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Provisional Peer Reviewed Toxicity Values for

Dimethyl methylphosphonate
(CASRN 756-79-6)

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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR DIMETHYL METHYLPHOSPHONATE (CASRN 756-79-6)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Neither RfD nor RfC values for dimethyl methylphosphonate are listed on IRIS (U.S. EPA, 2006) or the HEAST (U.S. EPA, 1997). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) includes an RfD of 0.2 mg/kg-day for dimethyl methylphosphonate that was derived in a Health Advisory document (U.S. EPA, 1992). The RfD was based on a LOAEL of 179 mg/kg-day for reproductive toxicity (increased resorptions in untreated female rats impregnated by subchronically-treated males) in a study by Dunnick et al. (1984a) and an uncertainty factor of 1000 (10 each for inter- and intra-species variability and 10 for use of a LOAEL). A cancer assessment for dimethyl methylphosphonate is not listed on IRIS (U.S. EPA, 2006) or the HEAST (U.S. EPA, 1997). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) includes a U.S. EPA (1986) cancer weight-of-evidence classification of Group C, possible human carcinogen that was derived in the Health Advisory document (U.S. EPA, 1992). This document also derived an oral slope factor of 5.0E-3 $(mg/kg\text{-}day)^{-1}$ based on the combined incidences of mononuclear cell leukemia and kidney tumors (pelvic transitional cell papilloma and carcinoma) in male rats exposed to dimethyl methylphosphonate. The CARA list (U.S. EPA, 1991b, 1994) does not include any documents for dimethyl methylphosphonate. No toxicological reviews are available from ATSDR (2006),

IARC (2006), or WHO (2006). ACGIH (2006), NIOSH (2006), and OSHA (2006) have not established occupational exposure limits for dimethyl methylphosphonate. The NTP (2003a,b) management status and health and safety reports were consulted for relevant information. Literature searches were conducted for the period from 1965 to March 2003 in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS. Additional literature searches from March 2003 through September 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure. No relevant data were located regarding toxicity or carcinogenicity in humans following oral exposure to dimethyl methylphosphonate.

Inhalation Exposure. No relevant data were located regarding toxicity or carcinogenicity in humans following inhalation exposure to dimethyl methylphosphonate.

Animal Studies

Oral Exposure. NTP (1987; partly described in Dunnick et al., 1988) conducted chronic and subchronic gavage assays in rats and mice. In the chronic rat bioassay, 0, 500 or 1000 mg/kg-day of dimethyl methylphosphonate (>98%) was administered to F344/N rats (50 per sex per group) by gavage in corn oil 5 days/week for 103 weeks. Rats were observed twice daily for clinical signs and weighed weekly for the first thirteen weeks and once every four weeks thereafter; the study was terminated at week 105. All rats received a gross necropsy examination and histopathological examination of gross lesions and more than 35 tissues. There was a dose-related trend for reduced survival in both sexes during the last year of the study; survival was significantly reduced in males at $\geq$500 mg/kg-day and in females at 1000 mg/kg-day. After week 80, mean body weights were reduced by 10-24% in high-dose males and by 8-12% in high-dose females compared to the controls. No compound-related clinical signs were recorded. There was a dose-related increase in the incidence of mononuclear cell leukemia in male rats that was statistically significant at the high dose: 10/50, 11/50, and 17/50 in the control, low- and high-dose groups, respectively. Compound-related nonneoplastic effects were observed in the kidney of male, but not female, rats. Although the incidence of nephropathy (degeneration of tubular epithelium, tubular dilatation with attenuation and atrophy of the epithelium, granular casts, thickening of basement membranes, and accumulation of interstitial collagen) was similar in control, low- and high-dose males (72, 86 and 82%, respectively), the severity was higher with treatment (1.9, 2.5 and 2.8, respectively, on a scale from 1 = minimal to 4 = marked). Increased incidences of calcification of the renal papilla and hyperplasia of cortical tubules and pelvic epithelia were also observed in males at $\geq$500 mg/kg-day. Various types of renal tumors developed in both treated male groups, but these were attributed to alpha $_{2u}$ globulin and are not considered to be relevant to humans (U.S. EPA, 1991c). There was no evidence of carcinogenicity in female rats. No treatment-related nonneoplastic lesions were observed in

female rats. Aside from the increased incidences of mononuclear cell leukemia and the renal effects in male rats, no specific target organs were identified. In this study, the dose of 500 mg/kg-day was associated with reduced survival in males, while the high dose of 1000 mg/kg-day was associated with reduced survival and reduced body weight gain in rats of both sexes.

