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Provisional Peer-Reviewed Toxicity Values for

Trimethyl Phosphate
(CASRN 512-56-1)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
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COMMONLY USED ABBREVIATIONS

BMC	Benchmark Concentration
BMD	Benchmark Dose
BMCL	Benchmark Concentration Lower bound 95% confidence interval
BMDL	Benchmark Dose Lower bound 95% confidence interval
HEC	Human Equivalent Concentration
HED	Human Equivalent Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
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
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 reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure (oral)
RfC	reference concentration (inhalation)
RfD	reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR TRIMETHYL PHOSPHATE (CASRN 512-56-1)

BACKGROUND

HISTORY

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA) 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 (PPRTVs) 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 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 a panel of six EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram 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 new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. 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 documents 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 Resource Conservation and Recovery Act (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 document 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

No RfD, RfC, or cancer assessment for trimethyl phosphate (see Figure 1 for the structure of trimethyl phosphate) is included on the IRIS database (U.S. EPA, 2009) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). The HEAST reported no RfD or RfC values (U.S. EPA, 1997). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) included a Health and Environmental Effects Profile (HEEP) for trimethyl phosphate (U.S. EPA, 1985) that declined to derive noncancer toxicity values due to inadequate noncancer data and potential carcinogenicity of the chemical (see below). The toxicity of trimethyl phosphate has not been reviewed by ATSDR (2009) or the World Health Organization (WHO, 2009). CalEPA (2009a,b) has not derived toxicity values for exposure to trimethyl phosphate. No occupational exposure limits for trimethyl phosphate have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2009), the National Institute of Occupational Safety and Health (NIOSH, 2009), or the Occupational Safety and Health Administration (OSHA, 2009).

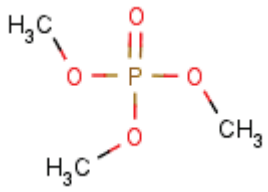


Figure 1. Chemical Structure of Trimethyl Phosphate

The HEAST (U.S. EPA, 1997) reported a cancer weight-of evidence classification of Group B2 (*Probable Human Carcinogen*) and an oral slope factor (OSF) of 3.7×10^{-2} $(mg/kg\text{-}day)^{-1}$ for trimethyl phosphate based on increased incidence of uterine tumors in female mice in a 103-week gavage study (NCI, 1978). The HEAST cited the HEEP (U.S. EPA, 1985) as the source of the OSF. Trimethyl phosphate has not been evaluated under

the 2005 *Guidelines for Carcinogen Assessment* (U.S. EPA, 2005). The International Agency for Research on Cancer (IARC, 2009) has not reviewed the carcinogenic potential of trimethyl phosphate. Trimethyl phosphate is not included in the *11th Report on Carcinogens* (NTP, 2005). CalEPA (2009b) has not prepared a quantitative estimate of carcinogenic potential for trimethyl phosphate.

Literature searches were conducted from the 1950s through August 2010 for studies relevant to the derivation of provisional toxicity values for trimethyl phosphate. Databases searched included MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months). The HEEP (U.S. EPA, 1985) was also reviewed for pertinent studies.

REVIEW OF PERTINENT DATA

HUMAN STUDIES

Oral Exposure

No data are available on the oral toxicity of trimethyl phosphate in humans.

Inhalation Exposure

Data on the inhalation toxicity of trimethyl phosphate in humans are limited to a single occupational exposure study (NIOSH, 1982). The study reported no medically significant cholinesterase depression among a group of 175 factory workers exposed via inhalation to a mixture of chemicals including trimethyl phosphate as well as dibromochloropropane, chloroform, Vapona, acetone, sodium hydroxide, hexane, methyl isobutyl ketone, methyl isocyanate, methyl thioacetoldoxime, Nudrin, and Azodrin during the manufacture or formulation of pesticide products (NIOSH, 1982). Levels of trimethyl phosphate at the plant (based on six personal air samples) were found to be below the detection limit of analysis by gas chromatography-mass spectrometry. These data do not support an inhalation toxicity value.

ANIMAL STUDIES

Oral Exposure

Available subchronic and chronic studies evaluating the oral toxicity of trimethyl phosphate in animals include a 9-week feeding study in rats (Oishi et al., 1982), 7-week gavage range-finding studies in rats and mice and follow-up lifetime gavage studies (NCI, 1978), a lifetime drinking water study in rats (Bomhard et al., 1997), an unpublished Japanese combined repeated dose/reproduction/developmental toxicity study (MHW, 1994), and several other studies specifically evaluating the effects on the male reproductive system and processes (Takizawa et al., 1998; Cho and Park, 1994; Hanna and Kerr, 1981; Harbison et al., 1976).

Subchronic Studies—Male JCL-Wistar rats (18 controls and 6 treated) were fed diets containing trimethyl phosphate (purity not reported) at concentrations of either 0 or 0.5% continuously for up to 9 weeks (Oishi et al., 1982). Based on a default body weight for male Wistar rats of 217 g and a default food consumption rate of 0.02 kg/day (U.S. EPA, 1988), the approximate equivalent doses are 0 and 461 mg/kg-day. Rats were weighed at study termination, and blood was collected for hematology (prothrombin time, kaolin-activated partial thromboplastin time [kaolin-PTT], leukocyte counts [white blood cells, or WBCs], erythrocyte

counts [red blood cells, or RBCs], hemoglobin concentration [Hgb], hematocrit [Hct], and mean corpuscular volume [MCV]) and clinical chemistry (total protein, urea nitrogen, cholesterol, glutamic oxaloacetic transminase [GOT or aspartate aminotransferase, AST] activity, glutamic pyruvic transaminase [GPT or alanine aminotransferase, ALT] activity, alkaline phosphatase [ALP] activity, total bile acids, sodium, and potassium). At sacrifice, liver, kidneys, spleen, and testes were removed and weighed; all but the testes were examined histologically.

As shown in Table 1, treated rats gained statistically significantly ($p < 0.05$) less weight than controls during the exposure period (12%). Significant differences ($p < 0.05$) in hematology and serum chemistry findings between treated and control rats included shorter prothrombin time and longer kaolin-PTT, lower RBC and Hgb, and decreased AST and ALT activities. In treated animals, both mean absolute and relative kidney weights were statistically significantly ($p < 0.05$) elevated over controls, and mean absolute testes weight was statistically significantly ($p < 0.05$) lower than controls. However, no treatment-related histological changes were reported. The only dose tested of 461 mg/kg-day is identified as a LOAEL for rats in this study based on a reduction in body weight and statistically significant ($p < 0.05$) hematological and biochemical changes as described above.

Table 1. Significant Changes in Male JCL-Wistar Rats Treated with Trimethyl Phosphate via Oral Administration for 9 Weeks

Parameter	Control	461 mg/kg-day
Number of animals examined	18	6
Terminal body weight	446.2 ± 10.7[a]	392.5 ± 3.9[b]
Hematology		
RBC ($\times 10^6$/mm^3)	6.94 ± 0.072	6.63 ± 0.076[b]
Hgb (g/100 mL)	13.3 ± 0.12	12.7 ± 0.13[b]
Prothrombin time (second)	20.1 ± 0.54	17.6 ± 0.4[b]
Kaolin-PTT (second)	37.4 ± 1.2 (17)	43.2 ± 1.0[b]
Clinical chemistry		
AST (Karmen units)	79 ± 4.9 (16)	59 ± 2.9[b]
ALT (Karmen units)	32 ± 1.8 (15)	25 ± 1.4[b]
Absolute organ weights		
Kidneys (g)	3.36 ± 0.11	3.73 ± 0.051[b]
Testes (g)	3.69 ± 0.051	3.01 ± 0.19[b]
Relative organ weights		
Kidneys (g/100 g bw)	0.75 ± 0.017	0.95 ± 0.017[b]

[a]Mean ± standard error (*n*, if different from group size).
[b]Significantly different from control at $p < 0.05$.

Source: Oishi et al. (1982).

The National Cancer Institute (NCI, 1978) conducted 7-week dose range-finding studies in rats and mice to estimate the low and high doses for use in chronic studies (described below). Trimethyl phosphate (purity >99% in one batch and >95% in a second batch) was administered by gavage in distilled water to groups of five rats or mice per sex at 0, 100 (rats only), 147, 215, 316, 464, 681, 1000, 1470, or 2150 (mice only) mg/kg-day, 3 days/week, for 7 weeks. Animals were monitored for survival and changes in body-weight gain. All rats dosed with trimethyl phosphate at ≥681 mg/kg-day died, and one male rat exposed to 464 mg/kg-day died. Distended bladders and gastrointestinal hemorrhage were observed in these rats. At 464 mg/kg-day, males gained approximately 44%, and females gained approximately 32% less weight than controls (data not shown). Male and female rats dosed with ≤316 mg/kg-day gained approximately 20% less weight than controls (data not shown). Five male mice and one female mouse died at 2150 mg/kg-day, and two female mice died at 1470 mg/kg-day. A slight depression in mean body weights was observed among male mice at ≥681 mg/kg-day (data not shown). According to the study authors, body weights among female mice were not greatly affected. Surviving animals were killed and necropsied 1 week after the end of the exposure period, but the results were not reported. Frank effect levels (FELs) of 464 and 1470 mg/kg-day are identified for rats and mice, respectively, based on mortality.

Chronic Studies—Lifetime exposure studies were conducted in rats and mice to assess the carcinogenicity of trimethyl phosphate (NCI, 1978). In the rat study, trimethyl phosphate (99% pure) was administered by gavage in distilled water to groups of F344 rats (50/sex) at doses of 50 or 100 mg/kg-day, 3 days/week, for 104 weeks. Rats were observed for an additional week following the exposure period. Vehicle control groups consisted of 20 male and 20 female rats given distilled water by gavage, 3 days/week, for 105 weeks.

Rats were observed twice daily for clinical signs, and body weights were measured at regular intervals, although the study does not report the frequency of this observation. At each weighing, rats were palpated for masses. Rats found in a moribund condition during the study and surviving rats at study termination were killed and necropsied. Pathological evaluation included gross and microscopic examination of all major tissues, organs, and gross lesions. No rats died prior to 52 weeks. Survival at the end of the study was 40% for control males, 56% for low-dose males, 35% for high-dose males, 60% for control females, 72% for low-dose females, and 55% for high-dose females. Statistical tests for a dose-related increase in mortality did not achieve significance in either sex ($p > 0.05$, Tarone's test).

NCI (1978) reported that the mean body weights among both sexes for both dose groups were slightly lower than controls. Body-weight data were presented as growth curves. Based on visual inspection of the growth curve, it appears that terminal body weights of high-dose rats of both sexes were more than 10% lower than controls. No clinical signs of toxicity were reported. Histopathology revealed a variety of degenerative and inflammatory conditions related to aging, but no treatment-related nonneoplastic lesions were observed. Doses of 50 and 100 mg/kg-day (duration-adjusted doses of 21 and 43 mg/kg-day by multiplying 3/7) are identified as NOAEL and LOAEL values, respectively, based on reduced body weights.

Table 2 shows a summary of tumor incidence as reported in NCI (1978) for F344 rats. There was a statistically significant dose-related increase ($p < 0.01$.by Cochran-Armitage test) for the incidence of subcutaneous fibromas in males across all dose groups, and the incidence of fibromas in high-dose males was statistically significantly ($p < 0.05$ by Fisher's exact test)

increased compared with controls. These benign tumors were characterized by layers of well-differentiated fibroblastic cells separated by dense bands of mature collagen. Apparent dose-related increases in the incidences of several other tumors were observed in male rats (as shown in Table 2), but none of these were statistically significant in trend or pairwise tests. Tumor incidence was not significantly different from controls among female rats, although the authors did note several 'unusual' tumors in female rats including glioblastoma multiforme in 1/48 high-dose females, myxosarcoma in 2/49 high-dose females, and malignant reticulosis in 1/50 low-dose females. NCI (1978) concluded that trimethyl phosphate treatment was associated with the induction of benign fibromas of the subcutaneous tissue in male rats and did not appear to be carcinogenic in female rats.

Table 2. Significant Changes in Male F344 Rats Treated with Trimethyl Phosphate via Oral Administration for up to 105 Weeks

Parameter	Control	50 mg/kg-day	100 mg/kg-day
Neoplastic lesions			
Subcutaneous tissue, fibroma	0/20 (0)[a,b]	2/50 (4)	9/49 (18)[c]
Alveolar/bronchiolar, adenoma or carcinoma	0/19 (0)	2/49 (4)	5/46 (11)
Hematopoietic system, leukemia or lymphoma	8/20 (40)	20/50 (40)	25/49 (51)
Adrenal, pheochromocytoma	1/20 (5)	4/48 (8)	7/47 (15)

[a]Number of tumor-bearing animals/number of animals examined at site (percent).
[b]Significant dose-related increase by Cochran-Armitage test at $p < 0.01$.
[c]Significant pairwise difference from control by Fisher's exact test at $p < 0.05$.

Source: NCI (1978).

In the corresponding mouse study, groups of B6C3F1 mice (50/sex/dose) were treated by gavage with trimethyl phosphate (99% pure) in distilled water at doses of 250 or 500 mg/kg-day, 3 days/week, for 103 weeks (NCI, 1978). No observation period followed treatment. Similar to the rat study, vehicle controls consisted of 20 male and 20 female mice treated by gavage with distilled water. Mice were evaluated for the same endpoints as outlined above in the rat study. Survival at the end of the study was 70% for control mice, 88% for low-dose males, 80% for high-dose males, 90% for control females, 62% for low-dose females, and 59% for high-dose females. Statistical tests for a dose-related increase in mortality did not achieve significance in either sex ($p > 0.05$, Tarone's test).

A dose-related decrease in mean body weights was observed among female mice, while mean body weights of male mice were comparable to controls throughout the study (data presented as growth curves). Based on an evaluation of the growth curve for this assessment, it appears that terminal body weight among high-dose female mice was at least 10% lower than controls. Most nonneoplastic lesions observed in treated mice were considered to be either spontaneous or common in mice in long-term studies. The 500 mg/kg-day dose (duration-adjusted dose of 214 mg/kg-day) is identified as a NOAEL for male mice and as a

LOAEL for female mice based on reduced body weights. The corresponding NOAEL for female mice is 250 mg/kg-day (duration-adjusted dose of 107 mg/kg-day by multiplying 3/7).

No significant increase in tumor incidence was observed in male mice (NCI, 1978). In female mice, a statistically significant ($p = 0.01$, Cochran-Armitage test) dose-related trend in the incidence of endometrial adenocarcinomas of the uterus was observed. The incidence of these tumors in the high-dose group was statistically significantly higher ($p = 0.01$, Fisher's exact test) than that in the controls (see Table 3). Microscopic examination of these tumors revealed vascular involvement and pulmonary metastases in four high-dose females and one low-dose female. NCI (1978) reported that this tumor has never been observed among historical controls (100 female B6C3F1 mice) at this laboratory. Extensive thrombosis of the pulmonary arteries was observed in three of these mice. In addition, endometrial squamous-cell carcinoma of the uterus occurred in one high-dose female, and leiomyosarcoma of the uterus was observed in one low-dose female. Hydronephrosis was observed in five of the female mice with uterine tumors. NCI (1978) concluded that trimethyl phosphate was carcinogenic to female mice in this study.

