

NATIONAL TOXICOLOGY PROGRAM
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TOXICOLOGY AND CARCINOGENESIS
STUDIES OF
DIMETHYL METHYLPHOSPHONATE
(CAS NO. 756-79-6)
IN F344/N RATS AND B6C3F₁ MICE
(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is made up of four charter DHHS agencies: the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF
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(GAVAGE STUDIES)



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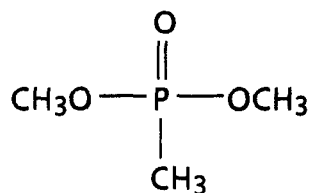
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NOTE TO THE READER

This study was performed under the direction of the National Institute of Environmental Health Sciences as a function of the National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for public peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

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DIMETHYL METHYLPHOSPHONATE

CAS No. 756-79-6

$\text{C}_3\text{H}_9\text{O}_3\text{P}$

Molecular weight 124.1

Synonyms: Fyrol DMMP; Methyl phosphonic acid, dimethyl ester; DMMP; Methanephosphonic acid dimethyl ester; Dimethyl methanephosphonate

ABSTRACT

Dimethyl methylphosphonate (98% pure) is one of four chemicals nominated by the U.S. Army for toxicology and carcinogenesis studies because it was being considered for use to simulate the physical and spectroscopic (but not the biologic) properties of anticholinesterase (nerve) agents. Dimethyl methylphosphonate is also used as a flame retardant, a preignition additive for gasoline, an antifoam agent, a plasticizer and stabilizer, a textile conditioner and antistatic agent, and an additive for solvents and low-temperature hydraulic fluids. The United States produces 0.2-2 million pounds (91,000-910,000 kg) of dimethyl methylphosphonate per year. Gavage was chosen as the route of administration for all four candidate "simulants" to mimic potential oral exposure.

Experimental Design: Dimethyl methylphosphonate was administered in corn oil by gavage to male and female F344/N rats and B6C3F₁ mice in single-administration, 15-day, and 13-week studies to obtain toxicity data, to establish dose levels for the 2-year studies, and to identify target tissues. Additional studies were also performed to determine toxicity to the reproductive system of male F344/N rats and B6C3F₁ mice and to study the potential for genetic damage in bacteria, mammalian cells, and *Drosophila*.

Single-Administration Studies: In the single-administration studies, dimethyl methylphosphonate was given to rats and mice at doses up to 6,810 mg/kg body weight. No compound-related deaths were seen in male or female rats or male mice; two high dose female mice died. Rats exhibited inactivity, unsteady gait, and prostration after dosing; mice were inactive after dosing.

Fifteen-Day Studies: Rats and mice received doses of 0, 1,250, 2,500, 5,000, 10,000, or 15,000 mg/kg dimethyl methylphosphonate per day. Compound-related deaths occurred in the three highest dose groups of rats and the two highest dose groups of mice. Rats receiving doses of 2,500 mg/kg or higher were inactive and at 5,000 or 10,000 mg/kg had an unsteady gait after dosing; mice exhibited inactivity, shallow breathing, and prostration at doses of 10,000 mg/kg and higher. No lesions were reported in rats. Nonneoplastic lesions of the stomach were seen in some male mice at doses of 1,250 mg/kg and higher and in some female mice at doses of 5,000 mg/kg and higher.

Thirteen-Week Studies: Dimethyl methylphosphonate was given at doses up to 8,000 mg/kg per day. Compound-related deaths occurred at 2,000, 4,000, and 8,000 mg/kg in rats and at 4,000 and 8,000 mg/kg in mice. Mean body weights of rats at 1,000 mg/kg and mice at 2,000 mg/kg were similar to those of the vehicle controls; decreased weight gain was seen at higher doses. No compound-related clinical signs were reported. Minimal to mild renal and testicular lesions were seen at all doses in male rats, but the severity of these lesions did not increase with increasing dose of the chemical. No apparent target tissues were identified in female rats or male and female mice.

Doses selected for the 2-year studies were based on body weight effects and mortality seen in the 13-week studies; the lesions seen in the kidney of male rats at the end of the 13-week studies were judged not to be life threatening. In the 2-year studies, dimethyl methylphosphonate was administered in corn oil by gavage at doses of 0, 500, or 1,000 mg/kg per day to groups of 50 F344/N rats of each sex and at 0, 1,000, or 2,000 mg/kg per day to groups of 50 B6C3F₁ mice of each sex. All animals were dosed 5 days per week for 103 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 5%-10% lower than those of the vehicle controls between weeks 28 and 76 and were 10%-24% lower between weeks 80 and 104. Mean body weights of high dose female rats were 8%-12% lower than those of the vehicle controls after week 80. Survival of male rats was greater than 50% in all groups until week 80, and after this time, survival decreased in both dose groups, with the survival at the end of the study being 27/50 in vehicle control, 17/50 in low dose, and 4/50 in high dose groups. Survival of low dose female rats was comparable to that of the vehicle controls, but final survival of high dose female rats was decreased (vehicle control, 30/50; low dose, 33/50; high dose, 23/50). No other compound-related clinical signs were observed.

Mean body weights of high dose male mice were 7%-16% lower than those of the vehicle control males between weeks 36 and 76, and those of high dose female mice were 6%-12% lower between weeks 88 and 103. Decreased survival between weeks 23 and 45 in high dose male mice was associated with fighting. Seventeen high dose male and 22 high dose female mice died during week 45; these deaths were associated with the accidental administration of a dose mixture that had a concentration 34% greater than the targeted amount. Eleven low dose male mice died on the same day during week 77. By the end of the study, 29/50 vehicle control, 12/50 low dose, and 0/50 high dose male mice were alive; 41/50, 30/50, and 2/50 female mice survived to the end of the study.

Renal Effects in the Two-Year Studies: Administration of dimethyl methylphosphonate to male rats increased the average severity of nephropathy and caused mineralization (calcification) of the collecting tubules in the renal papilla (12/50; 41/50; 36/49), hyperplasia of the transitional epithelium lining the renal pelvis and overlying the renal papilla (0/50; 23/50; 21/49), and focal hyperplasia of the renal tubular epithelium (0/50; 8/50; 9/49). Administration of dimethyl methylphosphonate to male rats was also associated with the occurrence of rare renal tubular cell adenocarcinomas (0/50; 2/50; 3/49) and papillomas of the transitional epithelium lining the renal pelvis (0/50; 7/50; 3/49); a transitional cell carcinoma occurred in a low dose male rat. There were no tubular cell or transitional cell neoplasms of the kidney in female rats.

Hematopoietic System Effects in the Two-Year Studies: The incidence of mononuclear cell leukemia was increased in high dose male rats (10/50; 11/50; 17/50).

Genetic Toxicity: Dimethyl methylphosphonate was not mutagenic when tested in the *Salmonella typhimurium*/microsome assay by the preincubation protocol with strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. The chemical did induce forward mutations in the mouse lymphoma L5178Y/TK^{+/-} assay system in the absence of metabolic activation. Treatment of cultured Chinese hamster ovary cells with dimethyl methylphosphonate did not induce chromosomal aberrations; however, sister chromatid exchanges were induced after exposure to this chemical in both the presence and absence of metabolic activation. When fed to *Drosophila*, dimethyl methylphosphonate induced a significant increase in the frequency of sex-linked recessive lethal mutations but did not induce reciprocal translocations. Dimethyl methylphosphonate caused a dominant lethal effect in male rats and mice.

Studies of Reproductive Effects: Dimethyl methylphosphonate caused a dose-related increase in the number of fetal resorptions in undosed female rats and mice mated with males that received the chemical by gavage in water 5 days per week for 13 weeks at doses of 0-2,000 mg/kg per day. After the 13-week dosing period, histopathologic changes were seen in the kidney and testis of male rats

but not in male mice; dosed male rats sired fewer litters and fewer pups per litter. Dose-related decreases in sperm count and sperm motility occurred in male rats but not in male mice. Toxic effects to the reproductive system of male rats and mice were reversible after a 13- to 14-week recovery period.

Data Audit: An audit of the experimental data was conducted for the 2-year studies on dimethyl methylphosphonate. No data discrepancies were found that influenced the final interpretations.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity** of dimethyl methylphosphonate for male F344/N rats as shown by increased incidences of tubular cell hyperplasia, tubular cell adenocarcinomas, hyperplasia of the transitional cell epithelium, and transitional cell papillomas of the kidney. There was an increased incidence of mononuclear cell leukemia in male rats at 1,000 mg/kg. Renal toxicity and decreased survival occurred in dosed male rats. There was *no evidence of carcinogenic activity* of dimethyl methylphosphonate for female F344/N rats given doses of 500 or 1,000 mg/kg. The study in male B6C3F₁ mice was an *inadequate study of carcinogenic activity* because of decreased survival in both dosed groups. There was *no evidence of carcinogenic activity* for female B6C3F₁ mice receiving dimethyl methylphosphonate at 1,000 mg/kg; decreased survival of female mice at 2,000 mg/kg made this group inadequate for determination of carcinogenic activity.

SUMMARY OF THE TWO-YEAR GAVAGE AND GENETIC TOXICOLOGY STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Male F344/N Rats | Female F344/N Rats | Male B6C3F ₁ Mice | Female B6C3F ₁ Mice |
|--|---|---|---|
| Doses 0, 500, or 1,000 mg/kg dimethyl methylphosphonate in corn oil | 0, 500, or 1,000 mg/kg dimethyl methylphosphonate in corn oil | 0, 1,000, or 2,000 mg/kg dimethyl methylphosphonate in corn oil | 0, 1,000, or 2,000 mg/kg dimethyl methylphosphonate in corn oil |
| Nonneoplastic effects Renal tubular cell hyperplasia; renal transitional cell hyperplasia | None | None | None |
| Neoplastic effects Renal tubular cell adenocarcinomas; renal transitional cell papillomas | None | None | None |
| Level of evidence of carcinogenic activity Some evidence | No evidence | Inadequate study | No evidence |
| Other considerations -- | -- | Reduced survival of dosed groups | -- |
| Genetic toxicology Not mutagenic in <i>S. typhimurium</i> strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation. Induced forward mutations in the mouse lymphoma L5178Y/TK ^{+/-} assay system in the absence of metabolic activation. Did not induce chromosomal aberrations in cultured CHO cells. SCEs significantly increased in both presence and absence of metabolic activation. Induced significant increase in frequency of sex-linked recessive lethal mutations in <i>Drosophila</i> . Caused dominant lethal effect in male rats and mice. | | | |

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 10.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans.

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

These considerations together with the definitions as written should be used as composite guidelines for selecting one of the five categories. Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the induction by chemicals of more neoplasms than are generally found, or the earlier induction by chemicals of neoplasms that are commonly observed. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on dimethyl methylphosphonate on August 19, 1986, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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**SUMMARY OF PEER REVIEW COMMENTS
ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF
DIMETHYL METHYLPHOSPHONATE**

On August 19, 1986, the draft Technical Report on the toxicology and carcinogenesis studies of dimethyl methylphosphonate received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. J.K. Dunnick, NTP, introduced the studies by reviewing the experimental design, results, and proposed conclusions (some evidence of carcinogenic activity for male rats; no evidence of carcinogenic activity for female rats; inadequate study of carcinogenic activity for male mice; no evidence of carcinogenic activity for female mice).

Dr. Crowley, a principal reviewer, agreed with the conclusions for male and female rats and male mice. He proposed that the conclusion for female mice be changed to inadequate study of carcinogenic activity based on there being reasonable survival only in the low dose group. Dr. Popp said that since there was one valid dose group, he thought the study was adequate.

As a second principal reviewer, Dr. Purchase commented that the substantial reduction in body weight and survival in high dose male rats indicated that the dose was excessive and made the findings difficult to interpret. Regarding mononuclear cell leukemia in male rats, he said that it would be appropriate to analyze stage-1 and stage-2 (nonlethal) leukemia by the incidental tumor test and to analyze stage-3 (lethal) leukemia by the life table test. Dr. J. Haseman, NIEHS, said such analyses were done but because most of the leukemia was stage 3, this analysis gave results similar to those obtained for the life table test. Further, he noted that the kidney lesions rather than the leukemia were the primary basis for the conclusion of some evidence of carcinogenic activity in male rats.

As a third principal reviewer, Dr. Gallo agreed with the conclusions as written. He thought that there could be some expanded discussion of the hypothesis regarding chemically induced renal lesions in male rats and increased renal tubular levels of α -2-microglobulin. [See page 57.]

Dr. Crowley moved that the Technical Report on dimethyl methylphosphonate be accepted with the conclusion as written for male rats (some evidence of carcinogenic activity). Dr. Hooper seconded the motion, and it was approved unanimously with eight votes. Dr. Crowley then moved for acceptance of the conclusion as written for female rats (no evidence of carcinogenic activity) and of the conclusion as written for male mice (inadequate study of carcinogenic activity). Dr. Hooper seconded both motions, and they were approved unanimously with eight votes. Dr. Crowley moved that the conclusion for female mice, no evidence of carcinogenic activity, be changed to inadequate study of carcinogenic activity. Dr. Chinchilli seconded the motion, which failed by six votes to two (Dr. Chinchilli and Dr. Crowley). Dr. Mirer moved that the conclusion as written be amended to state that higher doses might have been tolerated. Dr. Hooper seconded that motion, which failed by seven votes to one (Dr. Mirer). Dr. Purchase moved that the conclusion for female mice be accepted as written. Dr. Popp seconded the motion, and it was approved by five reviewers with three dissenting (Dr. Chinchilli, Dr. Crowley, and Dr. Mirer).

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Dimethyl Methylphosphonate is based on the first 13-week studies that began in December 1979 and ended in March 1979, the second 13-week studies (rats) that began in August 1980 and ended in November 1980, and the 2-year studies that began in July 1981 (rats) or November 1981 (mice) and ended in July 1983 (rats) or November 1983 (mice) at Litton Bionetics, Inc.