For the 103-week gavage study in $B6C3F_1$ mice, NTP (1987; partly described in Dunnick et al., 1988) followed the same protocol as described for the rat except that the doses were 0, 1000 or 2000 mg/kg-day, 5 days/week. The analysis of survival was complicated by deaths caused by aggression in high-dose males during weeks 23-44 (after which aggressive males were housed individually) and by deaths of 17 male and 22 female high-dose mice at week 45 (caused by the accidental administration of a high dose that was 34% higher than targeted). The dosing-error deaths were censored for the survival analysis. There was a dose-related trend for reduced survival in both sexes; for the control, low- and high-dose groups, survival to 103 weeks was 28/50, 12/50 and 0/50 for males and 41/50, 30/50 and 2/50 for females. The significant reductions in survival occurred in low-dose males after week 95, in high-dose males after week 23 and in high-dose females after week 52. Mean body weights of high-dose groups were reduced in both sexes compared to controls: by 7-16% in males transiently between weeks 36 and 76 and by 6-12% in females after week 88. Exposure to dimethyl methylphosphonate had no effect on the incidence of clinical signs. Treated male, but not female, mice exhibited an increase in centrilobular hepatocytomegaly (incidence of 5/50, 17/50 and 10/46 in the control, low- and high-dose males, respectively). No other nonneoplastic microscopic lesions were attributed to treatment in male or female mice. Increased centrilobular hepatocytomegaly in male mice at $\geq$1000 mg/kg-day was associated with reduced survival at these same dose levels. The analysis of carcinogenicity in both treated male groups and in the high-dose female group was inadequate because of poor survival. No carcinogenic effect was observed in the low-dose female group, for which survival was similar to the controls. Overall, this study is not an adequate test of the carcinogenic potential of dimethyl methylphosphonate in mice.

NTP (1987) conducted two subchronic gavage studies in rats because the first study was discovered to have been compromised by dosing errors; this first study is not considered further. In the second study, groups of F344/N rats (10/sex/dose) were given dimethyl methylphosphonate by gavage in corn oil at doses of 0, 250, 500, 1000, 2000 or 4000 mg/kg-day, 5 days/week for 13 weeks. All animals were observed twice daily for clinical signs and body weights were recorded weekly. At termination, necropsies were performed on all rats, liver weights were recorded, and more than 30 tissues, plus those with gross lesions, were preserved. Histopathology was evaluated in control and highest-dose rats and all rats dying prematurely. All rats receiving 4000 mg/kg-day died during the first week and are not considered further. At 2000 mg/kg-day, only 4/10 males and 7/10 females survived to the end of the study; the male deaths occurred between weeks 7 and 13, whereas the female deaths occurred between weeks 1 and 10. Terminal body weights were slightly reduced in rats treated at 2000 mg/kg-day, but the differences were not considered to be biologically significant (less than 10% change). Treatment had no effect on the occurrence of clinical signs in either sex. At 2000 mg/kg-day, relative liver weights were increased in both sexes and absolute liver weights were increased in males. Histopathological lesions of the kidney (nephrosis and hyaline droplet degeneration) were increased in all dosed male groups; however, these lesions are considered to be related to male rat-specific alpha $_{2u}$ globulin and not relevant to humans (U.S. EPA, 1991c).

Hypospermatogenesis (minimal to mild) was observed in the testis at 2000 mg/kg-day. No compound-related histopathology was observed in female rats. In this study, 1000 mg/kg-day is a NOAEL. Increased mortality, liver effects (increases in relative weights in both sexes and absolute weights in males), and testicular effects (hypospermatogenesis) were seen at 2000 mg/kg-day.