Table 3. Uterine Tumor Incidences in Female B6C3F1 Mice Treated with Trimethyl Phosphate via Oral Administration for 103 Weeks

Parameter	Control	250 mg/kg-day	500 mg/kg-day
Neoplastic lesions			
Uterus, adenocarcinoma	0/16 (0)	7/40 (18)[b]	13/37 (35)[c]

[a]Number of tumor-bearing animals/number of animals examined at site (percent).
[b]Significant dose-related increase by Cochran-Armitage test at $p < 0.01$.
[c]Significant pairwise difference from control by Cochran-Armitage test at $p < 0.01$.

Source: NCI (1978).

In a more recent chronic toxicity/carcinogenicity study, weanling Wistar rats (60/sex/dose) were exposed by gavage to trimethyl phosphate (99% pure) in drinking water at doses of 0, 1, 10, or 100 mg/kg-day for up to 30 months (Bomhard et al., 1997). Due to high mortality at 100 mg/kg-day, this dose was reduced to 50 mg/kg-day at Week 54. At 12 months, 10 rats/sex/dose were sacrificed and necropsied. At 24 months, surviving rats in the high-dose group were terminated and necropsied. All other surviving rats were terminated and necropsied at 30 months.

During the exposure period, rats were monitored for changes in appearance and behavior daily. Body weights were recorded weekly during the first 3 months and once every other week for the remainder of the study. Food and water consumption were monitored weekly. Ophthalmological examinations were performed in Weeks 98/99 and 128 on 10 rats/sex/dose. Clinical laboratory investigations were also conducted on 10 randomly selected animals per group. These included hematology (RBC, reticulocytes, leukocytes, differential leukocyte, and platelet counts, Hgb, Hct, MCV, mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and thromboplastin time) and urinalyses (volume, total protein, specific gravity, pH [Month 14 only], sediment [microscopically examined], and

semiquantitative measurements for blood, glucose, bilirubin, protein, ketone bodies, and pH [except Month 14]) on blood and urine samples collected at 6, 12, 14, 18, 23/24, and 30 months, and clinical chemistry (ALP, lactate dehydrogenase, AST, and ALT activity, total bilirubin, cholesterol, creatinine, albumin, total protein, urea nitrogen, triglycerides, inorganic phosphate, calcium, potassium, sodium, and chloride) on serum samples collected at 6, 12, and 14 months. Necropsies were performed on all rats found dead or terminated in extremis, and on 10 rats/sex/group 12 months after study initiation, all high-dose rats at 24 months, and on all other surviving rats at 30 months. Organ weights (adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, and testes) were recorded at scheduled necropsies. All rats were subjected to complete gross and histopathological evaluations.

Clinical signs characterized by the study authors as hind limb weakness, sunken flanks (especially in males), distended abdomen (especially in females), and poor general condition were observed in high-dose animals beginning in Week 46 (Bomhard et al., 1997). Reduction in the dose level for this group at Week 54 did not appear to alter these effects. Hind limb weakness was also observed in the other dose groups and controls starting around Week 120; this observation was attributed to old age. As mentioned above, high mortality rates were observed following treatment with 100-mg/kg-day trimethyl phosphate in both males and females. In both sexes, the increase in mortality became evident during the latter half of the first year of treatment (data presented as a survival curve), and continued, despite the dose reduction to 50 mg/kg-day at 54 weeks, until only approximately 30% of animals in this group survived to Week 100 (versus 85% or more of animals in other groups surviving to that point). Slightly more animals dosed at 10 mg/kg-day died toward the end of the study compared with controls, but a substance-related effect was questionable since Bomhard et al. (1997) reported that the mortality rate was within the range of historical controls, and no indications of substance-specific causes of death were reported. There was no effect on survival at 1 mg/kg-day. The authors reported reductions in body weights among high-dose males (-20%) and females (-15%) throughout the study and among mid-dose males towards the end of the study (about 10%) when compared with controls (body-weight data presented as growth curves). Food consumption was slightly decreased among high-dose males but was slightly higher than controls when adjusted to body weight.

No treatment-related effects were observed in any dose group upon ophthalmological examination. Hematology, clinical chemistry, and urinalysis data were not shown, but the authors reported that significant findings in the high-dose group (males and females unless otherwise noted) included reductions in Hgb, Hct, and RBC counts; increased proportion of reticulocytes (males up to Month 18) and higher thrombocyte counts (up to Month 18); and an increase in segmented neutrophils and a corresponding decrease of lymphocytes. At 12 months, a relative increase in the α1-globulin fraction above the historical range was observed, accompanied by significant decreases in albumin and γ-globulin fractions. A similar, but not statistically significant, trend was reported at 18 and 24 months. Increased urinary protein excretion (especially in males at Months 18 and 23) and decreased urine pH (females at 6 months) were also reported. No significant effects were noted in the other dose groups, with the exception of decreases in urine pH among all treated female groups at 12 months.

As shown in Table 4, absolute organ weights at interim sacrifice (12 months) among treated rats were comparable to controls except for a statistically significant ($p < 0.05$) decrease in liver weight among high-dose males and statistically significantly ($p < 0.01$) decreased lung

weight among mid-dose females (Bomhard et al., 1997). Relative heart, lungs, liver, and kidney weights were statistically significantly ($p < 0.05$) elevated over controls among high-dose males. Similarly, relative heart, liver, and kidney weights were statistically significantly ($p < 0.01$) elevated over controls among high-dose females at 12 months. The organ weight changes in high-dose rats occurred in parallel with decreased body weights in these rats. Statistically significant ($p < 0.05$) increases in relative liver and kidney weights were also observed among low-dose females. Since statistically significant differences between mid-dose females and controls in liver and kidney weights were not observed, the findings in low-dose females could possibly be related to sample size and may not be treatment-related. Gross examination of rats sacrificed at 12 months showed changes only in high-dose rats: muscle wasting and changes in lungs (mottled, reddish, pale), heart (thick, hard, abnormal color), liver (thick, scarring), and kidneys (scarring) in males and females; small seminal vesicles and testes in males; and skin edema in females. Microscopic examination at interim sacrifice revealed higher incidence of peripheral nerve and spinal cord degeneration in high-dose males and females only (see Table 4).

Table 4. Significant Changes in Wistar Rats Treated with Trimethyl Phosphate via Oral Administration for 12 Months

Parameter	Control	1 mg/kg-day	10 mg/kg-day	100 mg/kg-day
Males				
Number of animals examined	10	10	10	10
Absolute organ weights				
Liver (mg)	16,089 ± 1738.7[a]	16,728 ± 1750.1	14,850 ± 1499.1	14,160 ± 1528.1[b]
Relative organ weights				
Heart (mg/100 g bw)	253 ± 11.7	255 ± 21.3	251 ± 17.8	325 ± 43.4[c]
Lungs (mg/100 g bw)	336 ± 15.8	329 ± 28.7	352 ± 32.5	438 ± 44.8[c]
Liver (mg/100 g bw)	3801 ± 152.1	3850 ± 426.9	3609 ± 331.1	4241 ± 360.9[b]
Kidneys (mg/100 g bw)	659 ± 30.9	635 ± 47.9	656 ± 73.8	807 ± 88.7[c]
Nonneoplastic lesions				
Degeneration of peripheral nerve fiber	0/10[d]	0/10	0/10	8/10[e]
Degeneration of spinal cord nerve fiber	0/10	0/10	0/10	4/10
Females				
Number of animals examined	10	10	10	10
Absolute organ weights				
Lungs (mg)	1091 ± 102.0[a]	1005 ± 45.6	963 ± 112.6[c]	1057 ± 75.3
Relative organ weights				
Heart (mg/100 g bw)	301 ± 17.2	321 ± 29.6	316 ± 24.2	352 ± 21.0[c]
Liver (mg/100 g bw)	3668 ± 164.8	4034 ± 269.1[b]	3746 ± 231.0	4300 ± 449.0[c]
Kidneys (mg/100 g bw)	695 ± 52.4	760 ± 65.1[b]	745 ± 63.6	851 ± 43.6[c]
Nonneoplastic lesions				
Degeneration of peripheral nerve fiber	0/10[d]	0/10	1/10	9/10[e]
Degeneration of spinal cord nerve fiber	0/10	0/10	0/10	4/10

[a]Study did not indicate whether data reflect mean ± standard deviation or mean ± standard error.
[b]Significantly different from control at $p < 0.05$.
[c]$p < 0.01$.
[d]Number affected/number examined.
[e]Significantly different from control at $p < 0.05$ by Fisher's exact test performed for this review.

Source: Bomhard et al. (1997).

Necropsy of animals from the main study groups revealed a slight increase in the frequency of small hindlimbs, scarring of the kidneys, and small, soft testes in high-dose animals. No treatment-related gross changes were observed at necropsy in the low- and mid-dose animals. Microscopy revealed a statistically significantly ($p < 0.05$ by Fisher's exact test) increased incidence of degeneration and loss of spinal cord nerve fibers of high-dose male and female rats (see Table 5). Fiber damage in the peripheral nerves of these animals was associated with reactive cell proliferation, resulting in hypercellularity, which was statistically significantly increased in high-dose male and female rats ($p < 0.05$ by Fisher's exact test, see Table 5). Although incidence data were not shown, the authors described additional findings that they considered to be suggestive of dysfunction of the cardiovascular and respiratory system and possibly related to perturbation of the nervous system; these findings included chronic congestion of the lungs and kidneys, formation of thrombi in the heart atria in some males and females, necrosis and lymphocytic infiltration of the liver in some males, and edema of subcutaneous tissue and increased hematopoietic activity in the adrenal glands and bone marrow in some females at the high dose.

Table 5. Significant Changes in Wistar Rats Treated with Trimethyl Phosphate via Oral Administration for up to 24−30 Months

Parameter	Control	1 mg/kg-day	10 mg/kg-day	76 mg/kg-day[a]
Males				
Nonneoplastic lesions				
Peripheral nerve hypercellularity	0/50[b]	0/49	1/48	11/47[c]
Degeneration of spinal cord nerve fiber	0/50	2/49	1/48	6/47[c]
Loss of spinal cord nerve fiber	0/50	0/49	0/48	15/47[c]
Females				
Nonneoplastic lesions				
Peripheral nerve hypercellularity	0/49	2/49	1/50	6/50[c]
Loss of spinal cord nerve fiber	0/49	0/49	0/50	10/50[c]

[a]Time-weighted average (100 mg/kg-day for 54 weeks and 50 mg/kg-day for 50 weeks).
[b]Number affected/number examined.
[c]Significantly different from control at $p < 0.05$ by Fisher's exact test performed for this review.

Source: Bomhard et al. (1997).

No significant treatment-related differences in the incidence, time of occurrence, spectrum of types, or localizations of tumors were observed among treated rats when compared with concurrent controls (Bomhard et al., 1997). Although the early termination of the high-dose group limits the interpretation of those results, tumor incidence in this group was within the range of historical controls at this laboratory, and a survival-adjusted statistical analysis did not reveal any significant increase in any tumors in this group. Tumor incidences among the 10 and 1 mg/kg-day groups were also comparable to historical controls. Bomhard et al. (1997)

identified a NOAEL of 1 mg/kg-day for this study, based on suppression of body-weight gain in males at 10 mg/kg-day.

Reproductive/Developmental Studies—In an unpublished Japanese study, trimethyl phosphate (99.9% pure) was administered to groups of Sprague-Dawley rats (13/sex/dose) via gavage at 0, 40, 100, or 250 mg/kg-day, dosing commenced 14 days prior to mating and continued through the 2-week mating period and for an additional 2 weeks postmating. For females, dosing commenced 14 days before mating and was continued through mating, gestation, and until Lactation Day 3 (MHW, 1994). This study is reported in Japanese, but an English abstract and English data tables are available. This study was also submitted as a combined repeated dose/reproductive/developmental toxicity study under the Organisation for Economic Co-Operation and Development (OECD) high production volume (HPV) Chemicals Programme and was included in the SIDS Initial Assessment Report (IPCS, 1996). Based on the data tables in the Japanese report and the information included in the SIDS report, toxicological endpoints evaluated in this study appeared to include mortality, behavior, body weight, food consumption, organ weights (liver, kidneys, thymus, testes, epididymides) and histopathology (kidneys, liver, thymus, reproductive organs, and nerve fibers). Blood and serum samples were also collected from males for hematology (RBC, WBC, and platelet counts; Hgb; Hct; MCV; MCH; MCHC; and differentiation of leukocytes) and clinical chemistry (total protein, albumin, A/G ratio, ALP, GPT [ALT], GOT [AST], gamma-glutamyl transpeptidase [GGT], total bilirubin, cholinesterase, cholesterol, glucose, blood urea nitrogen [BUN], creatinine, inorganic phosphorous, sodium, potassium, chloride, and calcium). Reproductive endpoints evaluated in this study appeared to include copulation rate, fertility index, number of implantations, embryonic mortality, pup viability, pup weights, and morphological abnormalities among pups.

High-dose male rats gained significantly less weight ($p < 0.01$) and consumed significantly less food ($p < 0.01$) than controls starting on the first week of treatment (MHW, 1994). Clinical signs, including progressive paralytic gait and decreased motor activity, were observed in this group starting on the second week of exposure. Mortality in this group was high (12/13), with the first deaths occurring during the fourth week of treatment. No mortality or significant signs of toxicity were reported among low- or mid-dose males.

However, changes in hematology, clinical chemistry, organ weights, and histopathology were observed among these rats, as summarized in Table 6. As shown, the hematological changes observed in males at ≥100 mg/kg-day included decreased RBC counts, Hgb, and Hct, and an increase in platelet count and percent of segmented neutrophils. Statistically significant changes in clinical chemistry included decreases in A/G ratio and increases in cholinesterase, total cholesterol and calcium levels at ≥40 mg/kg-day and decreased glucose levels, increased total protein, and elevated sodium levels at 100 mg/kg-day. Males also exhibited significant increases in absolute and relative kidney weights at ≥40 mg/kg-day and significant increases in absolute and relative thymus and epididymis weights and relative liver weights at 100 mg/kg-day. In males, histopathology revealed nephropathy (slight to moderate), atrophy of the thymus (severe), liver (moderate), spleen (severe), and testis (moderate to severe); hypertrophy of the adrenal gland (slight); decreased sperm count (severe); and degeneration of peripheral nerve fibers (slight). Most of these lesions occurred only at the high dose of 250 mg/kg-day, but degeneration of sciatic nerve was increased also at 100 mg/kg-day, and renal

lesions were increased at all doses. Nephropathy in treated rats was characterized by slight-to-moderate tubular alterations, including increased eosinophilic droplets in the tubular epithelium, regenerated tubules, and increased eosinophilic bodies.