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I. INTRODUCTION

Use and Production

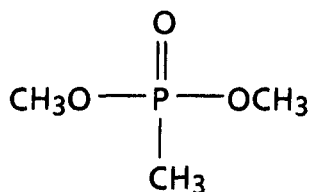
Worker Exposure and Environmental Fate

Studies in Animals

Genetic Toxicity

Study Rationale

I. INTRODUCTION



DIMETHYL METHYLPHOSPHONATE

CAS No. 756-79-6

$\text{C}_3\text{H}_9\text{O}_3\text{P}$

Molecular weight 124.1

Synonyms: Fyrol DMMP; Methyl phosphonic acid, dimethyl ester; DMMP; Methanephosphonic acid dimethyl ester; Dimethyl methanephosphonate

Use and Production

Dimethyl methylphosphonate is used as a flame retardant (Fyrol DMMP) in epoxy resins, acrylic latexes, unsaturated polyesters, urethane coatings, urethane rigid foam, and vinyl copolymers because it is a good solvent with low viscosity. Dimethyl methylphosphonate is used as a pre-ignition additive for gasoline, an antifoam agent, a plasticizer and stabilizer, a textile conditioner, and an antistatic agent and as an additive to solvents and low-temperature hydraulic fluids; it is also used in heavy metal extraction and solvent separation (Kirk-Othmer, 1980; USEPA, 1983a). Dimethyl methylphosphonate has been used experimentally as a nerve gas simulant to mimic the physical and spectroscopic (but not the biologic) properties of anticholinesterase agents (U.S. Naval Surface Weapons Center, 1982; Jones et al., 1948). The U.S. production range for dimethyl methylphosphonate in 1977 was 0.2-2 million pounds (91,000-910,000 kg). Dimethyl methylphosphonate is made by molecular rearrangement of trimethyl phosphite, which is catalyzed by a halogenated organic compound (USEPA, 1983a). Some properties of dimethyl methylphosphonate are summarized in Table 1.

Worker Exposure and Environmental Fate

No information is available on worker exposure to dimethyl methylphosphonate during the production process. Dimethyl methylphosphonate was identified in a liquid waste lagoon (Guzewich et al., 1983). The average half-life in soil is

estimated at 12 days and in water at 1-30 weeks, depending on temperature and initial concentration (USEPA, 1983b).

Studies in Animals

The acute oral LD_{50} value is estimated at greater than 3,000 mg/kg for rats and greater than 6,000 mg/kg for mice. The compound is an irritant to the skin and eyes of rabbits (USEPA, 1983b).

Male F344/N rats and B6C3F₁ mice administered dimethyl methylphosphonate by gavage in water, 5 days per week for 13 weeks at doses of 0, 250, 500, 1,000, or 2,000 mg/kg, showed dose-related toxicity to the reproductive system (Dunnick et al., 1984a,b; Chapin et al., 1984); a dominant lethal effect (increased fetal resorptions) was seen after male rats and mice, dosed for 13

TABLE 1. SOME PHYSICAL PROPERTIES OF DIMETHYL METHYLPHOSPHONATE (a)

| | |
|---------------------------------|---------------------|
| Boiling point | 181° C at 754 mm Hg |
| Melting point | 43° C |
| Density | 1.150 g/ml at 20° C |
| Solubility in water | >300 mg/ml |
| Index of refraction (n_D) | 1.4137 at 20° C |
| Flash point, Cleveland open cup | 220° F |
| Fire point, Cleveland open cup | 350° F |
| Viscosity (centistokes) | |
| 77° F | 1.81 |
| 100° F | 1.48 |
| 210° F | 0.84 |
| Vapor pressure (torr) | |
| 10°-65° C | <0.1-20 |

(a) USEPA, 1983b; Mobil Chemical Co.; MRI, 1982, 1986

weeks, were mated to undosed females. Decreased body weight gain and histopathologic changes (including vacuolization and necrosis of the testis and lack of spermatogenesis) were seen in male rats but not in male mice at 2,000 mg/kg. A dose-related decrease in sperm count and sperm motility was seen in male rats but not in male mice; dosed male rats sired fewer litters and fewer pups per litter than did vehicle control rats. The kidneys of dosed male rats (but not dosed male mice) had varying degrees of tubular cell regeneration, hyaline droplet degeneration, and cellular infiltrate. Toxicity to the reproductive system was reversible after a 13- to 14-week recovery period. No metabolism studies have been reported in the literature.

Hollingshaus et al. (1981) reported no delayed neurotoxicity in adult hens after daily intraperitoneal injections of dimethyl methylphosphonate at 50 mg/kg for 10 days. The NTP is currently conducting an acute neurotoxicity study of dimethyl methylphosphonate in hens, in which the chemical is administered by gavage at the LD₅₀ dose.

Genetic Toxicity

Dimethyl methylphosphonate was not mutagenic in the *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in either the presence or absence of S9 from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver when tested in the standard plate incorporation assay (Aerospace Medical Research Laboratory, 1983) or in a preincubation protocol (Appendix E, Table E1; Mortelmans et al., 1986). Dimethyl methylphosphonate induced forward mutations in the mouse lymphoma L5178Y/TK^{+/-} assay (Table E2) but not in the Chinese hamster ovary (CHO) cell/HGPRT assay (Aerospace Medical Research Laboratory, 1983); both assays were performed in the absence of S9.

In NTP studies, dimethyl methylphosphonate induced sister chromatid exchanges (SCEs) in CHO cells in the absence of S9 over a concentration range of 1.6-11 mg/ml (Table E3). No significant increases in SCEs were observed at doses up to 1 mg/ml in studies sponsored by the Air

Force. SCEs were also induced in the presence of S9 from Aroclor 1254-induced male Sprague Dawley rat liver, but only in the concentration range of 11-22 mg/ml. Dimethyl methylphosphonate did not induce neoplastic transformation in the BALB/c 3T3 cell assay (Aerospace Medical Research Laboratory, 1983).

In studies performed by the United States Air Force, dimethyl methylphosphonate induced chromosomal aberrations in CHO cells at the highest concentration tested (1 mg/ml) in the absence of exogenous metabolic activation (Aerospace Medical Research Laboratory, 1983), but in NTP studies, it did not induce chromosomal aberrations either in the presence or absence of Aroclor 1254-induced Sprague Dawley rat liver S9 when tested at concentrations up to 22 mg/ml (Table E4).

When tested for mutagenicity in vivo, dimethyl methylphosphonate significantly increased the frequency of sex-linked recessive lethal mutations in the germ cells of *Drosophila* males fed 23,735 ppm in a 5% sucrose solution (Table E5). This procedure did not induce reciprocal translocations (Table E6). The results with *Drosophila* and the dominant lethal effects seen in rats and mice (Dunnick et al., 1984a,b) demonstrate that dimethyl methylphosphonate induces chromosomal damage in postmeiotic germ cells.

Study Rationale

Dimethyl methylphosphonate was nominated in 1976 by the U.S. Army for toxicology and carcinogenesis studies because it was being considered for use as an anticholinesterase agent simulant to mimic the physical and spectroscopic (but not biologic) properties of these agents. Recently, toxicology and carcinogenesis studies have been completed on the three other candidate simulants nominated for testing by the U.S. Army: tris(2-ethylhexyl)phosphate (NTP, 1984), dimethyl morpholinophosphoramidate (NTP, 1986a), and dimethyl hydrogen phosphite (NTP, 1985a). All four chemicals were administered by the same route to facilitate comparison of results. Corn oil was chosen as a common vehicle because of the potential for hydrolysis in water of some of the chemicals in the group.

II. MATERIALS AND METHODS

**PROCUREMENT AND CHARACTERIZATION OF
DIMETHYL METHYLPHOSPHONATE**

**PREPARATION AND CHARACTERIZATION OF
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SINGLE-ADMINISTRATION STUDIES

FIFTEEN-DAY STUDIES

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SECOND THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

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Source and Specifications of Animals

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II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF DIMETHYL METHYLPHOSPHONATE

Dimethyl methylphosphonate was obtained in five lots from either Edgewood Arsenal or Stauffer Chemical Company (Table 2). All lots were obtained as clear colorless liquids. Identity and purity determinations were conducted by the analytical chemistry laboratory (Midwest Research Institute, MRI). (MRI reports on the analyses performed in support of the dimethyl methylphosphonate studies are on file at NIEHS.) The infrared, ultraviolet/visible, and nuclear magnetic resonance spectra of all lots were consistent with the spectra expected for the structure of dimethyl methylphosphonate (representative spectra presented in Figures 1 and 2). Purity of all lots studied was determined by elemental analysis, Karl Fischer water analysis, thin-layer chromatography, and gas chromatography. Thin-layer chromatography was performed with silica gel plates with iodine vapor visualization and a mobile phase of either acetone (100%) or methanol (100%). Gas chromatographic analysis was performed with flame ionization detection on either a 10% Carbowax 20M-TPA column (system 1) or a 20% SP2100/0.1% Carbowax 1500 column (system 2). Results of the purity analyses are presented in Table 3.

Stability studies with the gas chromatographic system described above for system 1 indicated that dimethyl methylphosphonate was stable as

a bulk chemical when kept for 2 weeks at temperatures of up to 60° C. Further confirmation of the stability of the bulk chemical during the toxicity studies (storage at 20° C) was obtained by gas chromatographic analysis with the same column as that described above for system 2. No deterioration was seen over the course of the studies. Identity of the chemical at the study laboratory was confirmed by infrared spectroscopy.

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

Dimethyl methylphosphonate was mixed with corn oil as described in Table 4. Studies conducted by the study laboratory indicated that the preparation of homogeneous dose mixtures required both blending with a Polytron® mixer and mechanical stirring and that dose mixtures could be resuspended adequately by handmixing and stirring with a magnetic stirrer. The analytical chemistry laboratory conducted stability studies of dose mixtures by gas chromatography with the same column as that described above for system 1. The results of this study indicated that dimethyl methylphosphonate at 0.6% in corn oil is stable when stored at room temperature for up to 7 days. A subsequent stability study performed at the study laboratory indicated that dimethyl methylphosphonate/corn oil mixtures are stable for 14 days under refrigeration. In the 2-year studies, dose mixtures were stored at 4° C for no longer than 13 days.

TABLE 2. IDENTITY AND SOURCE OF LOTS USED IN THE GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Single-Administration Studies | Fifteen-Day Studies | First Thirteen-Week Studies | Second Thirteen-Week Studies | Two-Year Studies |
|---|--|---|--|--|
| Lot Numbers EA113077 | EA113077 | EA113077 | 4182-2 | 4182-2; L120381; 1114L-6-1; 1114L-2-1 |
| Date of Initial Use 8/2/78 | Rats--8/31/78; mice--9/18/78 | 12/29/78 (1/8/79 for the 8,000 mg/kg mice) | 8/29/80 | 4182-2, 7/16/81; L120381, 1/82; 1114L-6-1, 9/82; 1114L-2-1, 10/83 |
| Supplier Edgewood Arsenal, Aberdeen Proving Ground, Aberdeen, MD | Same as single- administration studies | Same as single- administration studies | Same as single- administration studies | Same as single-administration studies; or Stauffer Chemical Co., Westport, CT (lot nos. 11146L-6-1 and 1114L-2-1) |

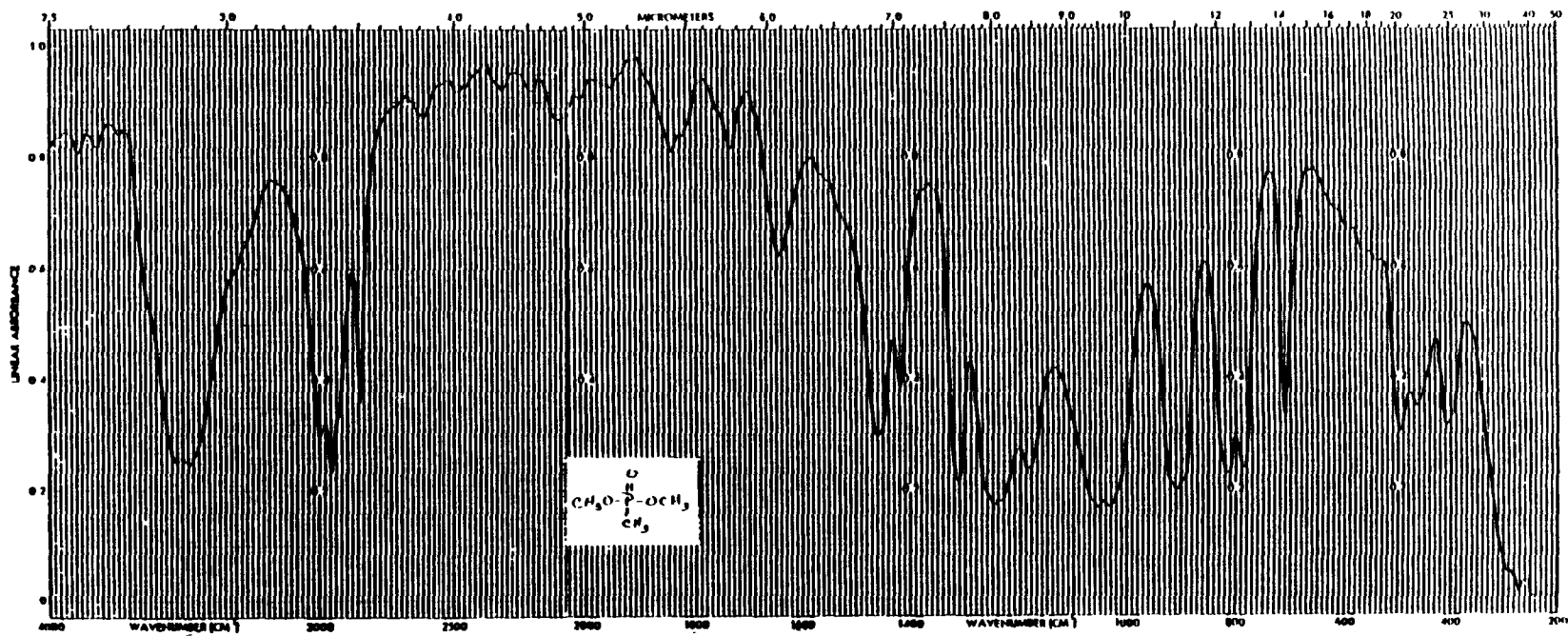


FIGURE 1. INFRARED ABSORPTION SPECTRUM OF DIMETHYL METHYLPHOSPHONATE (LOT NO. 1114L-6-1)