The subchronic NTP (1987) study in $B6C3F_1$ mice followed the protocol for rats except that groups of 10/sex were dosed with 0, 250, 500, 1000, 2000 and 4000 mg/kg-day and 7 males and 6 females were dosed at 8000 mg/kg-day, 5 days/week for 13 weeks. In addition, histopathology was conducted in the control and two highest dose groups. Nearly all mice died that were exposed at $\geq$4000 mg/kg-day, but no cause of death was reported. No deaths were observed in the lower dose groups. No significant treatment-related effects were observed in body weight gain, or the incidences of clinical signs, gross lesions or microscopic lesions. The NOAEL in this study is 2000 mg/kg-day; 4000 mg/kg-day is a FEL for increased mortality in male and female mice.

Ciba-Geigy (1977) fed diets containing 0, 2000, 6000 or 20,000 ppm of dimethyl methylphosphonate to groups of Sprague-Dawley rats (5/sex/dose) for four weeks. Intakes were reported as 0, 178, 535 or 1790 mg/kg-day. An additional group of high-dose rats was given a four-week recovery period following treatment. Clinical signs were monitored daily and body weight and food consumption were measured weekly. Ophthalmoscopic examinations were conducted prior to exposure and on 5 rats/sex in the 20,000 ppm group during study weeks 5 and 8. At termination, hematological and clinical chemistry tests and urinalysis were conducted on all rats. Gross necropsies were conducted on all animals and included weight measurements of selected organs: adrenals, kidneys, heart, brain, liver and gonads. All major organs were examined for histopathology. Treatment with dimethyl methylphosphonate had no effect on survival, body weight, food consumption, the incidence of clinical signs, hematology, clinical chemistry, urinalysis or ophthalmoscopic findings. Absolute and relative kidney weights were elevated in males at 20,000 ppm and females at $\geq$6000 ppm; absolute and relative liver weights were significantly elevated in males at 20,000 ppm. Histopathology in the kidney (hyaline droplets in the proximal convoluted tubules) was observed in male rats exposed at $\geq$2000 ppm; however, as this effect is related to male rat-specific alpha $_{2u}$ globulin, it is considered not relevant to humans. In the absence of liver histopathology, the increased liver weight in male rats at 20,000 ppm may be regarded as adaptive and not adverse. Therefore, the highest dietary level of 20,000 ppm (1790 mg/kg-day) was a NOAEL in this study.

In a developmental toxicity assay, Ciba-Geigy (1978) exposed groups of 25 pregnant female Sprague-Dawley rats with dimethyl methylphosphonate by gavage in 2% carboxymethylcellulose (CMC) at doses of 100, 1000 or 2000 mg/kg-day on GD 6-15. Dams were sacrificed on GD21. Feed intake was slightly reduced in dams at 1000 mg/kg-day; both feed intake and body weight gain were reduced in dams at 2000 mg/kg-day. Fetal toxicity (reduced fetal weight and delayed ossification of the skeleton) at $\geq$1000 mg/kg-day was attributed to maternal toxicity at those doses. Treatment had no effect on the incidence of fetal anomalies. The NOAEL for maternal toxicity was 100 mg/kg-day and the LOAEL was 1000 mg/kg-day for reduced food consumption. The NOAEL for fetal toxicity was 100 mg/kg-day and the LOAEL was 1000 mg/kg-day for reduced fetal weight and delayed ossification.

In another experiment, Ciba-Geigy (1978) exposed groups of 25 pregnant Sprague-Dawley rats to dimethyl methylphosphonate by gavage in 2% CMC at 2000 mg/kg-day on GD6-15 or at 2500 mg/kg-day on GD6-10. Both treatments reduced food consumption and the higher dose reduced maternal body weight gain. Fetal body weights were slightly reduced and ossification was delayed at both doses. No gross malformations or visceral or skeletal anomalies were observed at 2000 mg/kg-day. A few anomalies detected at 2500 mg/kg-day were considered to be in the normal range for the strain of rat. In this study, 2000 mg/kg-day was a LOAEL for maternal toxicity (reduced food intake) and fetal toxicity (reduced fetal weight and delayed ossification).