Table 6. Significant Changes in Male Sprague-Dawley Rats Treated with Trimethyl Phosphate via Gavage

Parameter	Control	40 mg/kg-day	100 mg/kg-day	250 mg/kg-day
Group size	13	13	13	13
Mortality	0	0	0	12
Terminal body weight (g)	479.7 ± 48.7[a]	480.7 ± 27.2	468.8 ± 28.8	244.8
Hematology				
Number examined	13	12	13	1
RBC count ($\times 10^4/mm^3$)	781 ± 37	768 ± 34	739 ± 24[b]	702
Hgb (g/dl)	14.5 ± 0.5	14.2 ± 0.5	13.7 ± 0.4[b]	13.2
Hct (%)	42.7 ± 1.5	41.4 ± 1.7	40 ± 1.1[b]	37.6
Segmented neutrophils (%)	8 ± 4	9 ± 4	16 ± 8[c]	52
Platelet count ($\times 10^4/mm^3$)	100.3 ± 6.7	105.9 ± 7.2	114.5 ± 10.8[b]	106.4
Biochemistry				
Number examined	13	12	13	1
Total protein (g/dl)	5.6 ± 0.3	5.8 ± 0.2	6.1 ± 0.3[b]	6.3
A/G ratio	1.17 ± 0.13	1.07 ± 0.07[c]	1.02 ± 0.10[b]	0.80
Cholinesterase (U/l)	288 ± 47	340 ± 42[c]	422 ± 93[b]	485
Total cholesterol (mg/dl)	56 ± 9	70 ± 13[c]	75 ± 16[b]	145
Glucose (mg/dl)	203 ± 19	190 ± 15	183 ± 11[b]	152
Na (mEq/l)	140.2 ± 0.5	140.9 ± 0.7	141.8 ± 1.3[b]	142.8
Ca (mg/dl)	8.9 ± 0.4	9.3 ± 0.2[c]	9.5 ± 0.3[b]	9.2
Absolute organ weights				
Number examined	13	13	13	1
Kidney (g)	2.97 ± 0.32	3.49 ± 0.44[b]	3.46 ± 0.34[b]	2.87
Thymus (mg)	337.7 ± 94.2	409.2 ± 91.1	458.7 ± 116.9[b]	222.6
Testes (g)	3.03 ± 0.19	3.27 ± 0.19[c]	3.07 ±0.35	1.30
Epididymides (g)	1.15 ± 0.11	1.08 ± 0.08	0.89 ± 0.07[b]	0.50

Table 6. Significant Changes in Male Sprague-Dawley Rats Treated with Trimethyl Phosphate via Gavage

Parameter	Control	40 mg/kg-day	100 mg/kg-day	250 mg/kg-day
Relative organ weights				
Number examined	13	13	13	1
Liver (g/100 g bw)	3.82 ± 0.28	4.07 ± 0.36	4.26 ± 0.29[c]	4.05
Kidney (g/100 g bw)	0.62 ± 0.04	0.73 ± 0.08[b]	0.74 ± 0.05[b]	1.17
Thymus (mg/100 g bw)	69.8 ± 15.9	85.6 ± 20.6	98.4 ± 27.2[b]	90.9
Epididymides (g/100 g bw)	0.24 ± 0.03	0.22 ± 0.02	0.19 ± 0.02[b]	0.20
Histopathology				
Thymus: atrophy	0/13[d]	0/13	0/13	12/13[b]
Liver: hepatocellular atrophy	0/13	0/13	0/13	12/13[b]
Kidney: eosinophilic droplet in tubular epithelium	1/13	13/13[b]	13/13[b]	2/13
Kidney: regenerated tubule	6/13	13/13[b]	13/13[b]	12/13[b]
Kidney: eosinophilic body	5/13	13/13[b]	13/13[b]	1/13
Adrenal: hypertrophy of cortical cell	0/13	0/13	0/13	8/13[b]
Spleen: atrophy of follicle	0/13	0/13	0/13	13/13[b]
Testes: atrophy	0/13	1/13	1/13	13/13[b]
Epididymis: decreased number of sperm	0/13	0/13	1/13	13/13[b]
Skeletal muscle: atrophy of myofiber	0/13	0/13	0/13	11/12[b]
Skeletal muscle: degeneration of nerve fiber	1/13	0/13	4/13	10/12[b]
Sciatic nerve: degeneration of nerve fiber	0/13	0/13	9/13[b]	12/12[b]

[a]Mean ± standard deviation.
[b]$p < 0.01$.
[c]Significantly different from controls at $p < 0.05$.
[d]Number affected/number examined.

Source: MHW (1994).

As in the males, high-dose females typically showed decreased motor activity starting on the second week of the study, but progressive paralytic gait was only infrequently reported, and only one animal died during the study (MHW, 1994). Fertility was significantly ($p < 0.01$) affected by trimethyl phosphate. As shown in Table 7, only two high-dose pairs copulated, and neither female became pregnant. Mid-dose pairs copulated, but only 2/13 females from this group became pregnant. Body weight of pregnant females was reduced compared to controls in both the low- (-12%) and mid-dose (-21%) groups on Gestation Day (GD) 20. Neither of the two pregnant mid-dose females achieved parturiency. In the low-dose group, the fraction of pregnant females delivering litters with live pups was lower than controls (10/12 versus 13/13),

and the average number of live pups per litter was markedly reduced (-43%, $p < 0.01$). The English abstract specifically describes a significant increase in intrauterine mortality in this group. There was no additional effect on pup viability between Days 0 and 4 of lactation, and pup weights were statistically significantly ($p < 0.01$) higher than controls from birth to terminal necropsy at Lactation Day 4. Terminal examination of the F0 females showed statistically significant ($p < 0.01$) increases in absolute and relative thymus weights in the low-dose group (not measured in mid- or high-dose groups) but without any corresponding histopathology. The only noteworthy histopathology finding in females was significant ($p < 0.01$) degeneration of nerve fibers in the high-dose group.

Table 7. Significant Changes in Female Sprague-Dawley Rats Treated with Trimethyl Phosphate via Gavage

Parameter	Control	40 mg/kg-day	100 mg/kg-day	250 mg/kg-day
Number mated	13	13	13	13
Number copulated	13	13	13	2[b]
Number pregnant	13	12	2[b]	0
Body weight on GD 20 (g)	404.2 ± 24.4[a]	357.2 ± 26.9[b]	319.5	NA
Day 0 of lactation (birth)				
Number of litters with live pups	13	10	0	0
Average number of live pups per litter	13.4 ± 3.7	7.6 ±3.8[b]	NA	NA
Pup weight—male (g)	6.3 ± 1.1	7.8 ± 0.6[b]	NA	NA
Pup weight—female (g)	5.9 ± 0.9	7.5 ±0.5[b]	NA	NA
Day 4 of lactation				
Number of litters with live pups	13	9	0	0
Average number of live pups per litter	12.8 ± 4.4	8.4 ±2.9[b]	NA	NA
Pup weight—male (g)	9.8 ± 2.0	12.8 ± 1.8[b]	NA	NA
Pup weight—female (g)	8.9 ± 2.4	12.5 ±1.8[b]	NA	NA
Terminal examination of F0				
Thymus wt (mg)	138.1 ± 70.2	261.9 ± 51.2[b]	NA	NA
Thymus wt—relative (mg/100 g bw)	44.7 ± 22.3	82.5 ± 15.4[b]	NA	NA
Skeletal muscle: degeneration of nerve fiber	0/13	0/13	0/13	9/13[b]
Sciatic nerve: degeneration of nerve fiber	0/13	0/13	0/13	11/13[b]

[a]Mean ± standard deviation.
[b]Significantly different from control at $p < 0.01$.

Source: MHW (1994).

Based on the effects described above, MHW (1994) identified the NOAEL as <40 mg/kg-day for both repeated dose toxicity and reproductive/developmental toxicity. OECD/Screening Information Data Sets (SIDS) (ICPS, 1996) also identified <40 mg/kg-day as the NOAEL and 40 mg/kg-day as the LOAEL for both repeated dose toxicity and reproductive/developmental toxicity.

Hanna and Kerr (1981) administered trimethyl phosphate (purity not reported) to male albino Sprague-Dawley rats (number not specified) at 0 or 250 mg/kg-day via gavage 5 days/week, for 30 days, or 6 days/week, for 60 days. After treatment, males were mated with untreated females to assess fertility. Upon cessation of treatment, semen was collected from two rats of the 30-day exposure group for evaluation of sperm presence and morphology. Testes from three animals from each treatment group and from controls were examined histologically. Following termination of treatment, virgin females mated with treated males lacked vaginal plugs, indicating impaired mating ability of the treated males. Spermatozoa from treated rats appeared abnormal, exhibiting detached heads and abnormalities of the head, middle piece, and principal piece. Histological examination of testes from rats exposed for 30 days revealed indications of impaired spermatogenesis due to abnormal spermiogenesis and depletion of the numbers of mature spermatids. Round spermatids showed vacuoles within their nuclei and extensive extracellular spaces between the germ cells and Sertoli cells. Evaluation of the testes from rats exposed for 60 days revealed absent germ cells in the seminiferous tubules, resulting in collapse and shrinkage of the tubules and the presence of only Sertoli cells. The only dose tested, 250 mg/kg-day (duration-adjusted dose of 179 mg/kg-day, based on exposure 5 days/week), is identified as a LOAEL for male reproductive effects.

Cho and Park (1994) administered trimethyl phosphate (99% pure) to male albino Sprague-Dawley rats (20/group) at 0, 400, 500, 750, 1000, or 1500 mg/kg-day via gavage 5 days/week for up to 5 weeks. Four surviving rats per group were sacrificed weekly. Testes were collected for microscopic examination and evaluation of spermatogenic stages, and seminiferous tubules were examined for maturation staging. Immediately following dosing, effects on spermatogenesis characterized by aggregation of multinucleated giant cells and maturation arrest at the spermatid level were observed. Peak frequency in the emergence of multinucleated giant cells occurred 1 week after treatment ended, and maturation arrest was prominent at 3 weeks following treatment. Dead rats found during the exposure period were subjected to gross and microscopic examination. Mortality rates were 0, 10, 90, 100, 100, and 100% at 0, 400, 500, 750, 1000, and 1500 mg/kg-day, respectively. Almost all dead rats were anuric and anorexic prior to death. Gross and microscopic examination of the kidneys, liver, lungs, and heart from these rats was unremarkable. However, gross examination revealed severely distended bladders. Microscopic evaluation of the bladders of these animals revealed multifocal ulceration, loss of urothelial epithelium with marked thinning, and atrophy of the muscle proper. The 400 mg/kg-day dose (lowest dose tested) is identified as a FEL for this study based on mortality.

Takizawa et al. (1998) administered trimethyl phosphate (purity not reported) to male Sprague-Dawley rats (10/dose) at 0 or 100 mg/kg-day via gavage once daily for 28 days. Rats were monitored for changes in body weight and food consumption during the exposure period. Twenty-four hours after the final dosing, the rats were sacrificed, and the testes, epididymides, seminal vesicles, and prostrate were removed and weighed. Sperm samples were collected from the cauda epididymis and analyzed for evaluation of sperm viability and counts (using flow

cytometry) as well as motility and morphology (using light microscopy). Testes and epididymides were also examined microscopically. No significant changes in body weights, food consumption, or organ weights were observed among treated rats. No significant effects were observed on sperm viability or counts, but sperm motility was reduced. Degenerative spermatogenic cells and degenerative sperm were observed in the ducts of the epididymides in 1/10 and 3/10 rats, respectively. No notable histological changes were observed in the testes, seminal vesicles, or prostate. The 100 mg/kg-day dose (the only dose tested) is identified as a LOAEL for reduction in sperm motility for this study.

Harbison et al. (1976) conducted numerous experiments in rats, mice, and rabbits, including short-term studies that are described in further detail below under the section ***Acute or Short-term Studies***, and longer-term studies in which trimethyl phosphate (purity 97%) was injected intraperitoneally (i.p). or administered by gavage. The report was unclear as to which method of administration was used in any given experiment. For all species, treated males were mated with untreated females. The females were later sacrificed for determination of pregnancy as a measure of male fecundity (calculated as the number of pregnancies per total breeding population). Control males were also mated with untreated females. Animals were monitored for differences between treated and control groups in general behavior, skeletal muscle activity, mating behavior, and frequency of vaginal plugs and mountings. Testicular biopsies and caudal spermatozoa were obtained at various periods during and following treatment. Treatment and findings reported by Harbison et al. (1976) for rats, mice, and rabbits are described briefly below.

Male albino Sprague-Dawley rats (number not specified) were treated with trimethyl phosphate at 0 and 100 mg/kg-day, 5 days/week, for 1 month or 0 and 750 mg/kg-day, 1 day/week, for 12 weeks (Harbison et al., 1976). In rats treated with 750 mg/kg-day, fecundity was reduced by 50% during the first week of treatment and, by 94−100%, on Weeks 3 through 12. Rats treated with 100 mg/kg-day for 1 month demonstrated a reduction in fecundity by about 71% during the first week following termination of treatment. Fertility returned to normal during the next week. There were no differences between treated and control groups in general behavior, skeletal muscle activity, mating behavior, or frequency of vaginal plugs. In addition, there were no significant histological changes in the testes of treated animals, and spermatogenesis appeared normal. The 100 mg/kg-day dose (the lowest dose tested, duration-adjusted dose of 71 mg/kg-day) is identified as a LOAEL for rats based on the effects on fecundity.

Male albino Swiss mice (number not specified) were treated with trimethyl phosphate at 1500 mg/kg-day, for 5 days/week, for 1 month (Harbison et al., 1976). In these mice, sterility persisted for 2 weeks following termination of treatment and gradually returned to normal 4 weeks later. There were no differences between treated and control groups in general behavior, skeletal muscle activity, mating behavior, or frequency of vaginal plugs. In addition, there were no significant histological changes in the testes of treated animals, and spermatogenesis appeared normal. The 1500 mg/kg-day dose (the only dose tested, duration-adjusted dose of 1071 mg/kg-day) is identified as a LOAEL for mice based on observed persistent sterility.

Male New Zealand white rabbits (number not specified) were treated with trimethyl phosphate at 200 mg/kg-day every 5 days, for 9 weeks, or at 325 mg/kg-day, once a week, for 13 weeks, and 750 mg/kg-day for dogs (Harbison et al., 1976). In rabbits treated with 200 mg/kg-day, fecundity was reduced by 50% by the 3rd week of treatment and by 75% by the 9th week. Among the rabbits treated with 325 mg/kg-day, fecundity was reduced by about 63% by the 2nd week of treatment and by 87% in the 750-mg/kg-day group in the first week following treatment. Sterility persisted from Week 5 after the start of treatment through Week 13 in these rabbits. Within 1 week following the end of the treatment (Week 13), fertility returned to normal in these animals. There were no differences between treated and control groups in general behavior, skeletal muscle activity, mating behavior, or frequency of vaginal plugs. In addition, there were no significant histological changes in the testes of treated animals, spermatogenesis appeared normal, and mating behavior was unaffected in any species tested. The 200 mg/kg-day dose (the lowest dose tested, duration-adjusted dose of 143 mg/kg-day) is identified as a LOAEL for rabbits based on the effects on fecundity.