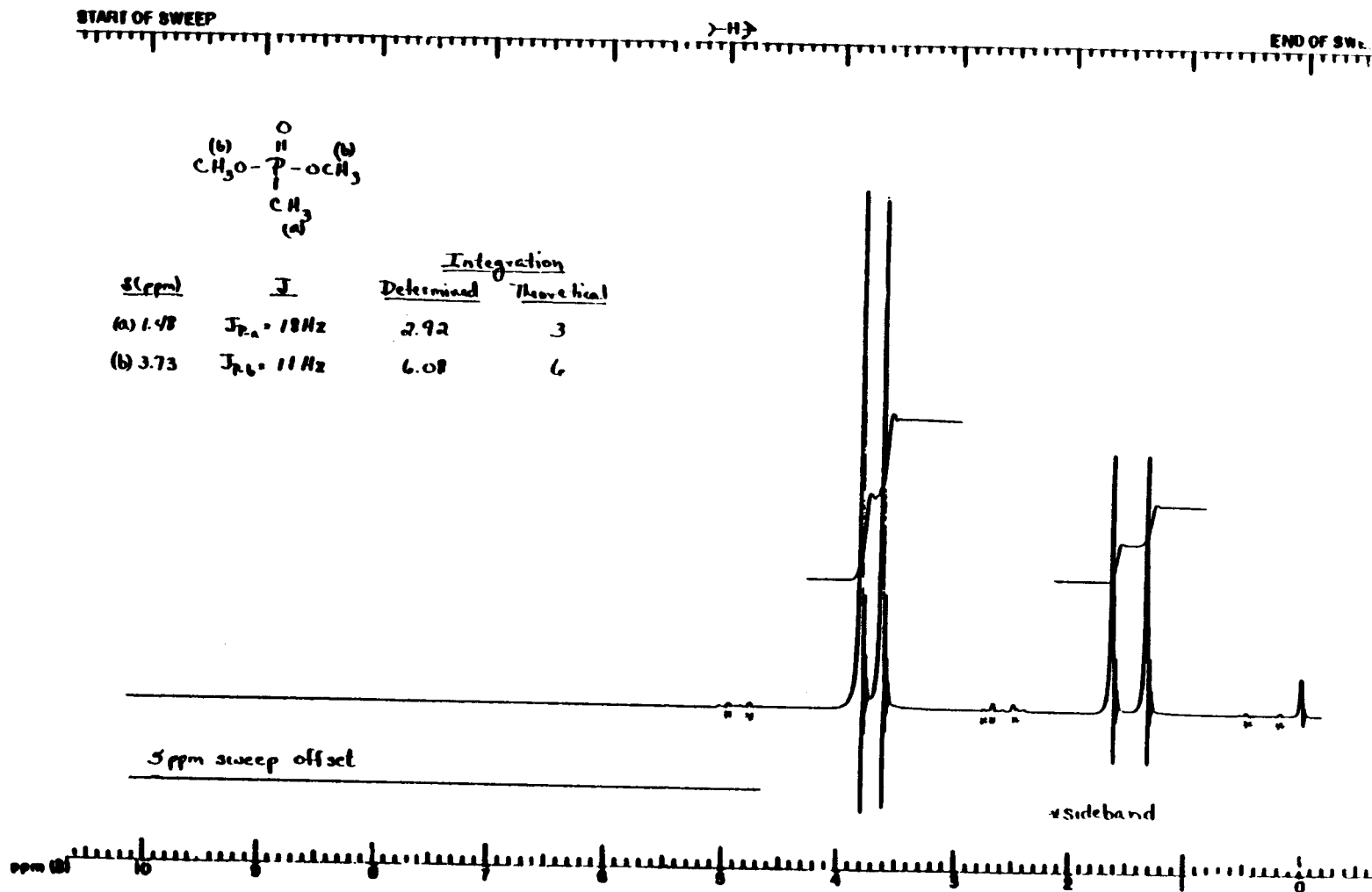


FIGURE 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF DIMETHYL METHYLPHOSPHONATE (LOT NO. 1114L-6-1)

TABLE 3. RESULTS OF PURITY ANALYSIS OF LOTS USED IN THE GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Lot Number | Determined Purity (percent) | Percent Water | Percent Total Impurities | |
|------------|-----------------------------|---------------|--------------------------|--------------|
| | | | System 1 (a) | System 2 (b) |
| EA113077 | > 98 | 0.11 | 1.5 | 0.5 |
| 4182-2 | ~ 98 | 0.25 | 1.35 | 2.01 |
| 1114L-6-1 | > 99 | 0.04 | 0.48 | 0.47 |
| 1114L-2-1 | ~ 99 | 0.06 | 1.1 | 1.1 |

(a) A 10% Carbowax 20M-TPA column

(b) A 20% SP2100/0.1% Carbowax 1500 column

TABLE 4. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Single-Administration Studies | Fifteen-Day Studies | First Thirteen-Week Studies | Second Thirteen-Week Studies | Two-Year Studies |
|--|---|--|--|--|
| Preparation Agitated in test tube; mixed for 2 min with stirring bar | Dimethyl methylphosphonate mixed with corn oil for 22 min with a vortex mixer and resuspended before dosing | Mixed in a graduated cylinder by inversion | Appropriate amount of corn oil added to dimethyl methylphosphonate; each dose mixture homogenized with a Polytron® mixer for at least 2 min at medium-high speed and then placed in five separate vials, one for each dose day of the week. Dose mixtures rehomogenized for 1 min with the Polytron® no more than 1 h before dosing. In animal room, each mixture stirred continuously with a magnetic stirrer throughout the dosing period to prevent separation. | Dimethyl methylphosphonate formulated with corn oil with a Polytron® mixer and resuspended daily with a magnetic stirrer |
| Maximum Storage Time N/A | 7 d | 1 wk | 1 wk | 13 d |
| Storage Conditions N/A | 4° C | 4° C | 4° C | 4° C |

II. MATERIALS AND METHODS

Periodic analysis of dimethyl methylphosphonate/corn oil dose mixtures were conducted at the study laboratory and the analytical chemistry laboratory by methanolic extraction of the dose mixtures followed by gas chromatographic analysis of the resultant extract with system 1. Dose mixtures were analyzed once during the second 13-week studies. The results ranged from 100% to 105% of the target concentration (Table 5).

During the 2-year studies, the dose preparations were analyzed at approximately 8-week intervals. Because 44/47 mixtures analyzed were within $\pm 10\%$ of the target concentration, it is estimated that the dose mixtures were prepared within specifications 94% of the time (Table 6). The three dose formulations determined to be out of specifications were within $\pm 13\%$ of the target concentrations. Referee analysis was periodically performed by the analytical chemistry

laboratory. Generally, good agreement was found between the results at the two laboratories (Table 7).

Deaths occurred after dosing in September 1982 in high dose mice and in April 1983 in low dose male mice. As a result, special analyses were performed on the contents of the dosing containers and their corresponding archive samples. The concentrations in the archive samples were within the specified limits, whereas that of the high-dose sample taken in the animal room was high (134% of target), and the low-dose sample taken in the animal room was low (79% of target). Because the archive samples were determined to be within specifications, it would appear that a dosing accident or misdosing due to improper handling or resuspending of the dose mixture occurred in the animal room on these days.

TABLE 5. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE SECOND THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Date Mixed | Target Concentration (mg/ml) | Determined Concentration (mg/ml) (a) | Determined as a Percent of Target |
|------------|------------------------------|--------------------------------------|-----------------------------------|
| 08/28/80 | 0 | 0 | -- |
| | 38.4 | 40.2 | 105 |
| | 77.0 | 77.9 | 101 |
| | 153.8 | 154 | 100 |
| | 307.5 | 307 | 99.8 |
| | 615.0 | 632 | 103 |

(a) Results of duplicate analysis

TABLE 6. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Date Mixed | Concentration of Dimethyl Methylphosphonate in Corn Oil (a) for Target Concentration (mg/ml) | | |
|------------------------------------|---|---------|---------|
| | 76.9 | 153.8 | 307.7 |
| 07/08/81 | 70.7 | (b) 204 | 285 |
| 07/09/81 | -- | (c) 155 | -- |
| 07/15/81 | 75.4 | 139 | 292 |
| 07/15/81 (d) | 75.7 | 152 | 306 |
| 09/02/81 | 78.4 | 149 | 301 |
| 10/28/81 | 75.2 | 146 | -- |
| 11/18/81 | 76.9 | 148 | 296 |
| 11/24/81 | 76.1 | 152 | 296 |
| 11/24/81 (d) | 76.0 | 151 | 294 |
| 01/13/82 | 74.1 | 141 | 289 |
| 03/10/82 | 76.3 | 142 | 291 |
| 05/05/82 | 69.1 | (b) 136 | 278 |
| 05/05/82 (d) | 72.7 | -- | 247 |
| 05/11/82 | -- | (c) 142 | -- |
| 05/11/82 (d) | -- | 148 | -- |
| 06/30/82 | 74.3 | 151 | 281 |
| 08/25/82 | 76.4 | 149 | (b) 274 |
| 08/27/82 | -- | -- | (c) 287 |
| 09/29/82 | -- | -- | 312 |
| 09/29/82 (d) | -- | -- | (e) 411 |
| 10/20/82 | 77.6 | 150 | 302 |
| 10/20/82 (d) | 77.3 | 153 | 307 |
| 12/16/82 | 75.3 | 148 | 301 |
| 02/09/83 | 78.4 | 156 | 302 |
| 04/06/83 | 74.1 | 152 | 293 |
| 04/06/83 (d) | 70.4 | 150 | 292 |
| 04/29/83 | -- | 143 | -- |
| 04/29/83 (d) | -- | (f) 121 | -- |
| 06/01/83 | 72.7 | 147 | 292 |
| 07/27/83 | -- | 144 | 291 |
| 09/21/83 | -- | 141 | 294 |
| 09/21/83 (d) | -- | 153 | 331 |
| Mean (mg/ml) | 75.1 | 149 | 293 |
| Standard deviation | 2.57 | 14.2 | 9.4 |
| Coefficient of variation (percent) | 3.4 | 9.5 | 3.2 |
| Range (mg/ml) | 69.1-78.4 | 136-204 | 274-312 |
| Number of samples | 16 | 19 | 18 |

- (a) Results of duplicate analysis
 (b) Out of specifications; not used in study.
 (c) Remix; not included in the mean.
 (d) Analysis of animal room samples; samples taken during dosing; not included in the mean.
 (e) Animal room sample; out of specifications; corresponds to deaths in high dose mice at week 45.
 (f) Animal room sample; out of specifications; corresponds to death in low dose male mice at week 77.

TABLE 7. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Date Mixed | Target Concentration (mg/ml) | Determined Concentration (mg/ml) | |
|------------|---------------------------------|----------------------------------|---------------------------|
| | | Study Laboratory (a) | Referee Laboratory (b) |
| 07/08/81 | 307.7 | 285 | (c) 245 |
| 05/05/82 | 76.9 | 69.1 | 69.8 |
| 10/20/82 | 153.8 | 150 | 144 |
| 02/09/83 | 153.8 | 156 | 144 |
| 09/21/83 | 307.7 | 294 | 280 |

- (a) Results of duplicate analysis
 (b) Results of triplicate analysis
 (c) The 16% difference in results between the study laboratory and the referee laboratory was attributed to possible differences in resuspension techniques.

II. MATERIALS AND METHODS

SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats and B6C3F₁ mice were obtained from Charles River Breeding Laboratories and held for 14 days before the studies began. Animals were 6 weeks old when placed on study. Groups of five males and five females were fasted and then administered a single dose of 1,470, 2,150, 3,160, 4,640, or 6,810 mg/kg dimethyl methylphosphonate in corn oil by gavage. Rats and mice were observed daily. A necropsy was performed on all animals that died before the end of the studies. Details of animal maintenance are presented in Table 8.

FIFTEEN-DAY STUDIES

Male and female F344/N rats and B6C3F₁ mice were obtained from Charles River Breeding Laboratories and held for 16 days (rats) or 18 days (mice) before the studies began. Rats were 6 weeks old when placed on study. Groups of five males and five females of each species were administered 0, 1,250, 2,500, 5,000, 10,000, or 15,000 mg/kg dimethyl methylphosphonate in corn oil by gavage for 15 consecutive days. The 15,000 mg/kg dose for mice was administered neat. Rats and mice were observed twice per day and were weighed on day 0, and mice were weighed also on day 15. A necropsy was performed on all animals. Stomachs of mice were examined microscopically. Details of animal maintenance are presented in Table 8.

FIRST THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of dimethyl methylphosphonate and to determine the doses to be used in the 2-year studies.

Five- to six-week-old male and female F344/N rats and B6C3F₁ mice were obtained from Charles River Breeding Laboratories, observed for 16 days, and assigned to groups according to a series of tables of random numbers. Groups of 10 rats and 10 mice of each sex were administered 0, 250, 500, 1,000, 2,000, or 8,000 mg/kg

dimethyl methylphosphonate, 5 days per week for 13 weeks. The 8,000 mg/kg group of mice had only seven males and six females. The original report does not explain the discrepancy in the number of animals, but it may have been due to a shortage of animals. The 250 mg/kg groups of rats and the 8,000 mg/kg groups of mice were started 1-2 weeks after the other groups.

Animals were housed five per cage. Feed and water were available ad libitum. Animals were checked two times per day; moribund animals were killed. Individual animal weights were recorded weekly.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 8.

SECOND THIRTEEN-WEEK STUDIES

Thirteen-week studies in rats were repeated because of gavage accidents in the lower dose groups in the first 13-week studies.

Five- to six-week old male and female F344/N rats were obtained from Charles River Breeding Laboratories, observed for 16 days, and assigned to groups according to a table of random numbers. Groups of 10 rats of each sex were administered 0, 250, 500, 1,000, 2,000, or 4,000 mg/kg dimethyl methylphosphonate, 5 days per week for 13 weeks.

Animals were housed five per cage. Feed and water were available ad libitum. Animals were checked two times per day; moribund animals were killed. Individual animal weights were recorded weekly.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Liver weights were taken, and liver weight to body weight ratios were calculated. Tissues and groups examined are listed in Table 8.