The only developmental toxicity study for mice was a single-dose level screening assay (Hardin et al., 1987). Groups of 50 pregnant female CD-1 mice were given dimethyl methylphosphonate by gavage in corn oil at a dose of 0 or 4175 mg/kg-day on gestational days (GD) 6-13. Dams were allowed to litter. Treatment had no effect on dams, the number of pups/litter, pup survival or fetal body weight gain. However, pup birth weights were significantly lower in the treated group compared to controls. In this study, 4175 mg/kg-day is a LOAEL for reduced birth weight in mice treated during gestation.

The reproductive toxicity of dimethyl methylphosphonate was evaluated in groups of 20 male F344 rats exposed by gavage in water at doses of 0, 250, 500, 1000 or 2000 mg/kg-day, 5 days/week over a 90-day period (Dunnick et al., 1984a). On day 84, each treated male was mated with two untreated female F344 rats (40/group). The males were sacrificed on day 90 and the kidneys, testes, epididymis and prostate were weighed and evaluated for histopathology. At sacrifice, plasma levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined and sperm samples were analyzed for sperm counts, motility and morphology. All females were sacrificed on day 100 and their uteri were removed for examination of the number of live and dead pups and the number of resorptions. Treatment had no effect on survival (unlike the results of the 90-day NTP study which used corn oil as a vehicle), and there were no clinical signs or overt indications of neurotoxicity. Body weight gain was significantly reduced by 11% in the 2000 mg/kg-day-group compared to the controls. Relative kidney weights were significantly increased in the $\geq$1000 mg/kg-day males and relative epididymal weights were reduced in the 2000 mg/kg-day-group. Alpha $_{2u}$ globulin-associated lesions were observed in the kidney of treated males at all doses. Testicular lesions in nearly all males treated at 2000 mg/kg-day included degeneration, vacuolization, necrosis and lack of spermatogenesis. Prostate changes in one rat at 1000 mg/kg-day and four rats at 2000 mg/kg-day included multifocal infiltration of lymphocytes and dilation of acini containing debris. Sperm parameters showed dose-dependent changes that were significant at the higher doses: decreased sperm motility at $\geq$1000 mg/kg-day, and decreased sperm counts and increased sperm head abnormalities at 2000 mg/kg-day. Male fertility was completely abolished at 2000 mg/kg-day and showed dose-related decreases in the other groups; fertility indices were 70, 75, 60, and 0% in the control and low-to-high treated groups. The percentage of pregnant females was significantly reduced at $\geq$1000 mg/kg-day: 50, 47.5, 42.5, 27.5 and 0% in the control and treated groups. The number of live fetuses per litter was significantly reduced at $\geq$500 mg/kg-day: 7.6, 7.8, 5.7, 0.82 and 0 in the control and treated groups. The percentage of resorptions was significantly increased at $\geq$250 mg/kg-day: 6.1, 14.9, 39.4, and 79.1 in the control and treated

groups that had pregnancies. The lowest dose in this study, 250 mg/kg-day, is a LOAEL for increased resorptions in untreated females mated with treated males.

Chapin et al. (1984) evaluated reproductive lesions in groups of 49 male F344 rats given dimethyl methylphosphonate by gavage in tap water at 1750 mg/kg-day, 5 days/week for up to 12 weeks; a group of fourteen control rats received tap water only. After 3, 4, 5, 7, 9 and 12 weeks, groups of seven treated and two control rats were sacrificed and the testes and epididymis were examined for microscopic lesions. The remaining group of nine rats were maintained for fourteen weeks and analyzed as above. Body weights were reduced after the fifth week of treatment. Epididymal weight gains were also impaired. Histopathology of the testis (focal exfoliation) and epididymis (decreased sperm density) was first observed after five weeks and increased in incidence and severity thereafter. Abnormalities were noted in Sertoli cells and sperm morphology. After 14 weeks of recovery, about 80% of seminiferous tubules were normal, but the remainder showed loss of normal epithelial organization. The single administered dose level of 1750 mg/kg-day is a LOAEL for reduced epididymal weight and increases in testicular pathology and sperm abnormalities in rats.