Based on the results across all of the experiments conducted by Harbison et al. (1976) (including the shorter-term studies described below), trimethyl phosphate was found to induce a dose-dependent reversible sterility, which was also dependent on duration of exposure, in male rats, mice, and rabbits. However, the lack of information regarding route of exposure limits the interpretation of these data for risk-assessment purposes.

Inhalation Exposure

No data are available on the inhalation toxicity of trimethyl phosphate in animals.

OTHER STUDIES

Acute or Short-term Studies

Acute oral studies (exposure duration of <5 days) have been conducted on male animals that support the findings from subchronic-duration and reproduction studies that trimethyl phosphate affects reproductive parameters and, in particular, have shown effects on the male reproductive tissues and processes (see Table 8). These studies have shown that acute oral exposure of male animals to trimethyl phosphate results in reversible functional infertility at doses of ≥100 mg/kg-day in rats and at 1000 mg/kg-day in mice. Trimethyl phosphate has also been shown to cause a significant reduction in sperm count and sperm motility; reduced prostate, testes, and epididymide weights; increased cauda weights; reduced testosterone levels; and increased numbers of immature Leydig cells in male rats. As shown in Table 8, the lowest dose associated with acute reproductive effects is 200 mg/kg-day in male rats.

Table 8. Acute Oral Studies of Male Reproductive Effects

Animal	Dose/Route/Duration	Parameters Examined	Results	Comments	Reference
Rats					
Male rats (5/group, strain not specified)	100 or 250 mg/kg-day via gavage for 5 d	Number of offspring from matings with untreated females (determined weekly)	Dose-related reduction in the number of offspring; offspring were absent or markedly reduced in number at Wks 2−5, but fertility was restored at Wks 6−12 in both dose groups.	No controls were reported; few data were presented.	Jackson and Jones, 1968
Male rats (number and strain not specified)	500 or 2500 mg/kg via an unspecified oral route for an unspecified duration	Effects on spermatogenesis	Sterility for 3 wks posttreatment at 500 mg/kg; complete disorganization of spermatogenesis without damage to the tubular architecture at 2500 mg/kg accompanied by infertility for 20−25 wks posttreatment.	Lacking study details; no further data were presented.	Jones and Jackson, 1969
Male Wistar rats (10−17/group)	0 or 100 mg/kg-day via gavage for 5 d	Organ weights and histology of testes, prostate, seminiferous tubules, pituitary, adrenal glands, liver, and kidney, testosterone levels in plasma and testes tissue, and histochemistry of the testes	Decreased prostate weight, decreased testosterone concentration in plasma and testes, positive histochemical reaction for 3β-hydroxysteroid dehydrogenase by the sperm tails, increased number of immature Leydig cells, and increased interstitial fluid in the testicular interstitial tissue.		Carstensen, 1971
Male Long-Evans hooded rats (20/group)	0, 100, 250, or 600 mg/kg-day, via gavage for 5 d	Body weight, organ weights (testis, cauda epididymis, epididymis), sperm counts, and sperm motion	Dose-related reduction in weight gain, increased cauda weight, decreased sperm count, and altered sperm motion		Toth et al., 1992

Table 8. Acute Oral Studies of Male Reproductive Effects

Animal	Dose/Route/Duration	Parameters Examined	Results	Comments	Reference
Male Wistar rats (number not given)	250 or 500 mg/kg-day via an unspecified oral route for 5 d	Sperm motility and count	Decreased sperm motility and count at 500 mg/kg-day.	This publication is an abstract.	Suzuki et al., 1996
Male Sprague-Dawley rats (number not given)	0 or 600 mg/kg single dose via an unspecified oral route	Testes weight and sperm counts, motility, swimming speed, and pattern	No effect on testes weight or sperm count. Significant decrease in sperm swimming speed, path velocity, straight-line velocity, curvilinear velocity, and lateral amplitude. Significant increase in beat frequency.	Rats were observed for 3 wks following treatment. This publication is an abstract.	Fukunishi et al., 2000
Male Sprague-Dawley rats (number not given)	0, 60, 200, or 600 mg/kg-day, via gavage for 5 d	Testes and epididymides weights, and sperm motion	Decreased testes and epididymides weights at 600 mg/kg-day, decreased sperm count at 600 mg/kg-day, and altered sperm motion at ≥200 mg/kg-day.	Data for trimethyl phosphate originally presented in an abstract presented at the 26th annual meeting of the Japanese Society of Toxicology.	Fukunishi et al., 1999 (as cited in Kato et al., 2001)
Mice					
Male mice, strain not specified (8/dose)	1000 mg/kg-day via gavage for 5 d	Number of offspring from matings with untreated females (determined weekly)	Reduced number of offspring.	Study is lacking information on controls and study methods; few data were presented.	Jones and Jackson, 1969; Jackson and Jones, 1968
Male Swiss mice (number not given)	0, 500, or 1000 mg/kg-day via gavage for 5 d	Number of pregnancies from matings with untreated females (determined weekly)	One male at 1000 mg/kg-day died; no other signs of systemic effects in males; reduced pregnancy rate at 1000 mg/kg-day.		Epstein et al., 1972, 1970

Additional acute studies by Harbison et al. (1976) in male rats, mice, and rabbits demonstrated that treatment with trimethyl phosphate daily for 5 days produced temporary infertility in rats at 600 mg/kg-day, and in mice at 750 and 1500 mg/kg-day. Rabbits treated with a single dose of trimethyl phosphate at 750 mg/kg also exhibited temporary infertility. However, these studies are limited because the authors do not indicate which animals or in which experiments gavage dosing was used instead of dosing by i.p. injection.

Neurotoxicity

In an older study specifically designed to assess the neurotoxicity of trimethyl phosphate in dogs, Schaeppi et al. (1984) fed gelatin capsules containing 1-mL trimethyl phosphate to five adult beagle dogs (two males and three females) daily for 1−4 months. Based on the reported individual body weights of the dogs during the course of the study, the approximate daily doses for each dog were 88 and 121 mg/kg-day for males exposed for 29 and 50 days, and 105, 89, and 106 mg/kg-day for females exposed for 71, 101, or 121 days, respectively. An additional female dog received a capsule containing 2-mL trimethyl phosphate orally 5 days/week, for 150 days. Based on an average body weight of 9.45 kg, the duration-adjusted equivalent dose for continuous daily exposure was approximately 181 mg/kg-day. No control group was tested simultaneously with these dogs. Neurological tests, including examination of tonic neck reflexes, righting response, standing on a straight line, pain reflex, cornea reflex, and pupil light response, were conducted weekly. Electrodiagnostic tests (maximum nerve conduction velocity [MNCV]) were conducted biweekly. Electrophysiological control values were available from pretest examination of the treated dogs and from previous studies on untreated control dogs. Treated dogs were also subjected to neuropathology examination.

All dogs treated daily with 1-mL trimethyl phosphate (88−121 mg/kg-day) developed signs of neurotoxicity including impaired gait, hopping, tactile placing and landing, persistence in abnormal posture, and decreased muscle tone; these signs became progressively more severe with duration of treatment (Schaeppi et al., 1984). The dogs that received ≥50 treatments had prolonged distal latency for neuromuscular impulse transmission compared with pretest values. Sensory MNCV was decreased for the dog that received 120 treatments. No changes in peripheral motor MNCV occurred in any dogs receiving 1 mL/day when compared with pretreatment control values or untreated dogs from previous studies. No neuropathologic changes were observed in dogs treated for ≤71 days. Neuropathology examination revealed degenerative changes in nerve fibers and demyelination of axons among the female dogs treated the longest (101 and 121 days).

The dog treated with 181 mg/kg-day exhibited notable weight loss after 85 days of treatment and inactivity after Day 88 (Schaeppi et al., 1984). Treatment was discontinued on Days 93−112, resumed during Days 113−149, and terminated following Day 149 due to severe morbidity. This dog was sacrificed on Day 151 in poor general condition. Signs of neurotoxicity in this animal increased in severity with increasing duration of exposure. These signs included enhanced patellar reflex (Day 18), attenuated extensor postural thrust (Day 25), atactic gait (Day 39), decreased muscle force and persistence in abnormal posture (Day 46), and decreased muscle tonus and impaired hopping and landing (Day 53). Neurophysiologic testing revealed attenuated sensory MNCV and a progressive decrease of central motor MNCV to as low as 50% of the pretreatment value by Day 150. Neuropathology examination revealed advanced distal degeneration of the long spinal tracts and the peripheral nerve fibers, and demyelination of nerve fibers.

The findings reported by Schaeppi et al. (1984) are uncertain due to the small number of animals, lack of concurrent control group, and variable dosing regimen; however, this study does provide suggestive evidence for neurotoxicity associated with oral trimethyl phosphate. The evidence for neurotoxicity is bolstered by the observations of Bomhard et al. (1997), who reported peripheral nerve damage manifested as muscle wasting of the hind limbs in Wistar rats treated with 100-mg/kg-day trimethyl phosphate in the drinking water for 54 weeks. This was the highest dose tested; however, this dose was reduced during the course of the study due to intolerance. Neurological effects were not observed at the lower test does of 1 and 10 mg/kg-day.

Genotoxicity

Trimethyl phosphate has been studied extensively in mutagenicity and genotoxicity assays in vivo and in vitro. As shown in Tables 9 (in vitro studies) and 10 (in vivo studies), trimethyl phosphate generally gave mixed or equivocal results in bacterial reverse-mutation assays, mixed results in bacterial tests of DNA repair, and consistently positive results for genotoxicity in mammalian cell systems and in mammals and *D. melanogaster* tested in vivo. Additionally, there are numerous in vivo studies where trimethyl phosphate has been used as a positive control substance (Sinha et al., 1983; Moutschen and Degraeve, 1981; Valencia, 1981; Hanna and Dyer, 1975; Legator et al., 1973).

As shown in Table 9, trimethyl phosphate was positive for reverse mutation in *Salmonella typhimurium* strain TA100 in the presence and absence of metabolic activation (Zeiger et al., 1992, 1982; De Flora et al., 1990, 1984; De Flora, 1981; Bruce and Heddle, 1979; Anderson and Styles, 1978; Farrow et al., 1976). For the most part, results in other *S. typhimurium* strains (TA98, TA1538, TA1537, TA1535, TA1530, TA1531, TA1532, TA1534, TA1536, TA1537, TA1538, GS46) were either equivocal or negative (De Flora et al., 1990, 1984; Zeiger et al., 1982; De Flora, 1981; Bruce and Heddle, 1979; Anderson and Styles, 1978; Farrow et al., 1976; Hanna and Dyer, 1975; MacPhee, 1973). However, some studies reported positive results in *S. typhimurium* strains TA102, TA2638 (Watanabe et al., 1996), TA98 (Farrow et al., 1976), hisC117 (Hanna and Dyer, 1975), LT2hisG46 (MacPhee, 1973), and TA1535 (Anderson and Styles, 1978). Positive results for reverse mutation were also obtained in *Escherichia coli* strains WP2, WP2uvrA, CM611, CM891, and WP12 (Watanabe et al., 1996; Li et al., 1993; Hanna and Dyer, 1975; Dean, 1972) and in the bacterium *Serratia marcesans* strains Hy/α13 and Hy/α21 (Dean, 1972). Trimethyl phosphate was positive with and without S9-activation in a DNA-repair assay using *E. coli* strains WP2 and WP67 or CM871 (DeFlora et al., 1990, 1984) and positive with S9-activation in strains uvrB+/recA/lac- and lvrB-/recA-/lac+ (Hellmer and Bolcsfoldi, 1992). Results were negative without activation in a similar assay using *E. coli* strains P3110 and P3478 (Fluck et al., 1976). Only a few assays using mammalian cell systems are available. A dose-related increase in the incidence of DNA single-strand breaks was observed in rat hepatocytes without metabolic activation (Sina et al., 1983). However, a similar assay was negative for DNA double-strand breaks (Storer et al., 1996). Trimethyl phosphate was positive for micronuclei in Chinese hamster lung cells (Ni et al., 1993) and chromosome aberrations in human lymphocytes (Soderman, 1972).

Table 9. Genotoxicity Studies of In Vitro Trimethyl Phosphate Exposure

Test System	Endpoint	Concentration	Activating System	Results[a]		Comments	Reference
				Without Activation	With Activation		
S. typhimurium TA1535, TA1538, TA98, TA100	Reverse mutation	2500 µg/plate	+S9	NA	+/−	Positive for TA1535 and TA100, negative for TA1538 and TA98.	Anderson and Styles, 1978
S. typhimurium TA1535, TA1537, TA98, TA100	Reverse mutation	0.05, 0.5, 5, 50, or 500 µg/plate	±S9	−/=	+/=	Positive for TA100 in presence of S9, equivocal for other strains; S9 from Aroclor-induced rats.	Bruce and Heddle, 1979
S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation	0.6−1.1 × 10^6 nmol/plate	±S9	+/−	+/−	Positive for TA100 (0.0003 revertants/nmol) and S9 slightly enhanced the response; negative for other strains; S9 from Aroclor-induced rats.	DeFlora et al., 1990, 1984; DeFlora, 1981
S. typhimurium LT2hisG46, LT2hisG46 (R-Utrecht), TA1530	Reverse mutation	Not reported	None	+/−	NA	Positive for LT2hisG46 and LT2hisG46 (R-Utrecht), negative for TA1530.	MacPhee, 1973
S. typhimurium GS46	Reverse mutation	1250, 1500, or 1700 mg/kg	Host-mediated	NA	−	Trimethyl phosphate did not induce revertants in the bacteria from the peritoneum, mouse host treated intramuscularly.	Farrow, 1975
S. typhimurium hisC117, hisG46, TA1530, TA1535, TA1531, TA1532, TA1534, TA1536, TA1537, TA1538	Reverse mutation	Not reported	None	+/−	NA	Positive for hisC117 strain only.	Hanna and Dyer, 1975
S. typhimurium GS46, TA92, TA100, TA98	Reverse mutation	125 mg/plate	Liver microsomes (mouse, rat, or monkey)	NA	+/−	Positive for TA100 (greatest number of revertants regardless of species) and TA92.	Farrow et al., 1976