TABLE 8. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Single-Administration Studies | Fifteen-Day Studies | First Thirteen-Week Studies | Second Thirteen-Week Studies | Two-Year Studies |
|---|--|--|---|--|
| EXPERIMENTAL DESIGN | | | | |
| Size of Study Groups | | | | |
| 5 males and 5 females of each species | 5 males and 5 females of each species | 10 males and 10 females of each species (high dose mice-- 7 males and 6 females) | 10 males and 10 females (rats only) | 50 males and 50 females of each species |
| Doses | | | | |
| 1,470, 2,150, 3,160, 4,640, or 6,810 mg/kg dimethyl methylphosphonate in corn oil by gavage | 0, 1,250, 2,500, 5,000, 10,000, or 15,000 mg/kg dimethyl methylphosphonate in corn oil by gavage (15,000 mg/kg dose administered neat) | 0, 250, 500, 1,000, 2,000, 4,000, or 8,000 mg/kg dimethyl methylphosphonate in corn oil by gavage; dose vol--rats: 6.5 ml/kg during wk 1 (double the intended dose); from wk 2, dose vol--3.33 ml/kg, except for the 8,000 mg/kg group: 6.5 ml/kg; mice: 3.3 ml/kg 12/29/78-1/5/79, then 6.5 ml/kg after 1/8/79 | 0, 250, 500, 1,000, 2,000, or 4,000 mg/kg dimethyl methylphosphonate in corn oil by gavage; dose vol--6.5 ml/kg | Rats--0, 500, or 1,000 mg/kg dimethyl methylphosphonate in corn oil by gavage; dose vol--6.5 ml/kg; mice--0, 1,000, or 2,000 mg/kg dimethyl methylphosphonate in corn oil by gavage; dose vol--6.5 ml/kg |
| Date of First Dose | | | | |
| 8/2/78 | Rats--8/31/78; mice--9/18/78 | Rats--12/29/78 (250 mg/kg group started at week 2); mice--12/29/78 (1/8/79 for the 8,000 mg/kg group) | 8/29/80 | Rats--7/16/81; mice--11/24/81 |
| Date of Last Dose | | | | |
| N/A | Rats--9/14/78; mice--10/2/78 | 3/28/79 | 11/28/80 | Rats--7/11/83; mice--11/18/83 |
| Duration of Dosing | | | | |
| Single dose only | 15 consecutive d | 5 d/wk for 12 or 13 wk | 5 d/wk for 13 wk | 5 d/wk for 103 wk |
| Type and Frequency of Observation | | | | |
| Observed immediately after dosing, at 1 h and 4 h, and 1 x d for 14 d | Rats--observed 2 x d; weighed on d 0; mice--observed 2 x d; weighed on d 0 and d 15 | Observed 2 x d; weighed 1 x wk | Same as first 13-wk studies | Observed 2 x d; palpated 1 x 4 wk; weighed 1 x wk for 13 wk, 1 x 4 wk thereafter |
| Necropsy and Histologic Examination | | | | |
| Necropsy performed on all animals that died during the studies | Necropsy performed on all animals; histologic exam of the stomach performed on all mice | Necropsy performed on all animals. Histologic exam performed on all animals that died before the end of the studies, on vehicle controls, on highest dose groups of rats, and on two highest dose groups of mice; tissues examined include: adrenal glands, brain, colon, esophagus, eyes (if grossly abnormal), gallbladder (mice), gross lesions and tissue masses, heart, kidneys, liver, lungs and mainstem bronchi, mammary | Same as first 13-wk studies | Necropsy and histologic exam performed on all animals; the following tissues were examined: adrenal glands, brain, cecum, colon, costochondral junction, duodenum, esophagus, eyes, gallbladder (mice), gross lesions and tissue masses, heart, ileum, jejunum, kidneys, larynx, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity, pancreas, parathyroids, pituitary gland, |

TABLE 8. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE (Continued)

| Single-Administration Studies | Fifteen-Day Studies | First Thirteen-Week Studies | Second Thirteen-Week Studies | Two-Year Studies |
|--|---|---|---------------------------------------|--|
| EXPERIMENTAL DESIGN (Continued) | | | | |
| | | gland, mandibular or mesenteric lymph node, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, regional lymph nodes, salivary glands, skin, small intestine, spinal cord (if neurologic signs present), spleen, sternbrae or femur or vertebrae including marrow, stomach, thymus, thyroid gland, trachea, and urinary bladder; tissues examined for other groups of rats: kidneys, salivary glands, and testes. | | prostate/testes/seminal vesicles or ovaries/uterus, rectum, regional lymph nodes, salivary glands, sciatic nerve, skin, spinal cord, spleen, sternum including marrow, stomach, thyroid gland, thigh muscle, thymus, trachea, and urinary bladder. |
| ANIMALS AND ANIMAL MAINTENANCE | | | | |
| Strain and Species F344/N rats; B6C3F ₁ mice | F344/N rats; B6C3F ₁ mice | F344/N rats; B6C3F ₁ mice | F344/N rats | F344/N rats; B6C3F ₁ mice |
| Animal Source Charles River Breeding Laboratories (Portage, MI) | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies | Frederick Cancer Research Center (Frederick, MD) |
| Study Laboratory Litton Bionetics, Inc. | Litton Bionetics, Inc. | Litton Bionetics, Inc. | Litton Bionetics, Inc. | Litton Bionetics, Inc. |
| Method of Animal Identification Ear punch | Ear notch | Rats--ear tag; mice--ear punch, toe clip | Ear tag | Same as first 13-wk studies |
| Time Held Before Study 14 d | Rats--16 d; mice--18 d | 16 d | 16 d | 20 d |
| Age When Placed on Study 6 wk | Rats--6 wk; mice--not available | 7-8 wk | 7-8 wk | Rats--male, 8 wk; female, 7 wk; mice--8 wk |
| Age When Killed 8 wk | Rats--8 wk; mice--not available | 21-22 wk | 21-22 wk | Rats--113 wk; mice--112-113 wk |
| Necropsy Dates 8/16/78 | Rats--9/15/78; mice--10/3/78 | Rats--4/3/79-4/4/79; mice--3/30/79-4/4/79 | 12/1/80-12/2/80 | Rats--7/19/83-7/20/83; mice--11/28/83-11/29/83 |
| Method of Animal Distribution Assigned to cages such that cage weights were approximately equal for each sex and species | Same as single-administration studies | Assigned to groups according to a series of tables of random numbers | Same as first 13-wk studies | Randomized to cages by one table of random numbers, then to groups by another table |

TABLE 8. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE (Continued)

| Single-Administration Studies | Fifteen-Day Studies | First Thirteen-Week Studies | Second Thirteen-Week Studies | Two-Year Studies |
|--|---|--|---|---|
| ANIMALS AND ANIMAL MAINTENANCE (Continued) | | | | |
| Feed Purina Lab Chow® meal (Ralston Purina Co., St. Louis, MO); available ad libitum | Same as single-administration studies | Purina Lab Chow® pellets (Ralston Purina Co., St. Louis, MO); available ad libitum | NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum | Same as second 13-wk studies |
| Bedding Absorb-Dri® (Lab Products, Garfield, NJ) | Same as single-administration studies | Same as single-administration studies | Absorb-Dri® (Lab Products, Inc., Gaithersburg, MD) | Rats--Absorb-Dri® heat-treated hardwood chips (Lab Products, Inc., Gaithersburg, MD) used until 9/23/81; Sani-Chips®, hardwood chip animal bedding (P.J. Murphy Forest Products Corp., Rochelle Park, NJ) used thereafter; mice--Sani-Chips® (P.J. Murphy Forest Products Corp., Rochelle Park, NJ) |
| Water Acidified to pH 2.5; available ad libitum in bottles | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies |
| Cages Polycarbonate (Lab Products, Inc., Garfield, NJ, and Rochelle Park, NJ) | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies | Polycarbonate (Lab Products, Inc., Garfield, NJ, and Hazleton Systems, Aberdeen, MD) |
| Cage Filters Nonwoven polyester (Snow Filtration, Cincinnati, OH) | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies |
| Animals per Cage 5 | 5 | 5 | 5 | 5 |
| Other Chemicals on Study in the Same Room None | Dimethyl hydrogen phosphite | None | None | None |
| Animal Room Environment Not available | Temp--22.2°-24.4° C; hum--30%-70%; fluorescent light 12 h/d; 12-15 room air changes/h | Temp--22.2°-24.4° C; hum--30%-70%; light 12 h/d; 15 room air changes/h | Same as first 13-wk studies except 12-15 room air changes/h | Temp--usually 22.2°-24.4° C; hum--30%-70%; fluorescent light 12 h/d; 12-15 room air changes/h |

II. MATERIALS AND METHODS

TWO-YEAR STUDIES

Study Design

Groups of 50 rats of each sex were administered 0, 500, or 1,000 mg/kg dimethyl methylphosphonate in corn oil by gavage, 5 days per week for 103 weeks. Groups of 50 mice of each sex were administered 0, 1,000, or 2,000 mg/kg dimethyl methylphosphonate on the same schedule. The mouse studies were started 4 months after the rat studies because the first 2-year mouse studies were terminated after 2 months due to the large number of deaths that were related to gavage technique.

Source and Specifications of Animals

The male and female F344/N rats and B6C3F₁ (C57BL/6N, female, × C3H/HeN MTV⁻, male) mice used in these studies were produced under strict barrier conditions at Frederick Cancer Research Center under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the study laboratory at 4-5 weeks of age and were quarantined for 20 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. Male rats and mice of each sex were placed on study at 8 weeks of age, and female rats at 7 weeks of age. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program (Appendix F).

Animal Maintenance

Animals were housed five per cage; neither the cages nor racks were rotated during the studies. Cages for each dose group were arranged in columns on the rack. Feed and water were available ad libitum. Further details of animal maintenance are given in Table 8.

Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded once per week. Body weights by cage were recorded once per week for the first 13 weeks of the studies and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed, cannibalized, missexed, or found missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 8.

When the pathology evaluation was completed, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnology was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG, which includes the laboratory pathologist, without knowledge of previously rendered diagnoses. When the

II. MATERIALS AND METHODS

consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with vehicle controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in the analysis of tumor incidence, and reported P values are one-sided.

*Life Table Analyses--*The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and vehicle control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of

II. MATERIALS AND METHODS

Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and vehicle control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See

Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of primary tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

III. RESULTS

RATS

SINGLE-ADMINISTRATION STUDIES

FIFTEEN-DAY STUDIES

FIRST THIRTEEN-WEEK STUDIES

SECOND THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

SINGLE-ADMINISTRATION STUDIES

FIFTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

III. RESULTS: RATS

SINGLE-ADMINISTRATION STUDIES

No compound-related deaths occurred at doses up to 6,810 mg/kg. Compound-related clinical signs were observed in all but the lowest dose groups and included transitory (1-4 hours after dosing) inactivity, unsteady gait, and prostration.

Because of a lack of dose-related mortality in the single-administration studies, doses selected for the 15-day studies were 0, 1,250, 2,500, 5,000, 10,000, and 15,000 mg/kg.

FIFTEEN-DAY STUDIES

All rats that received 10,000 or 15,000 mg/kg dimethyl methylphosphonate and 4/5 male and 4/5 female rats that received 5,000 mg/kg died before the end of the studies (Table 9). No compound-related gross pathologic effects were reported. Dosed rats that received 2,500 mg/kg or more were inactive after dosing; dosed animals at 5,000 and 10,000 mg/kg had an unsteady gait.

Doses selected for the 13-week studies were 0, 250, 500, 1,000, 2,000, 4,000, and 8,000 mg/kg because of the mortality at 10,000 and 15,000 mg/kg. Even though mortality occurred at 5,000 mg/kg in the 15-day studies, 8,000 mg/kg was selected as the high dose so that rats and mice would be administered the same doses in the 13-week studies.

FIRST THIRTEEN-WEEK STUDIES

All rats that received 8,000 mg/kg dimethyl methylphosphonate died before the end of the studies (Table 10). The final mean body weight of rats that received 4,000 mg/kg was more than 10% lower than that of the vehicle controls. Animals at 8,000 mg/kg had rough hair coats and decreased activity; decreased activity was observed by week 10 in 4,000 mg/kg males and females.

Lesions were seen in the kidney, testis, epididymus, or salivary gland (Table 11). Degeneration and atrophy of the testis (minimal severity) were observed in dosed male rats. The incidence and severity of the testicular atrophy were approximately the same in the three lowest dose groups. Degeneration of the epididymus was minimal or mild. A mild to moderate nephrosis of the kidney, characterized by hypertrophy and vacuolation of the proximal and distal tubular epithelium with accumulation of a finely granular proteinaceous material in the lumens, was seen at 8,000 mg/kg.

The 13-week studies were repeated because the deaths attributed to gavage error in lower dose groups prevented accurate dose selection for the 2-year studies. The doses selected for the second 13-week studies in rats were 0, 250, 500, 1,000, 2,000, and 4,000 mg/kg because all rats in the 8,000 mg/kg group died in the first 13-week studies.

TABLE 9. SURVIVAL OF RATS IN THE FIFTEEN-DAY GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Male | | Female | |
|--------------|--------------|--------------|--------------|
| Dose (mg/kg) | Survival (a) | Dose (mg/kg) | Survival (a) |
| 0 | 5/5 | 0 | 5/5 |
| 1,250 | (b) 4/5 | 1,250 | 5/5 |
| 2,500 | 5/5 | 2,500 | 5/5 |
| 5,000 | (c) 1/5 | 5,000 | (d) 1/5 |
| 10,000 | (e) 0/5 | 10,000 | (f) 0/5 |
| 15,000 | (g) 0/5 | 15,000 | (g) 0/5 |

- (a) Number surviving/number in group
(b) Day of death: 2 (probable gavage accident)
(c) Day of death: all 12
(d) Day of death: all 14
(e) Day of death: 1,7,9,9,9
(f) Day of death: 3,9,9,12,12
(g) Day of death: all 1

TABLE 10. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FIRST THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) | | | Final Weight Relative to Vehicle Controls (percent) |
|---------------|--------------|---------------------------|-------|--------|---|
| | | Initial (b) | Final | Change | |
| MALE | | | | | |
| 0 | 10/10 | 173 | 311 | +138 | -- |
| 250 | 10/10 | (c) | 307 | -- | 98.7 |
| 500 | 10/10 | 181 | 304 | +123 | 97.7 |
| 1,000 | 10/10 | 174 | 295 | +121 | 94.9 |
| 2,000 | (d) 3/10 | 176 | 295 | +119 | 94.9 |
| 4,000 | (e) 8/10 | 168 | 252 | +84 | 81.0 |
| 8,000 | (f) 0/10 | 176 | (g) | (g) | (g) |
| FEMALE | | | | | |
| 0 | 10/10 | 133 | 194 | +61 | -- |
| 250 | 10/10 | (c) | 192 | -- | 99.0 |
| 500 | 10/10 | 132 | 194 | +62 | 100.0 |
| 1,000 | (h) 8/10 | 134 | 188 | +54 | 96.9 |
| 2,000 | (i) 7/10 | 129 | 183 | +54 | 94.3 |
| 4,000 | (j) 3/10 | 130 | 172 | +42 | 88.7 |
| 8,000 | (k) 0/10 | 127 | (g) | (g) | (g) |

- (a) Number surviving/number in group
 (b) Initial group mean body weight
 (c) Initial body weight not recorded
 (d) Week of death: 6,9,9,9,10,11,12; 6/7 deaths attributed to gavage accidents.
 (e) Week of death: 7,10
 (f) Week of death: 1,1,1,1,1,1,1,1,2,5
 (g) No data are reported due to the 100% mortality in this group.
 (h) Week of death: 5,10; deaths attributed to gavage accidents.
 (i) Week of death: 9,9,10
 (j) Week of death: 1,2,2,7,9,9,9
 (k) Week of death: 1,1,2,2,2,2,2,3,4,5

TABLE 11. INCIDENCES OF RATS WITH SELECTED LESIONS IN THE FIRST THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Site/Lesion | Vehicle Control | 250 mg/kg | 500 mg/kg | 1,000 mg/kg | 2,000 mg/kg | 4,000 mg/kg | 8,000 mg/kg |
|------------------|-----------------|-----------|-----------|-------------|-------------|-------------|-------------|
| | MALE | | | | | | |
| Kidney nephrosis | 0/10 | (a) | (a) | (a) | 0/10 | 0/10 | (b) 4/10 |
| Testis | | | | | | | |
| Atrophy | 0/10 | 1/10 | 2/10 | 2/10 | 3/9 | 10/10 | (b) 2/9 |
| Degeneration | 0/10 | 0/10 | 0/10 | 0/10 | 0/9 | 0/10 | (b) 2/9 |
| Epididymus | | | | | | | |
| Degeneration | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 9/10 | (b) 3/9 |
| Salivary gland | | | | | | | |
| Atrophy | 0/10 | (a) | (a) | (a) | 0/10 | 0/10 | (b) 6/10 |
| FEMALE | | | | | | | |
| Salivary gland | | | | | | | |
| Atrophy | 0/10 | (a) | (a) | 0/2 | 0/10 | 0/10 | 4/10 |

- (a) Not examined
 (b) Nine of 10 rats dead by week 2; 10th rat dead by week 5.