Reproductive toxicity endpoints in mice were evaluated in a dominant lethal mutation assay (Dunnick et al., 1984b). Groups of 20 male $B6C3F_1$ mice received dimethyl methylphosphonate by gavage in water at doses of 0, 250, 500, 1000 or 2000 mg/kg-day, 5 days/week for 13 weeks. After 12 weeks of treatment, the males were mated during a five-day period to untreated CD-1 female mice. Females were sacrificed 16 days after the third mating day and the uterine contents were examined for the number of live and dead implants (resorptions). After 13 weeks of treatment, male mice were evaluated for body weight, plasma levels of cholinesterase, LH and FSH, sperm concentration and morphology, selected organ weights (kidney, prostate gland, testis and epididymis), and histopathology of the genitourinary system, pituitary gland, and thymus. No compound-related effects were observed on mortality, body weight, relative organ weights (testis, epididymis, prostate or kidney), histopathology findings, or sperm parameters (concentration and morphology). Plasma levels of cholinesterase, LH and FSH were also unaffected. Treatment had no effect on the fertilization rate. Treatment for 12 weeks at $\geq$1000 mg/kg-day significantly reduced the number of live implants per dam and increased the number of resorptions per dam and the percentage of resorptions (dead implants/total implants). The percentage of dominant lethal mutations was significantly increased at $\geq$1000 mg/kg-day. The NOAEL was 500 mg/kg-day and the LOAEL was 1000 mg/kg-day for effects on reproductive function in male mice leading to reduced live implants and increased resorptions indicative of increased dominant lethal mutations.

Inhalation Exposure. The database of available information regarding the effects of inhalation exposure to dimethyl methylphosphonate in animals is restricted to published abstracts only. Mattie et al. (1987) exposed an unspecified number of male F344 rats to vapors of dimethyl methylphosphonate at concentrations of 25 or 250 ppm (127 or 1269 mg/m^3) continuously for 90 days. No methodological details were available except that the testes were evaluated for histopathology and some animals were sacrificed one year after the end of exposure. Diffuse degeneration of the seminiferous epithelium was observed in the testes of treated rats and degeneration was still evident after a one-year recovery period. The abstract did not specify whether the effect was observed at 25 ppm in addition to 250 ppm. Another abstract by the

same investigators reported that male F344 rats developed hyaline droplet nephropathy following subchronic inhalation exposure to an unspecified concentration of dimethyl methylphosphonate (Mattie and Hixson, 1988). These reports do not provide sufficient detail to serve as the basis for an RfC for dimethyl methylphosphonate.

Other Studies

Dimethyl methylphosphonate is rapidly absorbed by the oral route and primarily eliminated in urine (Blumbach et al., 2000). Following administration of single gavage doses of 50 or 100 mg/kg to male and female F344 rats, the urinary elimination half-life was 3.3 hours for the parent compound and 3.4 hours for the hydrolysis product methyl methylphosphonate. The percentage of the applied dose eliminated in urine was 58.5-68.1% for male rats and 87.8-92.8% for female rats. Blumbach et al. (2000) confirmed using immunoblotting methods that alpha $_{2u}$ globulin protein accumulated in the kidney of male, but not female rats after gavage dosing on five consecutive days. Gas chromotography analysis verified that dimethyl methylphosphonate was sequestered in the alpha $_{2u}$ globulin fraction isolated from the kidney of male rats, which probably contributed to the lower recovery in the urine of male rats compared to females.

Results for *in vitro* genotoxicity assays of dimethyl methylphosphonate have been variable. Dimethyl methylphosphonate was not mutagenic to *Salmonella typhimurium* strains TA98, TA100, TA 1535 and TA 1537, with or without metabolic activation (U.S. EPA, 1992; Sivak, 1983). The compound induced forward mutations in mouse lymphoma L5187Y/TK$^{+/-}$ cells both in the absence and in the presence of metabolic activation (NTP, 1987; Tice, 1990). It did not induce mutations in Chinese hamster ovary (CHO) cells (HGPRT assay) without metabolic activation (Sivak, 1983). Dimethyl methylphosphonate induced chromosomal aberrations in CHO cells at the highest tested concentration (1 mg/mL) without activation in one assay (Sivak, 1983), but did not induce aberrations when tested at concentrations as high as 22 mg/mL in another assay, with or without activation (NTP, 1987). No significant increases in sister chromatid exchanges were obtained in CHO cells tested at concentrations as high as 1 mg/mL in one assay (Sivak, 1983), but positive results occurred in another assay, both in the absence (over a concentration range of 0.16-11 mg/mL) and presence (at 1.1-22 mg/mL) of metabolic activation (NTP, 1987). Dimethyl methylphosphonate did not induce neoplastic transformations in cultured BALB/c 3T3 cells (Sivak, 1983).