Table 9. Genotoxicity Studies of In Vitro Trimethyl Phosphate Exposure

Test System	Endpoint	Concentration	Activating System	Results[a]		Comments	Reference
				Without Activation	With Activation		
S. typhimurium GS46, TA92, TA100, TA98	Reverse mutation	1750 mg/kg administered to mice (urine added to *S. typhimurium* cultures)	NA	NA	+/−	Weakly positive for TA98 and TA100.	Farrow et al., 1976
S. typhimurium TA1535, TA100	Reverse mutation	0, 1, 10, 50, 100, or 250 mmol/plate	±S9	=/+	=/+	Equivocal for TA1535, positive for TA100; metabolic activation slightly enhanced the response in TA100; S9 from Aroclor-induced rats.	Zeiger et al., 1982
S. typhimurium TA98, TA100	Reverse mutation	0 (solvent control), 333, 1000, 3333, 6666, 10,000, or 15,000 µg/plate	±S9	NR	+/−	Positive for TA100, negative for TA98.	Zeiger et al., 1992
S. typhimurium TA97, TA98, TA100, TA102	Reverse mutation	Not reported	±S9	−	−	Negative in all strains; Chinese study.	Li et al., 1993
S. typhimurium TA102, TA2638	Reverse mutation	Not reported	±S9	+	NR	Results given for S9 mix.	Watanabe et al., 1996
E. coli WP2	Reverse mutation	Not reported	None	+	NA	Weakly mutagenic; paper disc method.	Dean, 1972
E. coli WP2, WP2uvrA, CM561, CM571, CM611, WP67, WP12	Reverse mutation	Not reported	None	+/−	NA	Positive for strains WP2, WP2uvrA, CM611, WP67, and WP12.	Hanna and Dyer, 1975
E. coli WP2, WP2uvrA, CM891, ND160, MR2-102	Reverse mutation	Not reported	±S9	+/−	+/−	Positive for strains WP2, WP2uvrA, and CM891; response potentiated by S9 activation; Chinese study.	Li et al., 1993

Table 9. Genotoxicity Studies of In Vitro Trimethyl Phosphate Exposure

Test System	Endpoint	Concentration	Activating System	Results[a]		Comments	Reference
				Without Activation	With Activation		
E. coli WP2/pKM101, WP2uvrA/pKM101	Reverse mutation	Not reported	±S9	+	NR	Results given for S9 mix.	Watanabe et al., 1996
E. coli WP2 (repair proficient) WP67 or CM871 (repair deficient)	DNA repair	Not reported	±S9	+	+	Positive for all strains; S9 from Aroclor-induced rats.	DeFlora et al., 1990, 1984
E. coli P3110 (polA+) P3478 (polA-)	DNA repair	25 µL	None	−	NA	Negative in both strains.	Fluck et al., 1976
E. coli uvrB+/recA/lac- (repair proficient) or 243/591uvrB-/recA-/lac+ (repair deficient)	DNA repair		±S9	−	+	S9 from Aroclor-induced rats.	Hellmer and Bolcsfoldi, 1992
Rat hepatocytes	DNA damage	0.03, 0.3, or 3 mM	None	+	NA	Evaluated for DNA single strand breaks; dose-related response; negative at lowest dose and positive at other doses; <30% cytotoxicity.	Sina et al., 1983
Rat hepatocytes	DNA damage	0.03, 0.1, 0.3, 1, 3, 7, or 10 mM	None	−	NA	Negative for DNA double-strand breaks.	Storer et al., 1996
Serratia marcesans HY/α13, HY/α21	Reverse mutation	25, 50, or 100 mg/mL	None	+	NA	Positive dose-related results in both strains, significant effect at all doses in HY/α13, significant effect at 50 and 100 mg/mL in HY/α21; paper disc method.	Dean, 1972

Table 9. Genotoxicity Studies of In Vitro Trimethyl Phosphate Exposure

Test System	Endpoint	Concentration	Activating System	Results[a]		Comments	Reference
				Without Activation	With Activation		
Chinese hamster lung cells	Micronucleus test	Not reported	None	+	NA	English data table; positive in vitro; no other details available in English.	Ni et al., 1993 (published in Chinese)
Human lymphocytes	Chromosome aberrations	0, 0.01, 0.1, 1, 2.5, 5, 10, 25, 50, 75, or 100 mM	None	+	NA	Dose-related increase in the percentage of anaphases with aberrations and in the number of metaphase breaks.	Soderman, 1972

[a]+ = positive; − = negative; = = equivocal; NA = not available; NR = not reported.

As shown in Table 10, gavage treatment and i.p. injection with trimethyl phosphate induced chromosomal aberrations in bone marrow cells of rats and mice (Sinha et al., 1983; Moutschen and Degraeve, 1981; Anderson and Richardson, 1981; Sheu, 1979; Weber et al., 1975; Farrow, 1975; Legator et al., 1973; Adler et al., 1971) and in spermatocytes of Chinese hamsters and mice (Katoh and Matsuda, 1985; Degraeve et al., 1984; Moutschen and Degraeve, 1981; Machemer and Lorke, 1975). Katoh and Matsuda (1985) suggested that trimethyl phosphate may act on the first cleavage metaphase of postmeiotic male germ cells to produce a high rate of heritable translocations in the F1 progeny males. Trimethyl phosphate induced micronuclei in vivo in mouse bone marrow cells (Bruce and Heddle, 1979; Farrow et al., 1976; Weber et al., 1975, 1974). However, in a study published in Chinese, Ni et al. (1993) reported no increased incidence of micronuclei in bone marrow cells of mice; however, few details were available in the English abstract.

During a recessive lethal test with *Drosophila melanogaster*, trimethyl phosphate fed to developing larvae resulted in reversible sterility in males (Dyer and Hanna, 1972). Trimethyl phosphate was used as a positive control in two other recessive lethal studies in *D. melanogaster* (Valencia, 1981; Hanna and Dyer, 1975). Other positive results in *D. melanogaster* included dose-dependent induction of somatic mutations in a wing-spot test (Graf et al., 1989) and in an eye mosaic assay (Vogel and Nivard, 1993).

Trimethyl phosphate was positive in dominant lethal assays in mice, resulting in increases in early fetal deaths and preimplantation losses within the first 3 weeks after mating (Moutschen and Degraeve, 1981; Degraeve et al., 1979; Newell et al., 1976; Lorke and Machemer, 1975; Farrow, 1975; Epstein et al., 1972, 1970; Dean, 1972; Dean and Thorpe, 1972). An increased incidence in translocation heterozygotes was observed in male ICR/SIM mice (Rushbrook et al., 1985), and a dose-related increase in the frequency of translocation carrier mice was observed in male C3H mice (Tezuka et al., 1985; Sasaki et al., 1984) following treatment with trimethyl phosphate during the postmeiotic stage of late spermatids.

Table 10. Genotoxicity Studies of In Vivo Trimethyl Phosphate Exposure

Test System	Endpoint/ Indicator	Application	Concentration or Dose	Response[a]	Comment	Reference
Male CD rats	Chromosome aberrations in bone marrow cells	i.p. injection	2000 mg/kg (single dose) or at single doses of 0, 500, 750, 1000, 1250, 1500, or 1750 mg/kg, or at 500 mg/kg-day for 4 d	+	Trimethyl phosphate induced chromatid aberrations including open breaks and reunion figures following single dose (≥750 mg/kg) and repeated exposure (500 mg/kg), a dose-response was observed with maximal response at 48 hrs.	Adler et al. 1971
Male Osborne-Mendel rats	Chromosome aberrations in bone marrow cells	i.p. injection or gavage	0 or 2000 mg/kg (single dose) or at 0 or 100 mg/kg-day for 5 d	+	Trimethyl phosphate used as a positive control by multiple laboratories; induced aberrations at both 1000 mg/kg-day for 5 days and 2000 mg/kg (single dose) by both routes.	Legator et al., 1973
Male Osborne-Mendel rats	Chromosome aberrations in bone marrow cells	i.p. injection or gavage	0 (solvent control) or single unspecified dose level as a single dose or as five daily doses	+	Trimethyl phosphate used as a positive control by multiple laboratories; induced chromosomal aberrations by both routes and with both single dose and repeated exposure.	Sheu, 1979
Male Wistar rats	Chromosome aberrations in bone marrow cells	i.p. injection	0 or 3000 mg/kg (single dose) or 0 or 1500 mg/kg 5 times in 1 d	+	Trimethyl phosphate induced chromosome aberrations including gaps, breaks, and fragments, and induced significantly greater numbers of abnormal cells following single and multiple doses.	Anderson and Richardson, 1981
Male and female Sprague-Dawley rats	Chromosome aberrations in bone marrow cells	Gavage	0 or 2000 mg/kg (single dose 24 hrs prior to sacrifice)	+	Trimethyl phosphate used as a positive control; induced chromatid gaps (males only), breaks, and exchanges, chromosome breaks in males, and severely damaged cells in both sexes; no effect on mitotic index.	Sinha et al., 1983

Table 10. Genotoxicity Studies of In Vivo Trimethyl Phosphate Exposure

Test System	Endpoint/ Indicator	Application	Concentration or Dose	Response[a]	Comment	Reference
Male mice (strain not specified)	Chromosome aberrations in bone marrow cells	Not reported	1250, 1500, or 1750 mg/kg	+	Abstract; no mention of control; maximum number of chromosome aberrations observed at 48 hrs and maximum changes (breaks, gaps, and fragments) seen at highest dose; data not shown.	Farrow, 1975
$B_6D_2F_1$/J mice (sex not specified)	Chromosome aberrations in bone marrow cells	i.p. injection	0, 500, 750, 1000, or 2000 mg/kg-day for 5 d	+	Dose-related increase in chromatid breaks (≥500 mg/kg-day); similar results at 750 and 1000 mg/kg-day; 2000 mg/kg-day was lethal.	Weber et al., 1975
Male mice (Q strain)	Chromosome aberrations in bone marrow cells	i.p. injection	Not reported	+	Trimethyl phosphate used as a positive control; induced chromosomal aberrations including breaks, exchanges, and gaps.	Moutschen and Degraeve., 1981
Male Chinese hamsters	Chromosome aberrations in spermatocytes	Gavage	0 or 500 mg/kg-day for 2 d, or 0 or 1000 mg/kg-day for 5 d	+	Significant increase in the number of aberrant metaphases when gaps were included, not significant (but still higher) when gaps excluded; three translocations were observed (500 mg/kg-day); marked mitotic inhibition (1000 mg/kg-day).	Machemer and Lorke, 1975
Male mice (Q strain)	Chromosome aberrations in spermatocytes	i.p. injection	Not reported	+	Trimethyl phosphate used as a positive control; induced chromosomal aberrations including breaks, exchanges, and gaps.	Moutschen and Degraeve,1981
Male mice (Q strain)	Chromosome aberrations in spermatocytes	i.p. injection	0 or 1000 mg/kg (single dose)	+	Primarily positive for breaks (24 total across Recovery Days 10−15, 1 exchange on Recovery Days 12−13, and 1 gap on Recovery Days 10−11 and 14−15).	Degraeve et al., 1984

Table 10. Genotoxicity Studies of In Vivo Trimethyl Phosphate Exposure

Test System	Endpoint/ Indicator	Application	Concentration or Dose	Response[a]	Comment	Reference
Male mice (strain not reported)	Chromosome aberrations in spermatocytes	i.p injection	3000 mg/kg	+	Abstract; no mention of control; chromosome aberrations induced in the postmeiotic stages, late spermatid stage was the most sensitive; data not shown.	Katoh and Matsuda, 1985
$B_6D_2F_1$/J mice (sex not specified)	Micronuclei in bone marrow cells	i.p. injection	0, 500, 750, 1000, or 2000 mg/kg-day for 5 d	+	Dose-related increase in the frequency of micronuclei (≥500 mg/kg-day); 2000 mg/kg-day was lethal.	Weber et al., 1974, 1975
Mice (sex and strain not reported)	Micronuclei in bone marrow cells	Not reported	0, 1250, 1500, or 1750 mg/kg	+	Abstract; time- and dose-related increase in micronuclei; data not shown.	Farrow et al., 1976
Female hybrid (C57BL/6 × C3H/He) mice	Micronuclei in bone marrow cells	i.p. injection	0−10,000 mg/kg	+	Doses were not explicitly reported, but the range on the figure plotting micronuclei was from 0 to 10,000 mg/kg; increases in micronuclei observed at and above 6000 mg/kg.	Bruce and Heddle, 1979
Mouse (sex and strain not specified)	Micronuclei in bone marrow cells	i.p. injection	Not reported	−	English data table; negative in vivo; no other details available in English.	Ni et al., 1993 (published in Chinese)
Male C3H mice	Heritable translocation assay	i.p. injection	0, 1000, or 1500 mg/kg (single dose)	+	Significant dose-related increases in the frequency of translocation carrier mice at ≥1000 mg/kg.	Tezuka et al., 1985; Sasaki et al., 1984
Male Swiss mice	Dominant lethal	i.p. injection or gavage	0, 200, 700, or 1000 mg/kg (single dose i.p.); 0, 500, 850, 1250, 1500, or 2000 mg/kg (single dose i.p.); 0, 500, or 1000 mg/kg-day for 5 d (gavage)	+	Early fetal deaths observed during first 2 wks of mating at ≥1000 mg/kg following i.p. injection; early fetal deaths at 700 mg/kg during Mating Wk 8 following i.p. injection; early fetal deaths during first 2 wks of mating at ≥500 mg/kg following oral exposure; reduction in total implants during first 3 wks of mating at ≥200 mg/kg following i.p. injection.	Epstein et al., 1972, 1970

Table 10. Genotoxicity Studies of In Vivo Trimethyl Phosphate Exposure

Test System	Endpoint/ Indicator	Application	Concentration or Dose	Response[a]	Comment	Reference
Male Swiss CF1 mice	Dominant lethal	i.p. injection	0 or 1000 mg/kg	+	Increased number of early fetal deaths at 2nd wk of mating.	Dean and Thorpe, 1972
Male NMRI mice	Dominant lethal	Oral (unspecified)	0 or 1000 mg/kg (single dose)	+	Used as a reference material; no effect on preimplantation loss; marked increase in postimplantation loss in the 2nd wk of mating.	Lorke and Machemer, 1975
Male mice (strain not specified)	Dominant lethal	i.p. injection or gavage	1250 mg/kg (i.p.) or 500 mg/kg-day for 5 d (gavage)	+	Abstract; no mention of controls; significant lethality occurred maximally in the 2nd wk of mating (i.p.); dominant lethality in the 1st and 2nd wks of mating (gavage); data not shown.	Farrow, 1975
Mouse (strain not specified)	Dominant lethal	Oral (unspecified)	Not reported, 5-d oral dosing	+	Abstract; no mention of controls; dominant lethal effects for 2 wks after 5-d treatment.	Newell et al., 1976
Mouse (strain not specified)	Dominant lethal	Not reported	1000 mg/kg	+	Abstract; no mention of control; high mutagenicity particularly at postmeiotic stages; data not shown.	Degraeve et al., 1979
Male mice (Q strain)	Dominant lethal	i.p. injection	0 or 1000 mg/kg	+	Trimethyl phosphate used as a positive control; significant increase in the frequency of preimplantation and postimplantation losses 2 wks after injection.	Moutschen-Dahmen et al., 1981
Male C3H mice	Dominant lethal	i.p. injection	0, 1000, 1250 or 1500 mg/kg (single dose)	+	Significant decreases in the number of implants and living embryos and increases in early fetal deaths at ≥1000 mg/kg; no significant effect on the number of corpora lutea.	Tezuka et al., 1985; Sasaki et al., 1984