III. RESULTS: RATS

SECOND THIRTEEN-WEEK STUDIES

All rats that received 4,000 mg/kg and 6/10 males and 3/10 females that received 2,000 mg/kg died before the end of the studies (Table 12). Final mean body weights of rats that received 2,000 mg/kg were 6% lower than that of the vehicle controls for males and 7% lower for females. No compound-related clinical signs were seen. The liver weight to body weight ratios were significantly increased ($P < 0.01$) for rats that received 2,000 mg/kg compared with those of the vehicle controls (Table 13).

Kidney lesions characteristic of spontaneous progressive nephropathy were seen in all groups of male rats (Table 14). Although the incidences of this lesion (diagnosed as nephrosis) were greater in all dosed groups except that receiving 4,000 mg/kg, the severity of the lesions in dosed and vehicle control rats was similar. Accumulation of hyaline droplets in the cytoplasm of epithelial cells was observed in convoluted tubules

of the renal cortex in all dosed groups of male rats. At the time of this study, these lesions were not considered to be life threatening.

Hypospermatogenic tubules (minimal to mild) were seen in the testis of dosed male rats in the higher dose groups. The severity of the lesion was not increased with increasing dose of chemical.

Inflammation of the salivary gland, suggestive of viral sialodacryoadenitis, was seen in some high dose male and female rats.

Dose Selection Rationale: Based on the incidence of deaths and weight gain depression observed in both 13-week studies, doses selected for the 2-year studies in rats were 500 and 1,000 mg/kg dimethyl methylphosphonate, administered in corn oil by gavage, 5 days per week for 103 weeks. The kidney lesions were not considered to be life threatening.

TABLE 12. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SECOND THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) | | | Final Weight Relative to Vehicle Controls (percent) |
|---------------|--------------|---------------------------|----------|------------|---|
| | | Initial (b) | Final | Change (c) | |
| MALE | | | | | |
| 0 | 10/10 | 125 ± 3 | 340 ± 5 | +215 ± 4 | -- |
| 250 | 10/10 | 123 ± 4 | 324 ± 14 | +201 ± 12 | 95 |
| 500 | 10/10 | 121 ± 4 | 336 ± 12 | +215 ± 10 | 99 |
| 1,000 | 10/10 | 126 ± 3 | 336 ± 4 | +210 ± 4 | 99 |
| 2,000 | (d) 4/10 | 126 ± 4 | 321 ± 15 | +190 ± 10 | 94 |
| 4,000 | (e) 0/10 | 126 ± 3 | (f) | (f) | (f) |
| FEMALE | | | | | |
| 0 | 10/10 | 104 ± 1 | 197 ± 3 | +93 ± 2 | -- |
| 250 | (e) 9/10 | 104 ± 3 | 194 ± 6 | +89 ± 5 | 98 |
| 500 | 10/10 | 105 ± 3 | 205 ± 6 | +100 ± 4 | 104 |
| 1,000 | (e) 9/10 | 100 ± 2 | 190 ± 6 | +91 ± 5 | 96 |
| 2,000 | (g) 7/10 | 105 ± 2 | 184 ± 2 | +77 ± 2 | 93 |
| 4,000 | (e) 0/10 | 103 ± 2 | (f) | (f) | (f) |

(a) Number surviving/number initially in the group

(b) Initial group mean body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study

(c) Mean body weight change of the survivors ± standard error of the mean

(d) Week of death: 7,7,11,12,13,13

(e) Week of death: 1

(f) No data are reported due to the 100% mortality in this group.

(g) Week of death: 1,6,10

TABLE 13. ABSOLUTE AND RELATIVE LIVER WEIGHTS OF RATS IN THE SECOND THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE (a)

| Dose (mg/kg) | No. of Animals Examined | Necropsy Body Weight (grams) | Liver Weight (mg) | Liver Weight/Body Weight (mg/g) |
|---------------|-------------------------|------------------------------|--------------------|---------------------------------|
| MALE | | | | |
| 0 | (b) 9 | 329 ± 17 | 11,165 ± 1,485 | 33.3 ± 3.99 |
| 250 | 10 | 310 ± 40 | 10,425 ± 1,432 | 33.7 ± 3.21 |
| 500 | 10 | 321 ± 34 | 11,721 ± 2,060 | 36.3 ± 4.21 |
| 1,000 | 10 | 322 ± 13 | 11,718 ± 1,396 | 36.4 ± 3.69 |
| 2,000 | 4 | 306 ± 30 | (c) 13,990 ± 2,767 | (d) 45.7 ± 7.98 |
| FEMALE | | | | |
| 0 | (e) 9 | 187 ± 11 | 5,845 ± 1,118 | 31.1 ± 4.87 |
| 250 | 9 | 187 ± 22 | 6,476 ± 1,009 | 34.5 ± 2.85 |
| 500 | 10 | 195 ± 17 | 6,450 ± 742 | 33.1 ± 3.20 |
| 1,000 | 9 | 179 ± 17 | 6,048 ± 517 | 34.0 ± 2.27 |
| 2,000 | 7 | 176 ± 6 | 6,519 ± 779 | (d) 37.0 ± 4.20 |

(a) Mean ± standard deviation; P values are versus the vehicle controls by Dunnett's test (Dunnett, 1955).

(b) Ten livers examined; one body weight not recorded.

(c) P < 0.05

(d) P < 0.01

(e) Ten body weights; one liver weight not recorded.

TABLE 14. INCIDENCES OF RATS WITH SELECTED LESIONS IN THE SECOND THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Site/Lesion | Vehicle Control | 250 mg/kg | 500 mg/kg | 1,000 mg/kg | 2,000 mg/kg | 4,000 mg/kg |
|------------------------------|-----------------|-----------|-----------|-------------|-------------|-------------|
| MALE | | | | | | |
| Kidney | | | | | | |
| Nephrosis | 2/10 | 9/10 | 10/10 | 10/10 | 5/9 | 0/10 |
| Hyaline droplet degeneration | 0/10 | 8/10 | 10/10 | 10/10 | 4/9 | 3/10 |
| Hemorrhage | 0/10 | 0/10 | 0/10 | 0/10 | 1/9 | 2/10 |
| Testis | | | | | | |
| Hypospermatogenesis | 0/10 | 1/10 | 0/10 | 2/10 | 4/9 | 1/10 |
| Giant cell degeneration | 0/10 | 0/10 | 0/10 | 1/10 | 0/9 | 0/10 |
| Salivary gland | | | | | | |
| Acute inflammation | 0/10 | (a) | (a) | 0/10 | 0/10 | 2/10 |
| FEMALE | | | | | | |
| Salivary gland | | | | | | |
| Acute inflammation | 0/10 | (a) | (a) | (a) | 1/10 | 1/10 |

(a) Not examined

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male rats were 5%-10% lower than those of the vehicle controls between weeks 28 and 76 and 10%-24% lower between weeks 80 and 104 (Table 15 and Figure 3). Mean body weights of high dose female rats were

8%-12% lower than those of the vehicle controls after week 80. Mean body weights of low dose male and female rats were comparable to those of the vehicle controls throughout most of the studies. No compound-related clinical signs were recorded.

TABLE 15. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Weeks on Study | Vehicle Control | | 500 mg/kg | | | 1,000 mg/kg | | |
|----------------|-----------------|------------------|-----------------|--------------------------------|------------------|-----------------|--------------------------------|------------------|
| | Av. Wt. (grams) | No. of Survivors | Av. Wt. (grams) | Wt. (percent) of veh. controls | No. of Survivors | Av. Wt. (grams) | Wt. (percent) of veh. controls | No. of Survivors |
| MALE | | | | | | | | |
| 0 | 163 | 50 | 162 | 99 | 50 | 158 | 97 | 50 |
| 1 | 194 | 50 | 194 | 100 | 50 | 194 | 100 | 50 |
| 2 | 222 | 50 | 219 | 99 | 50 | 219 | 99 | 50 |
| 3 | 244 | 50 | 241 | 99 | 50 | 239 | 98 | 50 |
| 4 | 261 | 50 | 258 | 99 | 50 | 256 | 98 | 50 |
| 5 | 278 | 50 | 272 | 98 | 50 | 269 | 97 | 50 |
| 6 | 289 | 50 | 285 | 99 | 50 | 283 | 98 | 50 |
| 7 | 298 | 50 | 295 | 99 | 50 | 289 | 97 | 50 |
| 8 | 310 | 50 | 303 | 98 | 50 | 300 | 97 | 50 |
| 9 | 311 | 50 | 305 | 98 | 50 | 302 | 97 | 50 |
| 10 | 325 | 50 | 319 | 98 | 50 | 314 | 97 | 50 |
| 11 | 335 | 50 | 333 | 99 | 50 | 327 | 98 | 50 |
| 12 | 343 | 50 | 341 | 99 | 50 | 333 | 97 | 50 |
| 13 | 348 | 50 | 346 | 99 | 50 | 341 | 98 | 50 |
| 16 | 371 | 50 | 369 | 99 | 50 | 357 | 96 | 50 |
| 20 | 391 | 50 | 387 | 99 | 50 | 370 | 95 | 50 |
| 24 | 410 | 50 | 408 | 100 | 50 | 392 | 96 | 49 |
| 28 | 424 | 50 | 419 | 99 | 50 | 402 | 95 | 49 |
| 32 | 436 | 50 | 430 | 99 | 50 | 413 | 95 | 49 |
| 36 | 439 | 49 | 437 | 100 | 50 | 417 | 95 | 49 |
| 40 | 450 | 49 | 439 | 98 | 50 | 418 | 93 | 49 |
| 44 | 456 | 49 | 448 | 98 | 49 | 425 | 93 | 49 |
| 48 | 464 | 49 | 455 | 98 | 49 | 428 | 92 | 49 |
| 52 | 467 | 48 | 463 | 99 | 48 | 435 | 93 | 49 |
| 56 | 475 | 47 | 466 | 99 | 45 | 438 | 92 | 46 |
| 60 | 484 | 43 | 472 | 98 | 41 | 445 | 92 | 43 |
| 64 | 487 | 42 | 478 | 98 | 39 | 450 | 92 | 43 |
| 68 | 485 | 40 | 480 | 99 | 38 | 445 | 92 | 40 |
| 72 | 488 | 40 | 478 | 98 | 37 | 439 | 90 | 37 |
| 76 | 487 | 39 | 478 | 98 | 34 | 437 | 90 | 35 |
| 80 | 489 | 39 | 477 | 98 | 32 | 434 | 89 | 27 |
| 84 | 484 | 38 | 471 | 97 | 30 | 423 | 87 | 24 |
| 88 | 479 | 37 | 466 | 97 | 25 | 413 | 86 | 17 |
| 92 | 480 | 36 | 468 | 98 | 24 | 406 | 85 | 10 |
| 96 | 473 | 35 | 457 | 97 | 23 | 386 | 82 | 10 |
| 100 | 468 | 29 | 459 | 98 | 19 | 369 | 79 | 8 |
| 104 | 451 | 27 | 416 | 92 | 17 | 343 | 76 | 4 |
| FEMALE | | | | | | | | |
| 0 | 119 | 50 | 126 | 106 | 50 | 123 | 103 | 50 |
| 1 | 134 | 50 | 141 | 105 | 50 | 136 | 101 | 50 |
| 2 | 145 | 50 | 151 | 104 | 50 | 149 | 103 | 50 |
| 3 | 153 | 50 | 158 | 103 | 50 | 156 | 102 | 50 |
| 4 | 163 | 50 | 167 | 102 | 50 | 163 | 100 | 50 |
| 5 | 168 | 50 | 173 | 103 | 50 | 170 | 101 | 50 |
| 6 | 174 | 50 | 178 | 102 | 50 | 173 | 99 | 50 |
| 7 | 176 | 50 | 181 | 103 | 50 | 176 | 100 | 50 |
| 8 | 179 | 50 | 185 | 103 | 50 | 178 | 99 | 50 |
| 9 | 181 | 50 | 186 | 103 | 50 | 182 | 101 | 50 |
| 10 | 185 | 50 | 191 | 103 | 50 | 187 | 101 | 50 |
| 11 | 188 | 50 | 193 | 103 | 50 | 189 | 101 | 50 |
| 12 | 192 | 50 | 195 | 102 | 50 | 192 | 100 | 50 |
| 13 | 193 | 50 | 198 | 103 | 50 | 193 | 100 | 50 |
| 16 | 200 | 50 | 202 | 101 | 50 | 198 | 99 | 50 |
| 20 | 206 | 50 | 209 | 101 | 50 | 204 | 99 | 50 |
| 24 | 213 | 50 | 214 | 100 | 50 | 208 | 98 | 50 |
| 28 | 219 | 50 | 218 | 100 | 50 | 215 | 98 | 50 |
| 32 | 227 | 50 | 225 | 99 | 50 | 223 | 98 | 50 |
| 36 | 229 | 50 | 225 | 98 | 50 | 223 | 97 | 50 |
| 40 | 231 | 50 | 227 | 98 | 50 | 226 | 98 | 49 |
| 44 | 238 | 50 | 231 | 97 | 50 | 230 | 97 | 49 |
| 48 | 245 | 49 | 236 | 96 | 50 | 235 | 96 | 49 |
| 52 | 254 | 49 | 247 | 97 | 50 | 245 | 96 | 49 |
| 56 | 262 | 49 | 254 | 97 | 49 | 252 | 96 | 45 |
| 60 | 266 | 49 | 258 | 97 | 46 | 254 | 95 | 42 |
| 64 | 276 | 49 | 269 | 97 | 46 | 265 | 96 | 42 |
| 68 | 283 | 49 | 276 | 98 | 45 | 274 | 97 | 40 |
| 72 | 291 | 48 | 283 | 97 | 44 | 277 | 95 | 40 |
| 76 | 298 | 48 | 286 | 96 | 44 | 285 | 96 | 37 |
| 80 | 303 | 47 | 287 | 95 | 44 | 278 | 92 | 30 |
| 84 | 304 | 46 | 294 | 97 | 44 | 276 | 91 | 29 |
| 88 | 306 | 44 | 293 | 96 | 44 | 283 | 92 | 27 |
| 92 | 310 | 43 | 301 | 97 | 42 | 283 | 91 | 26 |
| 96 | 313 | 37 | 296 | 95 | 39 | 281 | 90 | 26 |
| 100 | 314 | 34 | 293 | 93 | 35 | 283 | 90 | 24 |
| 104 | 318 | 30 | 293 | 92 | 33 | 280 | 88 | 23 |