Dimethyl methylphosphonate gave positive results in *in vivo* genotoxicity assays. When fed to *Drosophila*, the compound induced significant increases in the frequency of sex-linked recessive lethal mutations, but did not induce reciprocal translocations (NTP, 1987; Foureman et al., 1994). Dominant lethal mutations were induced in male F344/N rats and B6C3F$_1$ mice following oral exposure for 5 days/week for 13 week (Dunnick et al., 1984a,b). After mating with untreated females, the mutations were manifest as significant increases in early resorptions in rats at doses as low as 250 mg/kg-day and in mice at doses of 1000 or 2000 mg/kg-day.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR DIMETHYL METHYLPHOSPHONATE

No data are available for the toxicity of dimethyl methylphosphonate in humans exposed orally. Impaired reproductive function is the most sensitive relevant effect of oral exposure to dimethyl methylphosphonate in male rats (Dunnick et al., 1984a). After mating unexposed females with males treated 5 days/week for 13 weeks by gavage in water, statistically significant effects included a dose-related increase in resorptions at ≥250 mg/kg-day, reductions in the number of live fetuses per litter at ≥500 mg/kg-day, reductions in the percent of pregnant females at ≥1000 mg/kg-day and the total loss of male fertility at 2000 mg/kg-day. These effects are undoubtedly related to the overt effects on the testis and sperm observed at the higher doses: the significant loss of sperm motility at ≥1000 mg/kg-day, and the reduced sperm counts and increased sperm head abnormalities and testicular histopathology at 2000 mg/kg-day. The data are supported by the reproductive effects in male rats exposed at 1750 mg/kg-day, 5 days/week for 12 weeks (Chapin et al., 1984). Mice appear to be less vulnerable to reproductive toxicity than rats, since the only effects noted in the parallel experiment by Dunnick et al. (1984b) were decreases in live implants and increases in resorptions at ≥1000 mg/kg-day.

The standard chronic and subchronic rodent bioassays do not provide usable data for deriving a provisional RfD for dimethyl methylphosphonate. Renal toxicity in male rats was the most sensitive systemic effect observed in the NTP (1987) and Dunnick et al. (1988) bioassays, but this is a species-specific effect related to alpha $_{2u}$ globulin and not relevant to humans (U.S. EPA, 1991c). Reduced body weight gain was observed in female rats and male and female mice at relatively high doses, but these were levels that also reduced survival. It appears that the use of corn oil as a vehicle contributed to the higher mortality rates in the NTP (1987) and Dunnick et al. (1988) systemic toxicity assays compared to the reproductive toxicity assays (Dunnick et al., 1984a,b) that employed an aqueous vehicle.

The LOAEL of 250 mg/kg-day for increased resorptions from the study by Dunnick et al., (1984a) is selected as the basis for the provisional RfD. The LOAEL is first multiplied by 5/7 to average the exposure over seven days per week, resulting in a duration-adjusted LOAEL of 179 mg/kg-day. An uncertainty factory of 3000 is applied to the duration-adjusted LOAEL (10 to account for interspecies extrapolation, 10 for human variability, 10 for the use of a LOAEL, and 3 for database deficiencies, including the lack of reproductive toxicity testing in females and the limited developmental toxicity testing in mice). As the critical effect is based on male reproductive toxicity and rats were exposed for 84 days (sufficient for approximately 6.5 13-day cycles of seminiferous epithelium) prior to testing, the derivation of the chronic RfD does not require an additional uncertainty factor to account for less-than-chronic duration of exposure. Therefore, the provisional **subchronic and chronic RfD is 6E-2 mg/kg-day** for dimethyl methylphosphonate.