Table 10. Genotoxicity Studies of In Vivo Trimethyl Phosphate Exposure

Test System	Endpoint/ Indicator	Application	Concentration or Dose	Response[a]	Comment	Reference
Male *D. melanogaster*	Recessive lethal (second chromosome)	Feeding	0, 0.01, 0.015 or 0.2 M	+	Dose-related increase in the percentage of males with lethal mutations; significant increase in lethal mutations at ≥0.01 M.	Dyer and Hanna, 1972
Male *D. melanogaster*	Recessive lethal (second chromosome)	Feeding	Not reported	+	Used as a positive control; induced a high level of accumulated mutations (83%) compared with negative control (9%).	Hanna and Dyer, 1975
Male *D. melanogaster*	Recessive lethal	Feeding	0, 100, 300, or 1000 mg/kg	+	Used as a positive control; dose-response; negative at 100 and 300 ppm, positive at 1000 mg/kg.	Valencia, 1981
Male and female *D. melanogaster* Mwh-flr^3 cross (larvae)	Somatic mutation (Wing-spot test)	Feeding	0, 5, 10, or 20 mM for 48 hrs	+	Dose-dependent induction of all types of spots (small, large, and twin); results inconclusive at 5 mM and positive at ≥10 mM.	Graf et al., 1989
Male and female *D. melanogaster*	Eye mosaic assay	Feeding	2 or 10 mM for 3 d or 10, 50, 100, or 200 mM on the surface of food given for 48 hrs	+	Positive at both doses following the 3-d treatment; positive at ≥50 mM following surface treatment.	Vogel and Nivard, 1993

[a]+ = positive; − = negative

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RFD VALUES FOR TRIMETHYL PHOSPHATE

Oral studies of trimethyl phosphate include a subchronic-duration feeding study in rats (Oishi et al., 1982), subchronic-duration range-finding gavage studies in rats and mice (NCI, 1978), chronic-duration gavage studies in rats and mice (NCI, 1978), a chronic-duration drinking water study in rats (Bomhard et al., 1997), a neurotoxicity study in dogs (Schaeppi et al., 1984), a combined repeated dose/reproductive/developmental study in rats (MHW, 1994), and numerous acute, short-term, and subchronic-duration studies evaluating effects on male reproduction (Fukunishi et al., 2000, 1999, as cited in Kato et al., 2001; Takizawa et al., 1998; Suzuki et al., 1996; Cho and Park, 1994; Toth et al., 1992; Hanna and Kerr, 1981; Harbison et al., 1976; Carstensen, 1971; Jones and Jackson, 1969; Jackson and Jones, 1968). These studies reported effects on body weight in rats and mice, neurotoxic effects in dogs, and reproductive effects in rats, mice, and rabbits following oral treatment with trimethyl phosphate. Male animals appeared particularly susceptible to the reproductive effects of trimethyl phosphate. Oral studies considered adequate for derivation of a subchronic or chronic p-RfD are summarized in Table 11.

SUBCHRONIC AND CHRONIC p-RfD

As shown in Table 11, the lowest LOAEL among the subchronic-duration and reproductive toxicity studies is the LOAEL of 40 mg/kg-day in rats (MHW, 1994). This value is from an unpublished, combined systemic/single-generation reproduction study in rats that evaluated a sufficient number of animals and endpoints (MHW, 1994). The most prominent effects at this dose level were decreased gestational body weights in females at GD 20, decreased fraction of pregnant dams delivering litters with live pups, and markedly decreased number of live pups per litter. Also, at this dose level, treated males evaluated at study termination showed increases in absolute and relative kidney weights accompanied by histological changes (regenerated tubules and eosinophilic bodies). This study is limited by the fact that it has not been published and is available only in Japanese with an English abstract and data tables.

The lowest dose in the chronic and reproductive toxicity studies is 10 mg/kg-day for depressed body-weight gain in male rats given drinking water with trimethyl phosphate for 30 months (Bomhard et al., 1997). Body-weight was also depressed in female rats at a higher dose in this study; this body-weight change (10%) at this dose is considered a biologically relevant critical endpoint; thus, it is considered the POD for derivation of oral p-RfDs for both subchronic- and chronic-durations. BMD modeling cannot be conducted because the body-weight data were not provided in the principal study (the data were shown graphically). The NOAEL of 10 mg/kg-day is protective of the reproductive effects observed at ≥40 mg/kg-day and was chosen as the POD for deriving the chronic p-RfD.

Table 11. Summary of Noncancer Oral Dose-response Information Considered Adequate for Toxicity Value Derivation

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Duration-adjusted[a] NOAEL (mg/kg-day)	Duration-adjusted[a] LOAEL (mg/kg-day)	Responses at the LOAEL	Comments	Reference
Subchronic oral animal studies								
Rats (6 males/group) Diet	0, 461 mg/kg-day, 7 d/wk for 9 wks	NA	461	NA	461	Significant reduction in body-weight gain, significant changes in hematology and serum chemistry, elevated absolute and relative kidney weights and absolute testes weight	No treatment-related histological changes were observed.	Oishi et al., 1982
Chronic oral animal studies								
Rats (50/sex/group) Gavage	0, 50, or 100 mg/kg-day, 3 d/wk for 104 wks	50	100	21	43	Significant reduction in body weights		NCI, 1978
Rats (60/sex/group) Drinking water	0, 1, 10, 100 mg/kg-day for 30 mos (100 mg/kg-day dose reduced to 50 mg/kg-day in Wk 54)	10	100	10	100	Significant reduction in body weight of males		Bomhard et al., 1997
Mice (50/sex/group) Gavage	0, 250, or 500 mg/kg-day, 3 d/wk for 103 wks	250	500	107	214	Significant reduction in body weight of females		NCI, 1978

Table 11. Summary of Noncancer Oral Dose-response Information Considered Adequate for Toxicity Value Derivation

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Duration-adjusted[a] NOAEL (mg/kg-day)	Duration-adjusted[a] LOAEL (mg/kg-day)	Responses at the LOAEL	Comments	Reference
Oral studies of male reproductive effects								
Rats (13/sex) Gavage	0, 40, 100, or 250 mg/kg-day, 2 wks prior to mating through mating for a total of 42 d (males); 2 wks prior to mating through gestation to Postpartum Day 3 (females)	NA (parental) NA (repro)	40 (parental) 40 (repro)	NA (parental) NA (repro)	40 (parental) 40 (repro)	Parental: evidence of renal toxicity in males characterized by significant changes in clinical chemistry and kidney weights accompanied by histological changes Reproduction: decreased gestational body weights in females at GD 20, decreased fraction of pregnant dams delivering litters with live pups; markedly decreased number of live pups per litter	Unpublished Japanese combined repeated dose/reproductive/ developmental toxicity.	MHW, 1994
Rats Male (number not given) Gavage	0 or 250 mg/kg-day, 5 d/wk for 30 d or 6 d/wk for 60 d	NA	250	NA	179	Impaired mating ability, abnormal sperm, and impaired spermatogenesis		Hanna and Kerr, 1981

Table 11. Summary of Noncancer Oral Dose-response Information Considered Adequate for Toxicity Value Derivation

Species and Study Type (*n*/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Duration-adjusted[a] NOAEL (mg/kg-day)	Duration-adjusted[a] LOAEL (mg/kg-day)	Responses at the LOAEL	Comments	Reference
Rats Male (number not given) Gavage	0 or 100 mg/kg-day, 5 days/week, for 4 wks	NA	100	NA	71	Reduced sperm motility		Takizawa et al., 1998

NA = not applicable.

[a]Adjusted for continuous exposure as follows: $NOAEL_{adj}$ = NOAEL × exposure days/7 days.

A **chronic p-RfD** was derived for trimethyl phosphate by dividing the NOAEL of 10 mg/kg-day by a UF of 1000, as shown below. The value derived for the chronic p-RfD is also adopted for the **subchronic p-RfD**.

Chronic and subchronic p-RfD = NOAEL ÷ UF
= 10 mg/kg-day ÷ 1000
= **0.01 or 1×10^{-2} mg/kg-day**

The composite UF of 1000 is composed of the following UFs:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating a susceptible human response are insufficient.
- UF_A: A factor of 10 is applied for animal-to-human extrapolation because data for evaluating relative interspecies sensitivity are insufficient.
- UF_D: A factor of 10 is applied for database inadequacies because no studies evaluating developmental or multi-generational reproductive toxicity are available. The available reproduction study is not multigenerational.
- UF_L: A factor of 1 is applied for extrapolation from a LOAEL to a NOAEL because a NOAEL was used for the POD.

Confidence in the principal study (Bomhard et al., 1997) is medium. The chronic study evaluated multiple dose levels using an adequate number of rats (60/sex/dose) and evaluated several endpoints for assessing chronic toxicity. However, only limited data was reported. Confidence in the database is low. In addition to the principal chronic rat study, NCI (1978) evaluated the chronic effects of trimethyl phosphate in rats and mice and also observed effects on body-weight gain. The chronic database also consists of a single-generation reproduction study in rats and two studies specifically designed for evaluating effects on the male reproductive system. There are no developmental or multi-generational reproductive toxicity studies available. Available data indicate the occurrence of neurotoxicity; but, no comprehensive neurotoxicity study is available, and the neurotoxicity study in dogs is limited by the lack of a concurrent control group. Overall confidence in the chronic and subchronic p-RfD is low.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RFC VALUES FOR TRIMETHYL PHOSPHATE

No data are available on the effects of trimethyl phosphate in humans or animals exposed via inhalation. Derivation of provisional RfC values for trimethyl phosphate is precluded by the absence of data.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR TRIMETHYL PHOSPHATE

WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), trimethyl phosphate is "*Likely to be Carcinogenic to Humans*." This descriptor is appropriate when an

agent "has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" (U.S. EPA, 2005). No information was located on the carcinogenicity of trimethyl phosphate in humans. NCI (1978) observed a significant positive dose-related increase in the incidence of subcutaneous fibromas in male rats (see Table 2) given trimethyl phosphate by gavage at 50- and 100-mg/kg-day (duration-adjusted doses of 21 and 43 mg/kg-day) for 104 weeks. No historical control data were provided for this tumor in the study, but a study published a year later indicated that the background rate of this tumor during that time period was only 2.6% (Goodman et al., 1979). This compares with incidences of 0, 4, and 18% at the control, low-, and high-doses in the NCI (1978) study. In a more recent drinking water study, no evidence of carcinogenicity was observed among rats dosed with trimethyl phosphate up to 100 mg/kg-day (Bomhard et al., 1997). Significant mortality occurred at the highest dose tested in this study (100 mg/kg-day), but survival-adjusted statistical analysis performed by the study authors did not indicate evidence of carcinogenicity in this group. NCI (1978) also observed a significant increase in the incidence of uterine endometrial adenocarcinomas among female mice administered trimethyl phosphate by gavage at 500 mg/kg-day (duration-adjusted dose of 214 mg/kg-day) for 103 weeks (see Table 3). NCI (1978) reported that this tumor had never been observed in female mice based on historical controls. Genotoxicity data on trimethyl phosphate generally resulted in mixed or equivocal findings in bacterial mutagenicity assays and consistently positive findings in genotoxicity tests among animals exposed in vivo. In in vitro tests, trimethyl phosphate was mutagenic in bacterial mutation assays with *S. typhimurium* and *E. coli* (Watanabe et al., 1996; Li et al., 1993; Zeiger et al., 1992, 1982; De Flora et al., 1990, 1984; De Flora, 1981; Bruce and Heddle, 1979; Anderson and Styles, 1978; Farrow et al., 1976; Hanna and Dyer, 1975; Dean, 1972), impaired DNA repair in *E. coli* (DeFlora et al., 1990, 1984; Hellmer and Bolcsfoldi, 1992), and induced DNA damage in rat hepatocytes (Sina et al., 1983). A dose-related increase in the frequency of anaphases with aberrations and metaphase breaks was observed in human lymphocytes following trimethyl phosphate treatment at concentrations ranging from 0.01 to 100 mM. In vivo, trimethyl phosphate induced chromosome aberrations and micronuclei in rat and mouse bone marrow cells and aberrations in mouse sperm cells. Trimethyl phosphate was found to produce dominant lethal effects in several strains of mice (Moutschen and Degraeve, 1981; Degraeve et al., 1979; Newell et al., 1976; Lorke and Machemer, 1975; Farrow, 1975; Epstein et al., 1972, 1970).

MODE-OF-ACTION DISCUSSION

The EPA (2005) *Guidelines for Carcinogen Risk Assessment* defines mode of action as "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes and resulting in cancer formation." Examples of possible modes of carcinogenic action include mutagenic, mitogenic, antiapoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation, and immunologic suppression.

Apart from genotoxicity data, there is no other information on the potential mode(s) of action by which trimethyl phosphate induces subcutaneous fibromas or uterine adenocarcinomas. There is strong evidence that trimethyl phosphate induces micronuclei and chromosomal aberrations in laboratory animals tested in vivo; it is often used as a positive control substance in such assays. In vitro tests for mutagenicity have given mixed results.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Oral Exposure

Data for trimethyl phosphate are sufficient to perform dose-response modeling. The incidences of subcutaneous fibromas in male F344 rats and endometrial adenocarcinomas in female B6C3F1 mice administered trimethyl phosphate by gavage for up to 104 weeks (NCI, 1978) were modeled. In order to determine a POD for OSF derivation, animal doses in the NCI (1978) study were first adjusted (see Table 12) for continuous exposure as follows:

$$\text{Dose}_{\text{ADJ}} = \text{dose (mg/kg-day)} \times (\text{days per week} \div 7)$$
$$= \text{dose (mg/kg-day)} \times (3 \div 7)$$

The duration-adjusted values were then converted to human equivalent doses (HEDs) by adjusting for differences in body weight between humans and rats. In accordance with EPA (2005) *Guidelines for Carcinogen Risk Assessment,* a factor of $bw^{3/4}$ was used for cross-species scaling. Using this scaling factor, the dose (mg) in humans is obtained by multiplying the animal dose (mg) by the ratio of human:animal body weight raised to the 3/4 power. For doses expressed per unit of body weight (mg/kg or mg/kg-day), the relationship is reciprocal, and the human dose (mg/kg) is obtained by multiplying the animal dose (mg/kg) by the ratio of animal:human body weight raised to the 1/4 power. Because NCI (1978) did not report any information on the body weights of the animals used in the principal study, default body-weight values (0.380 kg for male F344 rats exposed chronically and 0.0353 kg for female B6C3F1 mice exposed chronically, as reported by U.S. EPA, 1988) were used to calculate the animal:human body-weight ratios. The equation used to calculate the HED values (see Table 12) is shown below, and the Table 12 presents HED.

$$\text{Dose}_{[\text{HED}]} = \text{Dose}_{[\text{ADJ}]} \times (\text{animal bw:human bw})^{1/4}$$

where:

$\text{Dose}_{[\text{ADJ}]}$ = average daily animal dose adjusted for continuous exposure (mg/kg-day)
animal bw = average rat or mouse body weight (kg), based on default values (U.S. EPA, 1988)
human bw = reference human body weight, 70 kg (U.S. EPA, 1988)

Appendix A describes the modeling approach and results. Table 13 shows the $BMD_{10[HED]}$ and $BMDL_{10[HED]}$ values predicted by the multistage cancer model for these tumor data. The results of modeling the two tumor types are very similar. A BMDL of 5.74 mg/kg-day associated with adenocarcinoma in female mice is considered the POD for the cancer assessment. The endometrial adenocarcinoma in mice is most likely associated with the carcinogenic potential of trimethyl phosphate exposure. As the mode of action of trimethyl phosphate-induced tumorigenicity is not clearly defined, a linear extrapolation from the POD to the origin was applied.

