 250 ppm continuously for 90 days induced histologic changes in the testes and epididymis (Mattie et al., 1987).

DMMP was not found to be teratogenic. No developmental effects were observed in mice following oral administration of 4,175 mg/kg/day on gestation days 6-13 (Hardin et al., 1987). DMMP induced minimal fetotoxicity (reduced fetal weight and delayed skeletal maturation) only at maternally toxic (1,000 or 2,000 mg/kg/day) doses in rats dosed during gestation days 6-15. No maternal toxicity, embryotoxicity, or fetotoxicity was observed at the 100-mg/kg/day dose. DMMP did not alter fetal anomalies (Ciba-Geigy, 1978b).

No delayed neurotoxicity (based on histopathology) was seen in hens following oral administration of 1,999 or 3,998 mg DMMP/kg initially and after 21 days (Ciba-Geigy, 1978d). Birds exhibited acute toxicity including ataxia, curved position, apathy or sedation, and fluttered feathers. In another study, an intraperitoneal injection of 50 mg DMMP/kg for 10 days produced no delayed neurotoxicity in hens (Hollingshaus et al., 1981).

Based on the adverse liver effects of rats administered DMMP via gavage, the Longer-term Health Advisory (HA) for exposure in a 10-kg child has been determined to be 70 mg/L (70,000 μg/L). In the absence of adequate animal data to determine a One-day or Ten-day Health Advisory, the Longer-term HA for a 10-kg child, 70 mg/L (70,000 mg/L), is used as a conservative estimate of the One-day or Ten-day HA. The Longer-term HA for an adult is 250 mg/L (250,000 μg/L). A Lifetime HA of 0.1 mg/L (100 μg/L) for a 70-kg adult was determined based on a Drinking Water Equivalent Level (DWEL) of 7 mg/L (7,000 μg/L). The DWEL is based on a Reference Dose (RfD) of 0.2 mg/kg/day. The RfD is based on a LOAEL of 179 mg/kg/day where the toxic endpoint was adverse reproductive effects in rats following oral (gavage) administration for 90 days.

C. DISCUSSION

Available data on the pharmacokinetics, health effects, analysis, and treatment of DMMP have been reviewed.

No data were available on the pharmacokinetics of DMMP either in humans or animals. Acute, subchronic, and chronic toxicity studies in mice and rats indicate that DMMP is absorbed via the oral route. Therefore, the data gap on pharmacokinetics needs to be filled in evaluating the pharmacokinetics of DMMP.

Human exposure data were not available. No studies evaluating the systematic toxicity of DMMP were available. Only one study evaluating the skin irritation and sensitization potential of DMMP in humans was available. Further studies are needed to fill the data gap in humans.

Acute toxicity studies for DMMP administered via the oral, inhalation, intravenous, and dermal routes in mice, rats, and rabbits were available. DMMP was practically nontoxic via the oral and dermal routes and slightly toxic via the intravenous route.

Fifteen-day oral toxicity studies in mice and rats were available. Effects were seen in the stomachs of both sexes of mice, with males being more sensitive than females. However, similar treatment-related effects were not seen in mice treated for 13 or 103 weeks in followup studies.

A 30-day dietary study with rats showed renal effects in male rats; no renal or other effects were seen in female rats.

Five longer term studies (90 days) were available: four examined the effects of DMMP following gavage administration (mice and rats) and, in the fifth, rats were exposed via inhalation to DMMP vapors. In the study with mice, except for mortality at high levels, no treatment-related systemic toxicity was observed. Histopathology from three of the studies revealed kidney nephrosis and accumulation of hyaline droplets in the convoluted tubules of the renal cortex in treated males; no kidney lesions were seen in females. The kidney lesions are associated with $alpha_{2\mu}$globulin found only in male rats

and, therefore, are not relevant to humans. These studies indicated a LOAEL of 250 mg/kg/day for reproductive toxicity. An inhalation study showed testicular lesions.

Two lifetime studies (mice and rats) evaluated the chronic toxicity and carcinogenicity of DMMP following oral (gavage) administration for 103 weeks. Except for an increase in the incidence of hepatocytomegaly in male mice, no chronic toxicity or carcinogenicity was observed in mice. However, the study was considered inadequate owing to poor survival in both sexes; this was attributed to fighting among males and dosing errors in females. DMMP did not induce chronic toxicity and was not carcinogenic to female rats. It was, however, carcinogenic to male rats, inducing hyperplasia of the transitional epithelium, and transitional cell papillomas or carcinomas (combined) of the pelvic epithelium in the kidneys and mononuclear cell leukemia. Renal tubular cell hyperplasia and carcinoma are considered to be associated with $alpha_{2\mu}$-globulin and not relevant to humans. Based on significant body weight decreases in male and female rats, the lifetime studies indicated a LOAEL of 1,000 mg/kg/day and a NOAEL of 500 mg/kg/day.

Three studies in male rats (two gavage studies and one inhalation study) examined the reproductive toxicity of DMMP. DMMP was shown to be a reproductive toxin. It induced lesions in the testes, epididymis, and prostate glands and affected reproductive functions in males. The LOAEL established was 250 mg/kg/day.

Available developmental toxicity studies in mice and rats found DMMP not to be a teratogen. Not all categories of genotoxicity were investigated. A mutagenicity test in an eukaryotic microorganism with and without activation and tests for DNA damage/repair would fulfill this data.

Available acute neurotoxicity studies (oral and intraperitoneal) in hens showed that DMMP was not a neurotoxin. However, additional studies (21 days) are needed to substantiate this finding.

D. CONCLUSIONS/RECOMMENDATIONS

Based on the above discussion, the following conclusions/recommendations can be made:

1. The available studies on DMMP toxicity are adequate for development of Longer-term and Lifetime Health Advisories and estimating One-day and Ten-day Health Advisories which are useful in dealing with the potential contamination of drinking water. It is recommended that appropriate subacute studies be performed to address emergency exposure for One- and Ten-day Health Advisories.

2. It is recommended that a 21-day neurotoxicity study be performed in hens and that brain and blood (RBC and plasma) cholinesterase studies be performed for a minimum of 14 days in appropriate animal models.

3. It is recommended that toxicokinetic studies be performed in animals and humans.

4. It is recommended that a teratology study be performed in rabbits.

5. It is recommended that additional genotoxicity studies be performed in microbial and nonmicrobial cell systems.

6. It is recommended that epidemiological studies of people exposed occupationally to DMMP be conducted to elucidate specific health effects.

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