Confidence in the critical reproductive study is medium since it was well-conducted and adequately reported, but did not identify a NOAEL. Confidence in the database is medium because of the technical errors in the chronic mouse study, the lack of reproductive toxicity testing in females and the fact that the assessment of developmental toxicity in mice is limited to a screening assay. There is medium confidence in the p-RfD results.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR DIMETHYL METHYLPHOSPHONATE

Subchronic inhalation exposure to dimethyl methylphosphonate vapor at concentrations of 25 or 250 ppm (127 or 1269 mg/m^3) resulted in toxicity to the testis (degeneration of the seminiferous epithelium) of male rats (Mattie et al., 1987). Kidney effects (hyaline droplet nephropathy) also occurred in male rats in a subchronic inhalation study (Mattie and Hixson, 1988), but the exposure levels were not reported. The kidney effects are related to alpha $_{2u}$ globulin accumulation, which is specific to male rats and not considered to be predictive of renal effects in humans (U.S. EPA, 1991c). Since most experimental details are lacking for these studies, data are insufficient for deriving a p-RfC for dimethyl methylphosphonate.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR DIMETHYL METHYLPHOSPHONATE

Weight-of-evidence Classification

There are no data for the carcinogenicity of dimethyl methylphosphonate in humans. U.S. EPA (1992) derived an oral cancer slope factor of 5.0E-3 (mg/kg/day)$^{-1}$ for dimethyl methylphosphonate based on combined increased incidences of mononuclear cell leukemia and renal tumors in male F344/N rats treated by gavage for 103 weeks (NTP, 1987; Dunnick et al., 1988). However, more recent evidence indicates that the kidneys of treated male rats accumulate alpha $_{2u}$ globulin, a male rat-specific protein implicated in renal toxicity and carcinogenicity in male rats (U.S. EPA, 1991c; Blumbach et al., 2000). Since the renal tumor data are not relevant to humans, they should not be considered for the carcinogenicity assessment. Mononuclear cell leukemia (also called “large granular lymphocytic leukemia” or LGL leukemia) is a spontaneous, rapidly fatal neoplasm that is common (average incidence of 28.1%) in aged untreated F344 rats in 2-year carcinogenicity studies by the NTP (Haseman et al., 1998). Caldwell (1999) has argued that the increased incidences of mononuclear cell leukemia in F344 rats treated with phthalates were not relevant to humans because the equivalent cell type (based on morphological criteria) does not exist in humans or other animals, the course of disease differed between species, and because phthalates are not genotoxic and induce leukemia only at high dose levels. However, the species-dependent arguments are weakened by a reliance on relatively old literature (largely pre-dating 1990) and an overly strict definition of the apparently related LGL leukemia in humans. Reviews of human LGL leukemia concluded that the disease has a diverse origin (NK or T cells) and a wide spectrum of acute or chronic clinical presentations (Lamy and Loughran, 1998; Wong, 2002). Canine LGL leukemias also may present as acute or chronic diseases, either of which may be caused by NK or T cells (Vernau and Moore, 1999). Thus, there is no definitive evidence that mononuclear cell leukemia in rats is not relevant to humans. Furthermore, the example of the phthalates may not be applicable here because, dimethyl methylphosphonate could be genotoxic since it not only increased the incidence of mononuclear cell leukemia in rats, but also reduced tumor latency. In this carcinogenicity assessment for dimethyl methylphosphonate, increases in the incidence of mononuclear cell leukemia in treated rats are considered relevant to the evaluation of potential carcinogenicity to humans.

Under the current guidelines for carcinogen risk assessment (U.S. EPA, 2005), the compound is characterized as having *suggestive evidence of carcinogenic potential* based on a significant positive trend and significant increases over concurrent and historical controls for the incidence of mononuclear cell leukemia in male rats exposed to 1000 mg/kg-day by gavage, 5 days/week over 103 weeks (NTP, 1987). There was no evidence of treatment-induced tumors in female rats treated at 500 or 1000 mg/kg-day or in female mice treated at 1000 mg/kg-day; assessments of carcinogenicity in male mice treated at 1000 or 2000 mg/kg-day and female mice treated at 2000 mg/kg-day were inadequate because of reduced survival.

Similar carcinogenicity results were reported for a related phosphorus-containing compound, dimethyl morpholinophosphoramidate, which caused increased incidences of mononuclear cell leukemia in male and female F344/N rats, but not in B6C3F1 mice exposed by gavage (NTP, 1986).