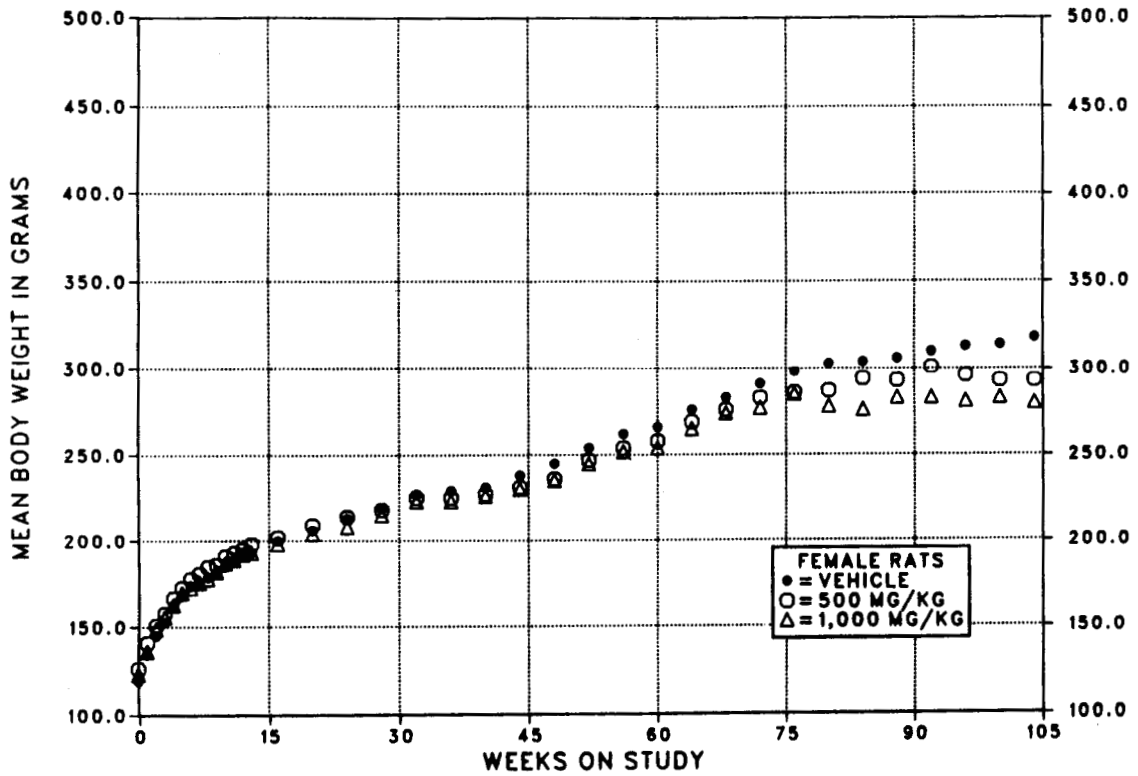
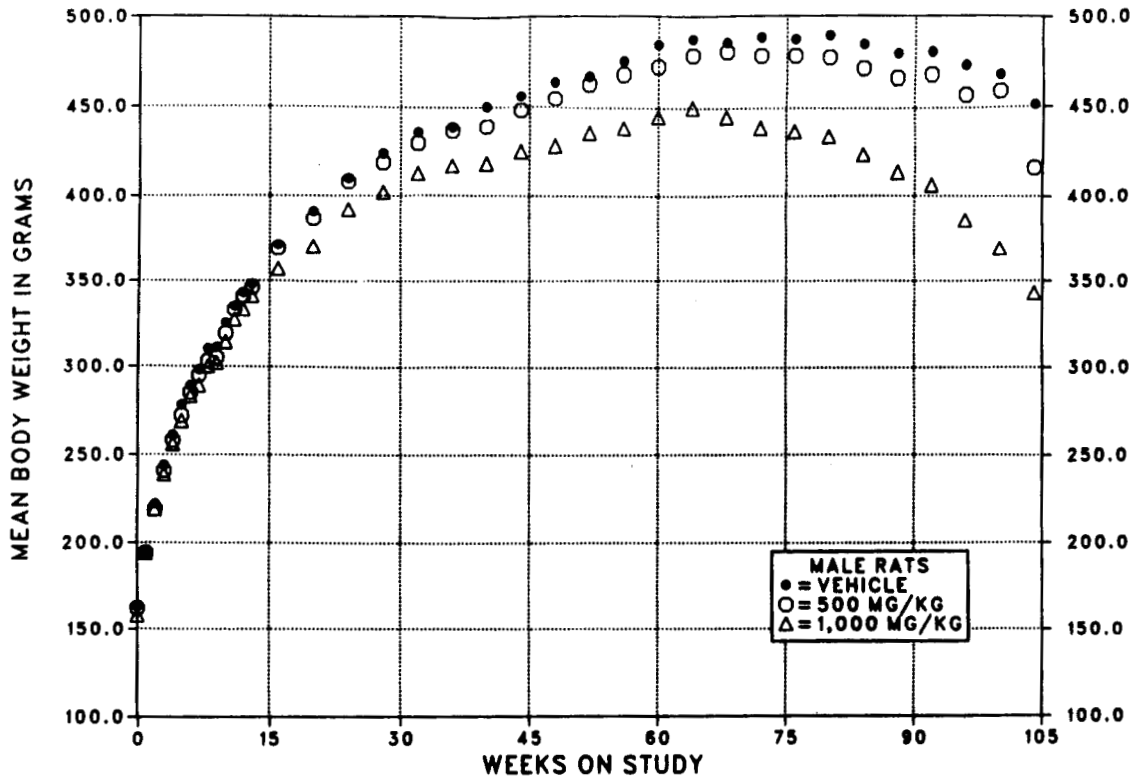


FIGURE 3. GROWTH CURVES FOR RATS ADMINISTERED DIMETHYL METHYLPHOSPHONATE IN CORN OIL BY GAVAGE FOR TWO YEARS

III. RESULTS: RATS

Survival

Estimates of the probabilities of survival for male and female rats administered dimethyl methylphosphonate at the doses used in these studies and for vehicle controls are shown in the Kaplan and Meier curves in Figure 4. In male rats, the number of survivors in both the low dose group (after week 88) and the high dose group (after week 82) was significantly lower than that in the vehicle controls (Table 16). The survival of high dose female rats was significantly lower than that of the vehicle controls after week 63 ($P < 0.05$; $P < 0.01$ between weeks 76 and 101). Survival of low dose female rats was comparable to that of the vehicle controls.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the kidney, hematopoietic system, adrenal gland, oral cavity, thyroid gland, multiple organs, and nasolacrimal duct.

Lesions in male rats are summarized in Appendix A. Histopathologic findings on neoplasms are summarized in Table A1. Table A2 gives the survival and tumor status for individual male rats. Table A3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes). Historical incidences of tumors in corn oil vehicle control male rats are listed in Table A4. Findings on nonneoplastic lesions are summarized in Table A5.

Lesions in female rats are summarized in Appendix B. Histopathologic findings on neoplasms are summarized in Table B1. Table B2 gives the survival and tumor status for individual female rats. Table B3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table B3 (footnotes). Historical incidences of tumors in corn oil vehicle control female rats are listed in Table B4. Findings on nonneoplastic lesions are summarized in Table B5.

TABLE 16. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---|-----------------|-----------|-------------|
| MALE (a) | | | |
| Animals initially in study | 50 | 50 | 50 |
| Nonaccidental deaths before termination (b) | 22 | 33 | 45 |
| Accidentally killed | 1 | 0 | 1 |
| Killed at termination | 26 | 17 | 4 |
| Died during termination period | 1 | 0 | 0 |
| Survival P values (c) | <0.001 | 0.031 | <0.001 |
| FEMALE (a) | | | |
| Animals initially in study | 50 | 50 | 50 |
| Nonaccidental deaths before termination (b) | 20 | 17 | 27 |
| Killed at termination | 30 | 32 | 23 |
| Died during termination period | 0 | 1 | 0 |
| Survival P values (c) | 0.044 | 0.720 | 0.049 |

(a) Terminal-kill period: week 105

(b) Includes animals killed in a moribund condition

(c) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.

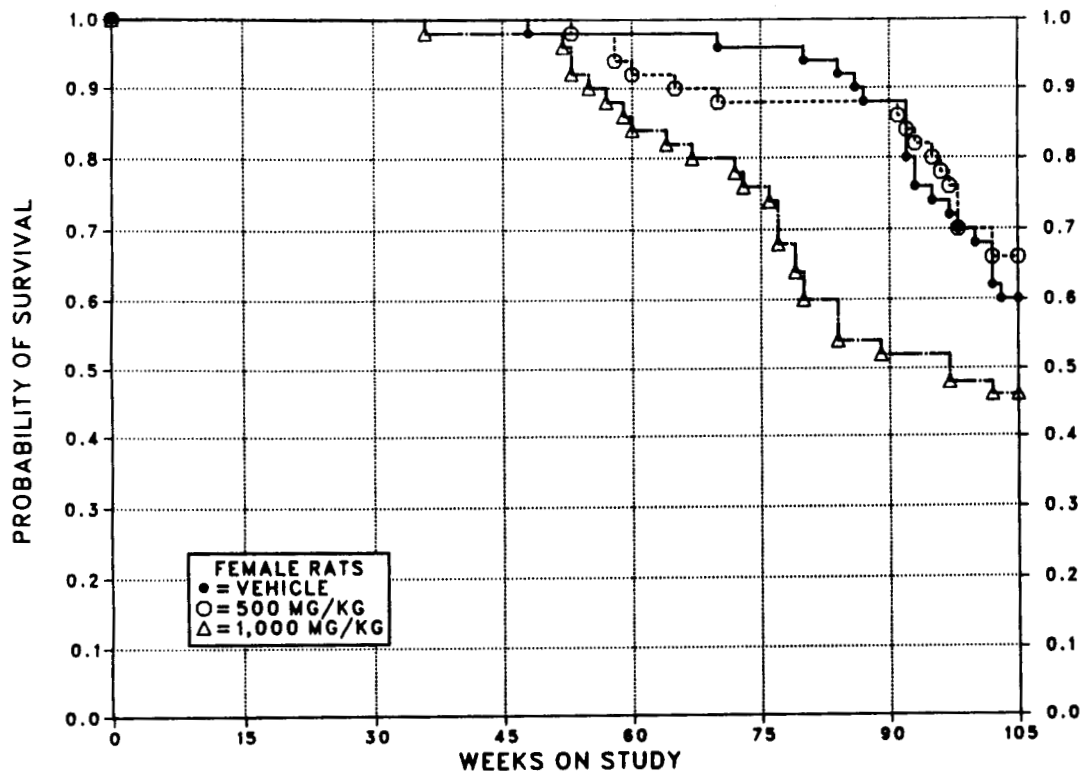
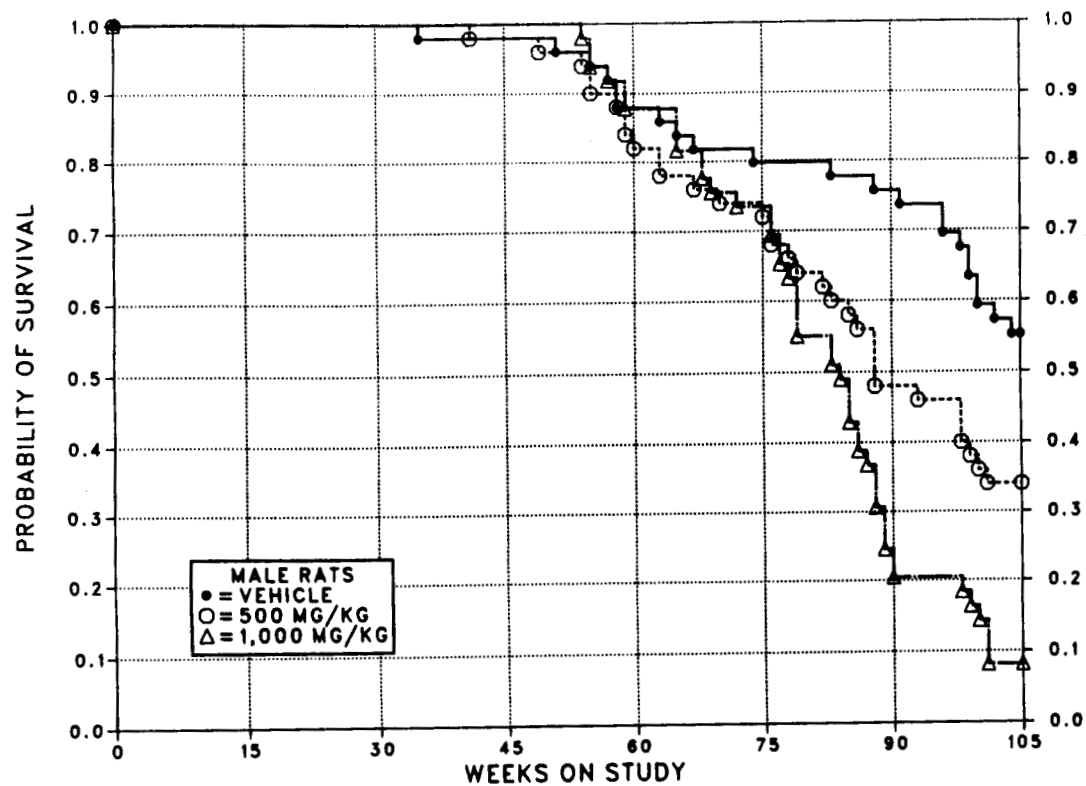


FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED DIMETHYL METHYLPHOSPHONATE IN CORN OIL BY GAVAGE FOR TWO YEARS

III. RESULTS: RATS

Kidney: Compound-related nonneoplastic and neoplastic lesions were seen in male but not in female rats (Table 17). The incidence of nephropathy was similar among groups of male rats, but the average severity of this lesion was greater in male rats receiving dimethyl methylphosphonate. The average severity for the vehicle control, low dose, and high dose groups was 1.9, 2.5, and 2.8, respectively (severity was graded from 1 to 4, minimal to marked). This lesion was characterized by the occurrence of several interrelated changes, including degeneration of

tubular epithelium, tubular dilatation with attenuation and atrophy of the epithelium, granular casts in tubules of the outer stripe of the outer medulla, thickening of basement membranes, minimal to mild accumulation of interstitial collagen, and minimal inflammatory cell infiltrates. There were increased incidences of mineralization (calcification) of collecting tubules in the renal papilla, focal hyperplasia of renal tubular epithelium, and hyperplasia of the pelvic epithelium overlying the renal papilla of dosed male rats compared with those of the vehicle

TABLE 17. ANALYSIS OF KIDNEY LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (a)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---|-----------------|-------------|-------------|
| Nephropathy | | | |
| Overall Rates | 36/50 (72%) | 43/50 (86%) | 40/49 (82%) |
| Severity (b) | 1.9 | 2.5 | 2.8 |
| Calcification of the Renal Papilla | | | |
| Overall Rates | 12/50 (24%) | 41/50 (82%) | 36/49 (73%) |
| Cortical Tubular Cell Hyperplasia | | | |
| Overall Rates | 0/50 (0%) | 8/50 (16%) | 9/49 (18%) |
| Tubular Cell Adenocarcinoma (c) | | | |
| Overall Rates | 0/50 (0%) | 2/50 (4%) | 3/49 (6%) |
| Adjusted Rates | 0.0% | 9.2% | 19.4% |
| Terminal Rates | 0/27 (0%) | 1/17 (6%) | 0/4 (0%) |
| Week of First Observation | | 88 | 77 |
| Life Table Tests | P=0.014 | P=0.160 | P=0.043 |
| Incidental Tumor Tests | P=0.091 | P=0.288 | P=0.167 |
| Pelvic Epithelial Hyperplasia | | | |
| Overall Rates | 0/50 (0%) | 23/50 (46%) | 21/49 (43%) |
| Transitional Cell Papilloma (d) | | | |
| Overall Rates | 0/50 (0%) | 7/50 (14%) | 3/49 (6%) |
| Adjusted Rates | 0.0% | 38.5% | 17.5% |
| Terminal Rates | 0/27 (0%) | 6/17 (35%) | 0/4 (0%) |
| Week of First Observation | | 99 | 85 |
| Life Table Tests | P=0.001 | P<0.001 | P=0.031 |
| Incidental Tumor Tests | P=0.014 | P=0.001 | P=0.301 |
| Transitional Cell Carcinoma | | | |
| Overall Rates | 0/50 (0%) | 1/50 (2%) | 0/49 (0%) |
| Transitional Cell Papilloma or Carcinoma | | | |
| Overall Rates | 0/50 (0%) | 8/50 (16%) | 3/49 (6%) |
| Adjusted Rates | 0.0% | 44.1% | 17.5% |
| Terminal Rates | 0/27 (0%) | 7/17 (41%) | 0/4 (0%) |
| Week of First Observation | | 99 | 85 |
| Life Table Tests | P<0.001 | P<0.001 | P=0.031 |
| Incidental Tumor Tests | P=0.010 | P<0.001 | P=0.301 |

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix A, Table A3 (footnotes).

(b) Severity of lesion graded from 1 to 4, minimal to marked

(c) Historical incidence of tubular cell adenomas or adenocarcinomas at study laboratory (mean): 3/450 (0.7%); historical incidence in NTP studies: 8/1,448 (0.6%)

(d) Historical incidence at study laboratory (mean): 0/450; historical incidence in NTP studies: 1/1,448 (<0.1%)

III. RESULTS: RATS

controls. The mineralization in vehicle control rats was minimal in severity and consisted of one or several small foci of mineral deposition. In dosed rats, there were many linear deposits of mineral, some extending almost the full depth of the papilla. Focal hyperplasia of the renal tubular epithelium consisted of single or multiple cross-sections of tubules filled or partially filled with stratified epithelial cells. The stratification of these cells and loss of basement membrane dependency differentiate this lesion from the epithelial regeneration occurring in response to the degenerative changes of spontaneous nephropathy. Hyperplasia of the renal pelvic epithelium was characterized by thickening and folding of the transitional epithelium to form small nodular structures protruding into the pelvic lumens.

Tubular cell adenocarcinomas in males occurred with a significant positive trend by the life table test, and the incidence in the high dose group was significantly greater than that in the vehicle controls by the life table test (Table 17). Transitional cell papillomas of the renal pelvis occurred in 7/50 low dose and 3/49 high dose rats; a transitional cell carcinoma occurred in a low dose male rat. Transitional cell papillomas and transitional cell papillomas or carcinomas (combined) in male rats occurred with significant positive trends; the incidences in the low dose group were greater than those in the vehicle controls.

Hematopoietic System: Mononuclear cell leukemia in male rats occurred with a significant positive trend; the incidence in the high dose group was significantly greater than that in the vehicle controls (Table 18); most of the leukemias were stage 3 and contributed to the deaths of the animals. Criteria for staging mononuclear cell leukemia are given below.

Stage 1. Spleen not enlarged or only slightly enlarged with small numbers of neoplastic mononuclear cells in the red pulp; no or very few mononuclear cells in the liver sinusoids. No identifiable neoplastic cells in other organs.

Stage 2. Spleen moderately enlarged with moderate to large numbers of mononuclear cells in the red pulp; architectural features including lymphoid follicles and periarteriolar lymphocytic sheaths remain intact. Minimal to moderate involvement of the liver. Mononuclear cells may be evident in blood vessels in other organs, but aggregates/masses of neoplastic cells generally limited to spleen and liver.

Stage 3. Advanced disease with multiple organ involvement. Spleen usually markedly enlarged with effacement of normal architectural features by accumulated neoplastic cells. Liver moderately to markedly enlarged and nodular; hepatic parenchyma shows variable degenerative changes associated with the accumulation of neoplastic cells. Accumulations of neoplastic mononuclear cells in other organs including lung, lymph nodes, kidney, brain, and adrenal gland.

TABLE 18. ANALYSIS OF MONONUCLEAR CELL LEUKEMIA IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (a)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|-------------------------------|-----------------|-------------|-------------|
| Overall Rates | 10/50 (20%) | 11/50 (22%) | 17/50 (34%) |
| Adjusted Rates | 30.0% | 38.5% | 100.0% |
| Terminal Rates | 4/27 (15%) | 4/17 (24%) | 4/4 (100%) |
| Week of First Observation | 91 | 63 | 76 |
| Life Table Tests | P<0.001 | P=0.188 | P<0.001 |
| Incidental Tumor Tests | P=0.048 | P=0.493 | P=0.032 |
| Stage | | | |
| 1 | 0 | 3 | 0 |
| 2 | 4 | 2 | 4 |
| 3 | 6 | 6 | 13 |
| Average stage leukemia | 2.60 | 2.27 | 2.76 |

(a) Historical incidence of leukemia at study laboratory (mean \pm SD): 85/450 (19% \pm 9%); historical incidence in NTP studies: 202/1,450 (14% \pm 8%)

III. RESULTS: RATS

Oral Cavity (mouth, palate, or tongue): Squamous cell papillomas or carcinomas (combined) in female rats occurred with a marginally significant positive trend by the life table test (vehicle control, 1/50; low dose, 0/50; high dose, 4/50; $P=0.045$). The incidence in the high dose group was not significantly greater than that in the vehicle controls.

Adrenal Gland: Pheochromocytomas and pheochromocytomas or malignant pheochromocytomas (combined) in male rats occurred with significant positive trends (Table 19).

TABLE 19. ANALYSIS OF ADRENAL GLAND LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---|-----------------|-------------|-------------|
| Medullary Focal Hyperplasia | | | |
| Overall Rates | 12/50 (24%) | 8/50 (16%) | 10/49 (20%) |
| Pheochromocytoma | | | |
| Overall Rates | 12/50 (24%) | 14/50 (28%) | 18/49 (37%) |
| Adjusted Rates | 35.5% | 57.8% | 91.3% |
| Terminal Rates | 7/27 (26%) | 8/17 (47%) | 3/4 (75%) |
| Week of First Observation | 55 | 70 | 65 |
| Life Table Tests | $P<0.001$ | $P=0.092$ | $P<0.001$ |
| Incidental Tumor Tests | $P=0.022$ | $P=0.284$ | $P=0.071$ |
| Malignant Pheochromocytoma | | | |
| Overall Rates | 0/50 (0%) | 4/50 (8%) | 0/49 (0%) |
| Adjusted Rates | 0.0% | 18.5% | 0.0% |
| Terminal Rates | 0/27 (0%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | | 86 | |
| Life Table Tests | $P=0.236$ | $P=0.026$ | (a) |
| Incidental Tumor Tests | $P=0.486$ | $P=0.052$ | (a) |
| Pheochromocytoma or Malignant Pheochromocytoma (b) | | | |
| Overall Rates | 12/50 (24%) | 18/50 (36%) | 18/49 (37%) |
| Adjusted Rates | 35.5% | 69.7% | 91.3% |
| Terminal Rates | 7/27 (26%) | 10/17 (59%) | 3/4 (75%) |
| Week of First Observation | 55 | 70 | 65 |
| Life Table Tests | $P<0.001$ | $P=0.012$ | $P<0.001$ |
| Incidental Tumor Tests | $P=0.017$ | $P=0.069$ | $P=0.071$ |

(a) No P value is reported because no tumors were observed in the 1,000 mg/kg and vehicle control groups.

(b) Historical incidence of pheochromocytomas or malignant pheochromocytomas at study laboratory (mean \pm SD): 98/449 (22% \pm 9%); historical incidence in NTP studies: 347/1,442 (24% \pm 9%)

III. RESULTS: RATS

Thyroid Gland: C-Cell carcinomas, considered a nonfatal tumor, occurred in male rats with a significant positive trend; the incidence in the high dose group was significantly greater than that in the vehicle controls, but the incidences of C-cell adenomas or carcinomas (combined) in dosed male rats were not significantly different from that in the vehicle controls by the incidental

tumor test (Table 20). The incidences of follicular cell adenomas or carcinomas (combined) in dosed male rats were increased by the life table trend test but only marginally by the incidental tumor trend test, the latter being the more appropriate test for nonfatal tumors (Table 21). The incidences of thyroid gland tumors were not increased in female rats.

TABLE 20. ANALYSIS OF THYROID GLAND C-CELL LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---------------------------------|-----------------|------------|-------------|
| Hyperplasia | | | |
| Overall Rates | 6/49 (12%) | 6/50 (12%) | 3/49 (6%) |
| Adenoma | | | |
| Overall Rates | 3/49 (6%) | 0/50 (0%) | 1/49 (2%) |
| Carcinoma | | | |
| Overall Rates | 1/49 (2%) | 4/50 (8%) | 4/49 (8%) |
| Adjusted Rates | 3.7% | 20.6% | 54.3% |
| Terminal Rates | 1/27 (4%) | 3/17 (18%) | 2/4 (50%) |
| Week of First Observation | 105 | 88 | 79 |
| Life Table Tests | P=0.002 | P=0.075 | P=0.004 |
| Incidental Tumor Tests | P=0.022 | P=0.122 | P=0.030 |
| Adenoma or Carcinoma (a) | | | |
| Overall Rates | 4/49 (8%) | 4/50 (8%) | 5/49 (10%) |
| Adjusted Rates | 14.8% | 20.6% | 57.3% |
| Terminal Rates | 4/27 (15%) | 3/17 (18%) | 2/4 (50%) |
| Week of First Observation | 105 | 88 | 79 |
| Life Table Tests | P=0.008 | P=0.387 | P=0.008 |
| Incidental Tumor Tests | P=0.112 | P=0.486 | P=0.118 |

(a) Historical incidence at study laboratory (mean \pm SD): 39/437 (9% \pm 3%); historical incidence in NTP studies: 181/1,417 (13% \pm 6%)

TABLE 21. ANALYSIS OF THYROID GLAND FOLLICULAR CELL LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---------------------------------|-----------------|------------|-------------|
| Hyperplasia | | | |
| Overall Rates | 0/49 (0%) | 1/50 (2%) | 0/49 (0%) |
| Adenoma | | | |
| Overall Rates | 0/49 (0%) | 0/50 (0%) | 2/49 (4%) |
| Carcinoma | | | |
| Overall Rates | 0/49 (0%) | 2/50 (4%) | 1/49 (2%) |
| Adenoma or Carcinoma (a) | | | |
| Overall Rates | 0/49 (0%) | 2/50 (4%) | 3/49 (6%) |
| Adjusted Rates | 0.0% | 11.8% | 22.9% |
| Terminal Rates | 0/27 (0%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | | 105 | 88 |
| Life Table Tests | P=0.003 | P=0.143 | P=0.014 |
| Incidental Tumor Tests | P=0.050 | P=0.143 | P=0.301 |

(a) Historical incidence at study laboratory (mean \pm SD): 6/437 (1% \pm 2%); historical incidence in NTP studies: 35/1,417 (2% \pm 3%)

III. RESULTS: RATS

Multiple Organs: Mesotheliomas in the tunica vaginalis in male rats occurred with a significant positive trend; however, the incidence of total mesotheliomas at all sites was only marginally increased when analyzed by the incidental tumor trend test, and the incidence of mesotheliomas (all sites) in dosed animals was not greater than the vehicle control incidence in

pairwise comparisons with the vehicle controls by the life table test (Table 22).

Nasolacrimal Duct: Chronic inflammation was observed at an increased incidence in high dose male rats (male: vehicle control, 1/50; low dose, 1/50; high dose, 8/50); this lesion was not notably increased in dosed females (0/50; 0/50; 2/50).