Table 12. Dose-response Data for the Incidence of Subcutaneous Fibromas in Male F344 Rats and Endometrial Adenocarcinomas in Female B6C3F1 Mice Administered Trimethyl Phosphate by Gavage for up to 104 Weeks

Male F344 rats				Female B6C3F1 mice			
Dose (mg/kg-day)	Adjusted Dose[a] (mg/kg-day)	HED[b] (mg/kg-day)	Incidence	Dose (mg/kg-day)	Adjusted Dose[a] (mg/kg-day)	HED[b] (mg/kg-day)	Incidence
0	0	0	0/20	0	0	0	0/16
50	21	6	2/50	250	107	16	7/40
100	43	12	9/49	500	214	32	13/37

[a]Adjusted for continuous (7 d/wk) exposure.
[b]HED = animal dose × (animal bw/human bw)$^{0.25}$, where animal body weights = 0.380 kg (male rats) and 0.0353 kg (female mice) (default values from U.S. EPA, 1988), and human body weight = 70 kg.

Source: NCI (1978).

Table 13. Summary of Human Equivalent BMDs and BMDLs Based on Incidence of Subcutaneous Fibromas in Male F344 Rats and Uterine Adenocarcinomas in Female B6C3F1 Mice Administered Trimethyl Phosphate by Gavage for up to 104 Weeks

	$BMD_{10[HED]}$ (mg/kg-day)	$BMDL_{10[HED]}$ (mg/kg-day)
Male rat subcutaneous fibromas	8.84	5.81
Female mouse uterine adenocarcinoma	8.13	5.74

The **p-OSF** for trimethyl phosphate was calculated as the ratio of the benchmark response (BMR) to the POD ($BMDL_{10[HED]}$) as shown below:

$$\begin{aligned} \textbf{p-OSF} &= BMR \div BMDL_{10[HED]} \\ &= 0.1 \div 5.74 \text{ mg/kg-day} \\ &= \textbf{0.017 or } \mathbf{2 \times 10^{-2}\ (mg/kg\text{-}day)^{-1}} \end{aligned}$$

The OSF for trimethyl phosphate should not be used with exposures exceeding the POD ($BMDL_{10[HED]}$ = 5.74 mg/kg-day) because, at exposures above this level, the fitted dose-response model better characterizes what is known about the carcinogenicity of trimethyl phosphate. Table 14 shows the doses associated with specific levels of cancer risk based on the p-OSF estimated herein.

Table 14. Doses of Trimethyl Phosphate Associated with Specific Levels of Cancer Risk	
Risk Level	**Dose (mg/kg-day)**
10^{-4}	0.006
10^{-5}	0.0006
10^{-6}	0.00006

Inhalation Exposure

Lack of suitable data precluded derivation of quantitative estimates of cancer risk following inhalation exposure to trimethyl phosphate.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2009) Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH.

Adler, ID; Ramarao, G; Epstein, SS. (1971) In vivo cytogenetic effects of trimethylphosphate and of TEPA on bone marrow cells of male rats. Mutat Res 13:263−273.

Anderson, D; Richardson, CR. (1981) Issues relevant to the assessment of chemically induced chromosome damage in vivo and their relationship to chemical mutagenesis. Mutat Res 90(3):261−272.

Anderson, D; Styles, JA. (1978) An evaluation of 6-short-term tests for detecting organic chemical carcinogens. Appendix 2. The bacterial mutation test. Br J Cancer 37:924−930.

ATSDR (Agency for Toxic Substances and Disease Registry). (2009) Toxicological Profile Information Sheet. U.S. Department of Health and Human Services, Public Health Service. Available online at http://www.atsdr.cdc.gov/toxpro2.html (accessed on September 15, 2009).

Bomhard, EM; Krinke, GJ; Rossberg, WM; et al. (1997) Trimethylphosphate: a 30-month chronic toxicity/carcinogenicity study in Wistar rats with administration in drinking water. Fundam Appl Toxicol 40(1):75−89.

Bruce, WR; Heddle, JA. (1979) The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays. Can J Genet Cytol 21(3):319−334.

CalEPA (California Environmental Protection Agency). (2009a) Search chronic RELs. Office of Environmental Health Hazard Assessment. Available online at http://www.arb.ca.gov/toxics/healthval/chronic.pdf http://www.oehha.ca.gov/air/chronic_rels/AllChrels.html (accessed September 15, 2009).

CalEPA (California Environmental Protection Agency). (2009b) Search toxicity criteria database. Office of Environmental Health Hazard Assessment. Available online at http://www.oehha.ca.gov/air/hot_spots/pdf/TSDlookup2002.pdf and http://www.oehha.ca.gov/air/hot_spots/pdf/Appendix%20I2002.pdf (accessed September 15, 2009).

Carstensen, H. (1971) Inhibiting effect of a methylating agent, trimethylphosphate, on testosterone synthesis in the male rat. Life Sci I 10(16):917−925.

Cho, NH; Park, C. (1994) Effects of dimethyl methylphosphonate (DMMP) and trimethylphosphate (TMP) on spermatogenesis of rat testis. Yonsei Med J 35(2):198−208.

De Flora, S. (1981) Study of 106 organic and inorganic compounds in the salmonella/microsome test. Carcinogenesis 2:283−298.

De Flora, S; Zanacchi, P; Camoirano, A; et al. (1984) Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat Res 133:161−198.

De Flora, S; Zanacchi, P; Bennicelli, C; et al. (1990) Genotoxicity biotransformations and interactions of marine pollutants as related to genetic and carcinogenic hazards. In: Grandjean, E; ed. Advances in applied biotechnology series, Vol. 5. carcinogenic, mutagenic, and teratogenic marine pollutants: impact on human health and the environment. Houston, TX: Gulf Publishing Co. pp. 3−32.

Dean, BJ. (1972) The mutagenic effects of organophosphorus pesticides on microorganisms. Arch Toxikol 30:67−74.

Dean, BJ; Thorpe, E. (1972) Studies with dichlorvos vapour in dominant lethal mutation tests on mice. Arch Toxikol 30:51−59.

Degraeve, N; Moutschen, J; Moutschen-Dahmen, M; et al. (1979) Genetic effects of organophosphate insecticides in mouse. Mutat Res 64(2):131.

Degraeve, N; Chollet, MC; Moutschen, J. (1984) Cytogenetic effects induced by organophosphorus pesticides in mouse spermatocytes. Toxicol Lett 21:315−319.

Dyer, KF; Hanna, PJ. (1972) Mutagenic and antifertility activity of trimethylphosphate in Drosophila melanogaster. Mutat Res 16(3):327−331.

Epstein, SS; Bass, W; Arnold, E; et al. (1970) Mutagenicity of trimethylphosphate in mice. Science 168(931):584−586.

Epstein, SS; Arnold, E; Andrea, J; et al. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 23(2):288−325.

Farrow, MG. (1975) The use of tri methyl phosphate as a positive control agent in the dominant lethal assay cyto genetic assay and host mediated assays. Mutat Res 31(5):314−315.

Farrow, MG; Barnett, J; Lassen, LJ; et al. (1976) The use of trimethyl phosphate as a positive control agent part 2 the salmonella microsome test the salmonella urine assay and the micro nucleus test. Mutat Res 38(6):381−382.

Fluck, ER; Poirier, LA; Ruelius, HW. (1976) Evaluation of a dna polymerase-deficient mutant of E. coli for the rapid detection of carcinogens. Chem Biol Interact 15:219−231.

Fukunishi, K; Teraoka, M; Awata, N. (1999) The effect of 5 days treatment with trimethyl phosphate on sperm motile and other characteristic parameters measured by CASA-system (IVOS). Proceedings of 26th Annual Meeting of the Japanese Society of Toxicology, p. 347 (as cited in Kato et al., 2001).

Fukunishi, K; Teraoka, M; Awata, N. (2000) Effect of a single treatment of trimethyl phosphate (TMP) on sperm motility and other characteristic parameters measured by the CASA-system. Teratology 62(3):46A.

Goodman, DG; Ward, JM; Squire, RA; et al. (1979) Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol Appl Pharmacol 48:237−248.

Graf, U; Frei, H; Kaegi, A; et al. (1989) Thirty compounds tested in the drosophila wing spot test. Mutat Res 222:359−373.

Hanna, PJ; Dyer, KF. (1975) Mutagenicity of organophosphorus compounds in bacteria and Drosophila. Mutat Res 28:405−420.

Hanna, PJ; Kerr, JB. (1981) Antifertility activity of trimethylphosphate in male rats. Experientia 37(9):999−1001.

Harbison, RD; Dwivedi, C; Evans, MA. (1976) A proposed mechanism for trimethylphosphate-induced sterility. Toxicol Appl Pharmacol 35(3):481−490.

Hellmer, L; Bolcsfoldi, G. (1992) An evaluation on the Escherichia coli K-12 uvrB/recA DNA repair host-mediated assay: I. In vitro sensitivity of the bacteria to 61 compounds. Mutat Res 272(2):145−160.

IARC (International Agency for Research on Cancer). (2009) IARC monographs on the evaluation of carcinogenic risks to humans. Available online at http://www.monographs.iarc.fr/ENGMonographs/allmonos90.php (accessed September 15, 2009).

IPCS (International Programme on Chemical Safety). (2006) Trimethyl phosphate. Organisation for Economic Co-operation and Development/Screening Information Data Set for High Production Volume Chemicals. Available online at http://www.inchem.org/documents/sids/sids/512561.pdf (accessed September 15, 2009).

Jackson, H; Jones, AR. (1968) Antifertility action and metabolism of trimethylphosphate in rodents. Nature 220(5167):591−592.

Jones, AR; Jackson, H. (1969) Chemosterilant action of trimethylphosphate in rodents. Br J Pharmacol 37:531.

Kato, M; Fukunishi, K; Ikegawa, S; et al. (2001). Overview of studies on rat sperm motion analysis using a Hamilton-Thorn sperm analyzer − collaborative working study. J Toxicol Sci 26(5):285−297.

Katoh, M; Matsuda, Y. (1985) Chromosome-type aberrations in postmeiotic male germ cells of mice administered trimethylphosphate. Mutat Res 147:258−259.

Legator, MS; Palmer, KA; Adler, ID. (1973) A collaborative study of in vivo cytogenetic analysis. 1. interpretation of slide preparations. Toxicol Appl Pharmacol 24:337−350.

Li, SQ; Liu, YQ; Ni, ZY; et al. (1993) Mutagenicity of 24 organophosphorus, pesticides determined by 6 short-term tests. Zhongguo Yaolixue Yu Dulixue Zazhi 7(1):73−77.

Lorke, D; Machemer, L. (1975) Testing mutagenic properties with the dominant lethal test on the male mouse. Environ Qual Saf Suppl 4:239−246.

Machemer, L; Lorke, D. (1975) Method for testing mutagenic effects of chemicals on spermatogonia of the Chinese hamster: results obtained with cyclophosphamide, saccharin, and cyclamate. Arzneimittelforschung 25(12):1889−1896.

MacPhee, DG. (1973) Salmonella typhimurium Hisg46 (R-Utrecht): possible use in screening mutagens and carcinogens. Appl Microbiol 26(6):1004−1005.

MHW. (1994) Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test of Trimethyl Phosphate by Oral Administration in Rats. Unpublished Japanese report. Available online at http://dra4.nihs.go.jp/mhlw_data/home/paper/paper512-56-1D.html.

Moutschen, N and J.Degraeve. (1981) Metrifonate and dichlorvos: cytogenetic investigations. Acta Pharmacol Toxicol Suppl (Copenh) 49(5):29−39.

NCI (National Cancer Institute). (1978) Bioassay of trimethyl phosphate for possible carcinogenicity. NCI Carcinogenesis Tech. Rep. Ser. No. 81. Available online at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr081.pdf (accessed September 15, 2009).

Newell, GW; Jorgenson, TA; Rushbrook, CJ; et al. (1976) Preliminary investigations of new orally active reference mutagens. Toxicol Appl Pharmacol 37:108.

Ni, Z; Li, S; Liu, Y; et al. (1993) [Induction of micronucleus by organophosphorus pesticides both in vivo and in vitro]. Hua Xi Yi Ke Da Xue Xue Bao 24(1):82−86.

NIOSH (National Institute for Occupational Safety and Health). (1982) Health hazard evaluation report No. HHE-80-137-1145, Shell Chemical Company, Denver, Colorado. HHE-80-137-1145.

NIOSH (National Institute for Occupational Safety and Health). (2009) NIOSH pocket guide to chemical hazards. Index by CASRN. Available online at http://www.cdc.gov/niosh/npg/npgdcas.html (accessed September 15, 2009).

NTP (National Toxicology Program). (2005) 11th Report on carcinogens. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. Available online at http://ntp-server.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932 (accessed September 15, 2009).

Oishi, H; Oishi, S; Hiraga, K. (1982) Toxicity of several phosphoric acid esters in rats. Toxicol Lett 13(1−2):29−34.

OSHA (Occupational Safety and Health Administration). (2009) OSHA Standard 1915.1000 for air contaminants. Part Z, toxic and hazardous substances. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10286 (accessed September 15, 2009).

Rushbrook, CJ; Jorgenson, TA; Blazak, WF. (1985) Induction of heritable translocations in mice by trimethylphosphate. Environ Mutagen 7(Suppl 3):65−66.

Sasaki, YF; Tezuka, H; Inoue, M; et al. (1984) Heritable translocation test on trimethylphosphate in C3H mice. Mutat Res 130:380.

Schaeppi, U; Krinke, G; Kobel, W. (1984) Prolonged exposure to trimethylphosphate induces sensory motor neuropathy in the dog. Neurobehav Toxicol Teratol 6(1):39−50.