Inconsistent results from mutagenic and genotoxic tests for dimethyl methylphosphonate (U.S. EPA, 1992; Sivak, 1983; NTP, 1987; and Tice, 1990) do not support a conclusive mode of action. Negative tests include, mutagenic results in *Salmonella typhimurium* strains TA98, TA100, TA 1535 and TA 1537, with or without metabolic activation (U.S. EPA, 1992; Sivak, 1983), and no induction of mutation in Chinese hamster ovary (CHO) cells (HGPRT assay) without metabolic activation (Sivak, 1983). Furthermore, dimethyl methylphosphonate did not induce neoplastic transformation in cultured BALB/c 3T3 cells (Sivak, 1983). Varying concentrations of dimethyl methylphosphonate also gave mixed results. Chromosomal aberrations were induced in CHO cells at the highest tested concentration (1 mg/mL) without activation in one assay (Sivak, 1983), but did not induce aberrations when tested at concentrations as high as 22 mg/mL in another assay, with or without activation (NTP, 1987). Positive tests included induction of forward mutations in mouse lymphoma L5187Y/TK$^{+/-}$ cells both in the absence and in the presence of metabolic activation (NTP, 1987; Tice, 1990). When fed to *Drosophila*, dimethyl methylphosphonate induced sex-linked mutations (NTP, 1987). Dominant lethal effects were reported in male rats and mice following oral exposure (Dunnick et al., 1984a,b). Based on mixed results of mutagenic and genotoxic testing, no mode of action can be determined for dimethyl methylphosphonate. According to the U.S. EPA (2005) cancer guidelines a linear extrapolation approach is recommended when the mode of action cannot be determined.

Quantitative Estimates of Carcinogenic Risk

Dose-response modeling was performed on the incidence of mononuclear cell leukemia in male rats in the two-year NTP (1987) gavage bioassay to obtain a potential point of departure for deriving an oral slope factor. In accordance with the U.S. EPA (2005) guidelines, the LED_{10} (lower bound on dose estimated to produce a 10% increase in tumor incidence over background) was estimated using the U.S. EPA (2000) benchmark dose methodology, and a linear extrapolation to the origin was performed by dividing the 10% (0.1) risk by the LED_{10}. The multistage model for dichotomous data in the EPA Benchmark Dose Software (version 1.3.2) was fit to the incidence data. Prior to modeling, rat gavage doses were adjusted by multiplying by 5/7 to average the exposure over 7 days/week; incidences were 10/50, 11/50 and 17/50 for the 0, 357 and 714 mg/kg-day groups (duration-adjusted). The multistage model, which estimated

an ED_{10} of 524.9 mg/kg-day and the lower 95% confidence intervals (LED_{10}) predicted by the model, was 216.4 mg/kg-day (x^2 p-value = 0.081 and AIC = 170.891).

The duration-adjusted LED_{10} of 216.4 mg/kg-day for mononuclear cell leukemia in male rats was converted to a human equivalent dose (HED) using a cross-species scaling factor of body weight raised to the 3/4 power (or the body weight ratio raised to the 1/4 power; U.S. EPA, 2005):

$$HED = \text{rat dose} \times (\text{rat time-weighted-average body weight}/\text{human reference body weight})^{1/4}$$

$$HED = 216.4 \text{ mg/kg-day} \times (0.393 \text{ kg} / 70 \text{ kg})^{1/4} = 216.4 \text{ mg/kg-day} \times 0.274$$

$$\text{Human equivalent } LED_{10} = 52.3 \text{ mg/kg-day}$$

A linear extrapolation to the origin (0.1 / 52.3 mg/kg-day) results in a provisional human **oral slope factor of 1.7E-3 (mg/kg-day)$^{-1}$** for dimethyl methylphosphonate. Although there is limited evidence for mutagenicity by dimethyl methylphosphonate, the data are not sufficient to establish a mutagenic mode of action (MOA) for the observed carcinogenicity. Consequently, the early-life exposure adjustment is not applied (U.S. EPA, 2005).

There are no human or animal inhalation data from which to derive a provisional inhalation unit risk for dimethyl methylphosphonate.

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