Sheu, CW. (1979) Collaborative studies on cytogenetic and dominant lethal assays. J Assoc Off Anal Chem 62:844−856.

Sina, JF; Bean, CL; Dysart, GR; et al. (1983) Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res 113:357−391.

Sinha, AK; Linscombe, VA; Gollapudi, BB; et al. (1983) In vivo cytogenetic study in rats maintained for eight days on diets containing probucol. Mutat Res 124(1):91−96.

Soderman, G. (1972) Chromosome breaking effect of a gasoline additive in cultured human lymphocytes. Hereditas 71(2):335−338.

Storer, RD; McKelvey, TW; Kraynak, AR; et al. (1996) Revalidation of the in vitro alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat Res 368(2):59−101.

Suzuki, H; Omotera, A; Ichiba, S; et al. (1996) Utility of a novel chamber for sperm analysis in rats. Teratology 54(4):38A−39A.

Takizawa, S; Katoh, C; Inomata, A; et al. (1998) Flow cytometric analysis for sperm viability and counts in rats treated with trimethylphosphate or pyridoxine. J Toxicol Sci 23(1):15−23.

Tezuka, H; Sasaki, YF; Inoue, M; et al. (1985) Heritable translocation study in male mice with trimethyl phosphate. Mutat Res 157(2-3):205−213.

Toth, GP; Wang, SR; McCarthy, H; et al. (1992) Effects of three male reproductive toxicants on rat cauda epididymal sperm motion. Reprod Toxicol 6(6):507−515.

U.S. EPA (Environmental Protection Agency). (1985) Health and Environmental Effects Profile (HEEP) for trimethyl phosphate. Environmental Criteria and Assessment Office, Cincinnati, OH. ECAO-CIN-P147. September.

U.S. EPA (U.S. Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Cincinnati, OH.

U.S. EPA (Environmental Protection Agency). (1991) Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA (Environmental Protection Agency). (1994) Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA (Environmental Protection Agency). (1997) Health Effects Assessment Summary Tables (HEAST). FY-1997 update. Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati OH for the Office of Emergency and Remedial Response, Washington, DC. EPA/540/R-97/036. NTIS PB97-921199.

U.S. EPA (U.S. Environmental Protection Agency). (1999) Benchmark dose software (BMDS) version 2.1.1 and 2.1.2 (last modified June 16, 2010). Available online at http://www.epa.gov/ncea/bmds/.

U.S. EPA (Environmental Protection Agency). (2005) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765−17817.

U.S. EPA (Environmental Protection Agency). (2006) 2006 Edition of the drinking water standards and health advisories. Office of Water, Washington, DC. EPA/822/R-06/013. Washington, DC. Available online at http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf (accessed September 15, 2009).

U.S. EPA (Environmental Protection Agency). (2009) Integrated Risk Information System (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/iris/ (accessed September 15, 2009).

Valencia, R. (1981) Mutagenesis screening of pesticides using drosophila. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC. EPA/600/S1-81/017.

Vogel, EW; Nivard, MJ. (1993) Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis 8(1):57−81.

Watanabe, K; Sakamoto, K; Sasaki, T. (1996) Comparisons on chemically-induced mutagenicity among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: collaborative study I. Mutat Res 361(2-3):143−155.

Weber, E; Bidwell, K; Legator, MS. (1974) An evaluation of the micronuclei test for routine cytogenetic analysis. Mutat Res 26:461.

Weber, E; Bidwell, K; Legator, MS. (1975) An evaluation of the micronuclei test using triethylenemelamine, trimethylphosphate, hycanthone and niridazole. Mutat Res 28(1):101−106.

WHO (World Health Organization). (2009). Online catalogs for the Environmental Health Criteria series. Available online at http://www.who.int/ipcs/publications/ehc/ehc_alphabetical/en/index.html (accessed September 15, 2009).

Zeiger, E; Pagano, DA; Nomeir, AA. (1982) Structure-activity studies on the mutagenicity of tris(2,3-dibromopropyl)phosphate (Tris-Bp) and its metabolites in Salmonella. Environ Mutagen 4(3):271−277.

Zeiger, E; Anderson, B; Haworth, S; et al. (1992) Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19 Suppl 21:2−141.

APPENDIX A. DETAILS OF BENCHMARK DOSE MODELING FOR ORAL SLOPE FACTOR

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model-fitting procedure for dichotomous cancer incidence data is as follows. The multistage cancer model in the EPA Benchmark Dose Software (BMDS) is fit to the incidence data using the extra risk option. The multistage cancer model is run for all polynomial degrees up to n - 1 (where n is the number of dose groups including control). Adequate model fit is judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response. Among all of the models providing adequate fit to the data, the lowest BMDL is selected as the point of departure when the difference between the benchmark dose lower bound 95% confidence intervals (BMDLs) estimated from these models are more than 3-fold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) is chosen. In accordance with EPA (1999 guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated.

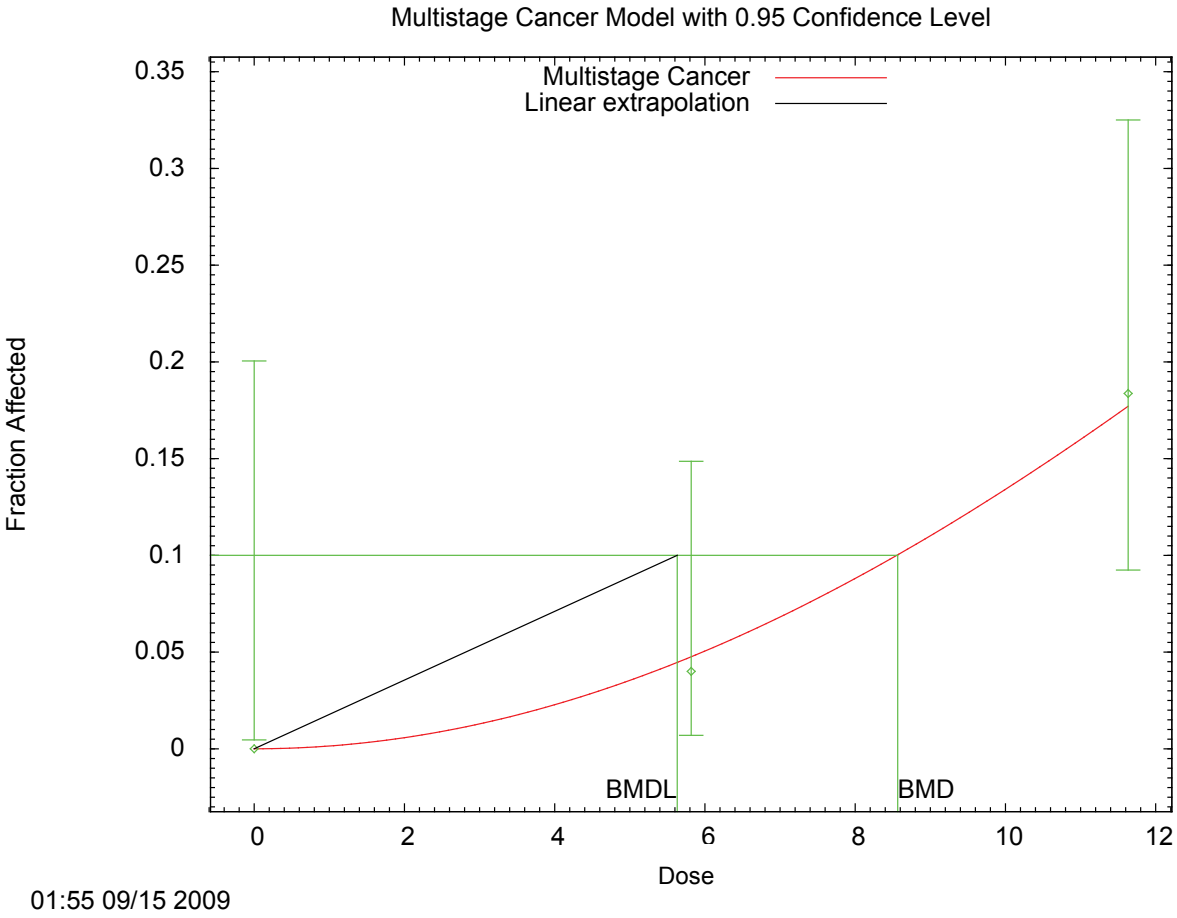
MODEL-FITTING RESULTS FOR SUBCUTANEOUS FIBROMAS IN MALE F344 RATS (NCI, 1978)

Table 12 shows the incidence data for subcutaneous fibromas in male F344 rats administered trimethyl phosphate via gavage 3 days/week, for 104 weeks (NCI, 1978). Modeling was performed according to the procedure outlined above using BMDS version 2.1 with default parameter restrictions based on the duration-adjusted human equivalent dose (HED) shown in Table 12. Table A-1 shows model predictions. The multistage cancer model provided adequate fit (goodness-of-fit p-value > 0.1), and the 2-degree polynomial model had the lowest AIC, yielding a $BMD_{10[HED]}$ value of 8.57 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10[HED]}$) of 5.63 mg/kg-day. The fit of the 2-degree multistage cancer model to the incidence data for male rats is shown in Figure A-1.

Table A-1. Model Predictions for Subcutaneous Fibromas in Male F344 Rats

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit p-Value[a]	AIC	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)
Multistage Cancer Model (2-degree polynomial)[b]	2	0.08	0.961	65.61	8.57	5.63
Multistage Cancer Model (1-degree polynomial)[b]	2	1.46	0.482	67.15	7.67	4.85

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Betas restricted to ≥0.



BMDs and BMDLs indicated are associated with an extra risk of 10% and are in units of mg/kg-day as HED.

Figure A-1. Fit of 2-Degree Multistage Cancer Model to Data on Incidence of Subcutaneous Fibromas in Male F344 Rats

Source: NCI (1978).

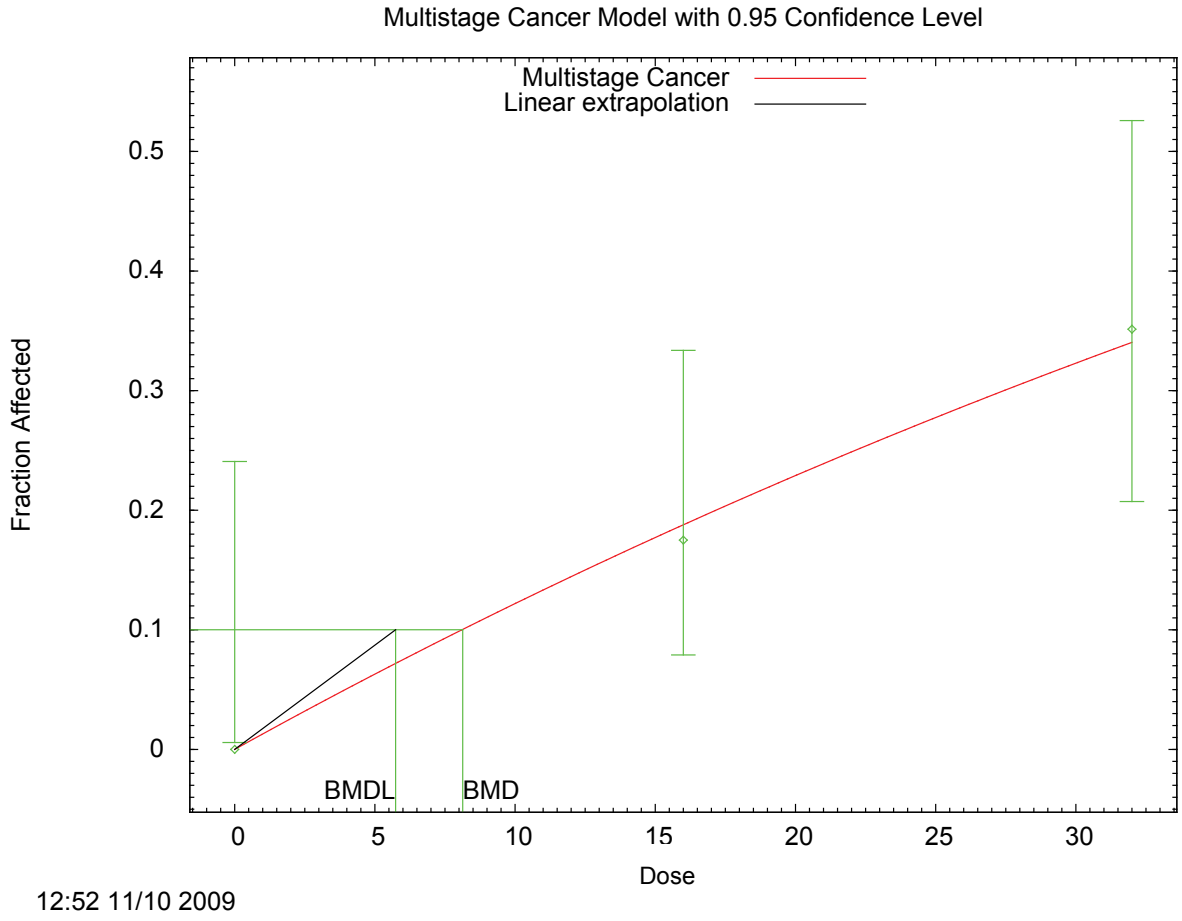
MODEL-FITTING RESULTS FOR ENDOMETRIAL ADENOCARCINOMAS IN FEMALE B6C3F1 MICE (NCI, 1978)

Table 12 shows the incidence data for endometrial adenocarcinomas in female B6C3F1 mice administered trimethyl phosphate via gavage 3 days/week, for 103 weeks (NCI, 1978). Modeling was performed according to the procedure outlined above using BMDS version 2.1 with default parameter restrictions based on the duration-adjusted HED shown in Table 12. Table A-2 shows model predictions. The multistage cancer model provided adequate fit (goodness-of-fit p-value > 0.1), and the 1-degree polynomial model had the lowest AIC, yielding a $BMD_{10[HED]}$ value of 8.13 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10[HED]}$) of 5.74 mg/kg-day. The fit of the 1-degree multistage cancer model to the incidence data for female mice is shown in Figure A-2.

Table A-2. Model Predictions for Uterine Adenocarcinomas in Female B6C3F1 Mice

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit *p*-Value[a]	AIC	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)
Multistage Cancer Model (2-degree polynomial)[b]	1	0	1	89.07	9.25	5.76
Multistage Cancer Model (1-degree polynomial)[b]	2	0.06	0.969	87.13	8.13	5.74

[a]Values < 0.10 fail to meet conventional goodness-of-fit criteria.
[b]Betas restricted to ≥ 0.



BMDs and BMDLs indicated are associated with an extra risk of 10% and are in units of mg/kg-day as HED.

Figure A-2. Fit of 1-Degree Multistage Cancer Model to Data on Incidence of Uterine Adenocarcinomas in Female B6C3F1 Mice.

Source: NCI (1978).