TABLE 22. ANALYSIS OF MESOTHELIOMAS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---------------------------|-----------------|------------|-------------|
| Tunica Vaginalis | | | |
| Overall Rates | 0/50 (0%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted Rates | 0.0% | 20.2% | 34.3% |
| Terminal Rates | 0/27 (0%) | 3/17 (18%) | 0/4 (0%) |
| Week of First Observation | | 82 | 68 |
| Life Table Tests | P<0.001 | P=0.025 | P=0.002 |
| Incidental Tumor Tests | P=0.007 | P=0.044 | P=0.046 |
| All Sites (a) | | | |
| Overall Rates | (b) 2/50 (4%) | 5/50 (10%) | 6/50 (12%) |
| Adjusted Rates | 6.4% | 25.9% | 34.3% |
| Terminal Rates | 1/27 (4%) | 4/17 (24%) | 0/4 (0%) |
| Week of First Observation | 96 | 82 | 68 |
| Life Table Tests | P=0.002 | P=0.083 | P=0.009 |
| Incidental Tumor Tests | P=0.034 | P=0.130 | P=0.141 |

(a) Historical incidence of mesotheliomas at all sites at study laboratory (mean \pm SD): 20/450 (4% \pm 3%); historical incidence in NTP studies: 55/1,450 (4% \pm 3%)

(b) Includes one malignant mesothelioma

III. RESULTS: MICE

SINGLE-ADMINISTRATION STUDIES

Two of five female mice in the highest (6,810 mg/kg) dose group died before the end of the studies; all other mice survived to the end of the studies. No gross pathologic effects were observed in the two female mice that died. Transitory inactivity (1-4 hours after dosing) was observed in the two highest dose groups. Higher doses (0, 1,250, 2,500, 5,000, 10,000, and 15,000 mg/kg) were selected for the 15-day studies because the oral LD₅₀ value was judged to be greater than 6,810 mg/kg.

FIFTEEN-DAY STUDIES

All the mice that received 15,000 mg/kg and 4/5 males and 5/5 females that received 10,000 mg/kg died before the end of the studies (Table 23). Inactivity, prostration, and shallow breathing were seen in animals dying after dosing in the 10,000 and 15,000 mg/kg groups. Various stomach lesions were observed at increased incidences in the three highest dose groups of females (gastropathy, gastritis, hyperkeratosis, or epithelial ulceration) and in all dosed groups of males (squamous atrophy, gastropathy, or gastritis) (Table 24). Based on the mortality at the 10,000 and 15,000 mg/kg doses, a high dose of 8,000 mg/kg was selected for the 13-week studies.

TABLE 23. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FIFTEEN-DAY GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) | | | Final Weight Relative to Vehicle Controls (percent) |
|-----------------|--------------|---------------------------|-------|--------|---|
| | | Initial (b) | Final | Change | |
| MALE | | | | | |
| 0 | 5/5 | 28 | 27 | -1 | -- |
| 1,250 | (c) 4/5 | 28 | 28 | 0 | 103.7 |
| 2,500 | 5/5 | 28 | 26 | -2 | 96.3 |
| 5,000 | 5/5 | 27 | 28 | +1 | 103.7 |
| 10,000 | (d) 1/5 | 27 | 22 | -5 | 81.5 |
| 15,000 | (e) 0/5 | 27 | (f) | (f) | (f) |
| FEMALE | | | | | |
| 0 | 5/5 | 20 | 20 | 0 | -- |
| 1,250 | 5/5 | 20 | 20 | 0 | 100.0 |
| 2,500 | 5/5 | 20 | 19 | -1 | 95.0 |
| 5,000 | 5/5 | 20 | 20 | 0 | 100.0 |
| 10,000 | (g) 0/5 | 20 | (f) | (f) | (f) |
| 15,000 | (h) 0/5 | 20 | (f) | (f) | (f) |

(a) Number surviving/number initially in group

(b) Initial group mean body weight

(c) Day of death: 8, death judged not compound related

(d) Day of death: 2,7,8,15

(e) Day of death: 2,2,2,2,3

(f) No data are reported due to the 100% mortality in this group.

(g) Day of death: 2,7,10,11,15

(h) Day of death: all 1

TABLE 24. NUMBERS OF MICE WITH STOMACH LESIONS IN THE FIFTEEN-DAY GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

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

III. RESULTS: MICE

THIRTEEN-WEEK STUDIES

Seven of seven males and 5/6 females that received 8,000 mg/kg and 9/10 males and 9/10 females that received 4,000 mg/kg dimethyl methylphosphonate died before the end of the studies (Table 25). The final mean body weights of the dosed and vehicle control groups were comparable. No compound-related clinical signs or gross or microscopic lesions were observed.

Dose Selection Rationale: Based on the incidences of deaths and body weight effects, doses selected for mice for the 2-year studies were 1,000 and 2,000 mg/kg dimethyl methyl-

phosphonate, administered in corn oil by gavage, 5 days per week for 103 weeks.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male mice were 7%-16% lower than those of vehicle controls between weeks 36 and 76 (Table 26, and Figure 5). Mean body weights of high dose female mice were 6%-12% lower than those of the vehicle controls between week 88 and week 103. No compound-related clinical signs were reported.

TABLE 25. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) | | | Final Weight Relative to Vehicle Controls (percent) |
|-----------------|--------------|---------------------------|-------|------------|---|
| | | Initial (b) | Final | Change (c) | |
| MALE | | | | | |
| 0 | 10/10 | 23 | 29 | +6 | -- |
| 250 | 10/10 | 23 | 30 | +7 | 103.4 |
| 500 | 10/10 | 24 | 32 | +8 | 110.3 |
| 1,000 | 10/10 | 24 | 32 | +8 | 110.3 |
| 2,000 | 10/10 | 24 | 32 | +8 | 110.3 |
| 4,000 | (d) 1/10 | 25 | 28 | +3 | 96.6 |
| 8,000 | (e) 0/7 | (f) | (g) | -- | (g) |
| FEMALE | | | | | |
| 0 | 10/10 | 19 | 24 | +5 | -- |
| 250 | 10/10 | 19 | 24 | +5 | 100.0 |
| 500 | 10/10 | 18 | 24 | +6 | 100.0 |
| 1,000 | 10/10 | 19 | 24 | +5 | 100.0 |
| 2,000 | 10/10 | 19 | 24 | +5 | 100.0 |
| 4,000 | (h) 1/10 | 19 | 23 | +4 | 95.8 |
| 8,000 | (i) 1/6 | (f) | 25 | -- | 104.2 |

(a) Number surviving/number initially in group

(b) Initial group mean body weight

(c) Mean body weight change of the survivors

(d) Week of death: 2,5,6,6,6,6,9,9

(e) Week of death: 1,3,3,3,3,4

(f) Initial weights not reported

(g) No data are reported due to the 100% mortality in this group.

(h) Week of death: 8,9,9,10,10,12,12,12,12

(i) Week of death: 2,6,6,10,11

TABLE 26. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| Weeks on Study | Vehicle Control | | 1,000 mg/kg | | | 2,000 mg/kg | | |
|----------------|-----------------|------------------|-----------------|--------------------------------|------------------|-----------------|--------------------------------|------------------|
| | Av. Wt. (grams) | No. of Survivors | Av. Wt. (grams) | Wt. (percent of veh. controls) | No. of Survivors | Av. Wt. (grams) | Wt. (percent of veh. controls) | No. of Survivors |
| MALE | | | | | | | | |
| 0 | 22.4 | 50 | 23.0 | 103 | 50 | 22.0 | 98 | 50 |
| 1 | 21.9 | 49 | 23.8 | 109 | 50 | 23.1 | 105 | 50 |
| 2 | 24.6 | 49 | 25.0 | 102 | 49 | 24.4 | 99 | 50 |
| 3 | 26.4 | 49 | 26.1 | 99 | 49 | 25.3 | 96 | 50 |
| 4 | 26.8 | 49 | 27.0 | 101 | 49 | 26.1 | 97 | 49 |
| 5 | 27.4 | 49 | 27.4 | 100 | 49 | 26.2 | 96 | 49 |
| 6 | 28.4 | 49 | 28.7 | 101 | 49 | 27.7 | 98 | 49 |
| 7 | 28.6 | 49 | 29.2 | 102 | 49 | 28.5 | 100 | 49 |
| 8 | 29.4 | 49 | 29.3 | 100 | 49 | 28.9 | 98 | 49 |
| 9 | 30.3 | 49 | 30.7 | 101 | 49 | 30.1 | 99 | 49 |
| 10 | 30.8 | 49 | 30.9 | 100 | 49 | 30.4 | 99 | 49 |
| 11 | 31.1 | 48 | 30.9 | 99 | 49 | 30.6 | 98 | 48 |
| 12 | 31.3 | 48 | 31.2 | 100 | 49 | 30.5 | 97 | 48 |
| 13 | 31.3 | 48 | 31.6 | 101 | 49 | 30.9 | 99 | 48 |
| 16 | 32.4 | 47 | 32.7 | 101 | 49 | 31.7 | 98 | 48 |
| 20 | 34.3 | 47 | 33.8 | 99 | 46 | 33.1 | 97 | 44 |
| 24 | 35.0 | 47 | 34.7 | 99 | 45 | 33.3 | 95 | 39 |
| 28 | 36.4 | 45 | 35.9 | 99 | 45 | 34.8 | 96 | 37 |
| 32 | 37.5 | 44 | 37.2 | 99 | 45 | 35.8 | 95 | 36 |
| 36 | 38.9 | 44 | 38.2 | 98 | 45 | 36.1 | 93 | 33 |
| 40 | 38.7 | 44 | 38.7 | 100 | 44 | 35.9 | 93 | 32 |
| 44 | 41.3 | 43 | 40.7 | 99 | 44 | 37.4 | 91 | 28 |
| 48 | 41.4 | 41 | 41.2 | 100 | 44 | 36.3 | 88 | 11 |
| 52 | 41.9 | 41 | 42.5 | 101 | 44 | 37.1 | 89 | 11 |
| 56 | 42.8 | 41 | 43.5 | 102 | 42 | 38.3 | 89 | 11 |
| 60 | 44.8 | 40 | 44.4 | 99 | 42 | 37.5 | 84 | 11 |
| 64 | 42.4 | 40 | 43.6 | 103 | 41 | 38.8 | 92 | 11 |
| 68 | 44.1 | 40 | 43.1 | 98 | 41 | 39.1 | 89 | 10 |
| 72 | 44.1 | 40 | 43.5 | 99 | 41 | 39.5 | 90 | 8 |
| 76 | 45.0 | 40 | 43.1 | 96 | 40 | 39.5 | 88 | 6 |
| 80 | 44.8 | 38 | 43.7 | 98 | 29 | 42.8 | 96 | 4 |
| 84 | 44.3 | 38 | 43.4 | 98 | 27 | 45.3 | 102 | 3 |
| 88 | 44.1 | 36 | 42.3 | 96 | 26 | 45.0 | 102 | 3 |
| 92 | 43.0 | 35 | 43.0 | 100 | 25 | 45.3 | 105 | 3 |
| 96 | 41.3 | 32 | 41.1 | 100 | 19 | -- | -- | -- |
| 100 | 40.6 | 31 | 42.3 | 104 | 13 | -- | -- | -- |
| 104 | 39.4 | 30 | 39.8 | 101 | 12 | -- | -- | -- |
| FEMALE | | | | | | | | |
| 0 | 17.6 | 50 | 18.3 | 104 | 50 | 18.0 | 102 | 50 |
| 1 | 18.1 | 50 | 19.1 | 106 | 50 | 18.5 | 102 | 50 |
| 2 | 18.9 | 50 | 19.7 | 104 | 50 | 19.2 | 102 | 50 |
| 3 | 20.0 | 50 | 20.2 | 101 | 50 | 19.8 | 99 | 50 |
| 4 | 20.0 | 50 | 20.4 | 102 | 50 | 20.0 | 100 | 50 |
| 5 | 20.2 | 50 | 20.5 | 101 | 50 | 20.3 | 100 | 50 |
| 6 | 21.1 | 50 | 21.2 | 100 | 50 | 21.1 | 100 | 50 |
| 7 | 21.1 | 50 | 21.7 | 103 | 50 | 21.4 | 101 | 50 |
| 8 | 21.6 | 50 | 22.1 | 102 | 50 | 21.4 | 99 | 50 |
| 9 | 21.9 | 50 | 22.8 | 104 | 50 | 22.3 | 102 | 50 |
| 10 | 22.2 | 50 | 22.8 | 103 | 50 | 22.7 | 102 | 50 |
| 11 | 22.5 | 50 | 23.0 | 102 | 50 | 22.8 | 101 | 50 |
| 12 | 22.4 | 50 | 22.9 | 102 | 50 | 22.5 | 100 | 50 |
| 13 | 22.5 | 50 | 23.3 | 104 | 50 | 22.9 | 102 | 50 |
| 16 | 23.5 | 50 | 23.7 | 101 | 50 | 23.4 | 100 | 50 |
| 20 | 24.5 | 50 | 25.0 | 102 | 49 | 24.3 | 99 | 47 |
| 24 | 24.7 | 50 | 25.3 | 102 | 49 | 24.6 | 100 | 47 |
| 28 | 25.6 | 50 | 26.0 | 102 | 49 | 25.9 | 101 | 47 |
| 32 | 27.2 | 50 | 27.6 | 101 | 49 | 26.9 | 99 | 46 |
| 36 | 28.3 | 50 | 28.7 | 101 | 47 | 27.9 | 99 | 45 |
| 40 | 28.4 | 50 | 29.1 | 102 | 47 | 28.1 | 99 | 45 |
| 44 | 30.6 | 48 | 30.8 | 101 | 47 | 29.7 | 97 | 43 |
| 48 | 30.7 | 48 | 31.4 | 102 | 47 | 30.4 | 99 | 21 |
| 52 | 31.5 | 48 | 32.4 | 103 | 47 | 31.0 | 98 | 21 |
| 56 | 33.4 | 48 | 34.0 | 102 | 47 | 32.4 | 97 | 20 |
| 60 | 35.0 | 48 | 35.3 | 101 | 47 | 33.1 | 95 | 19 |
| 64 | 34.2 | 47 | 35.8 | 105 | 46 | 34.0 | 99 | 19 |
| 68 | 35.2 | 47 | 36.6 | 104 | 45 | 35.5 | 101 | 18 |
| 72 | 36.5 | 47 | 37.1 | 102 | 45 | 35.1 | 96 | 10 |
| 76 | 37.4 | 47 | 38.4 | 103 | 44 | 36.1 | 97 | 8 |
| 80 | 38.0 | 47 | 38.9 | 102 | 44 | 37.4 | 98 | 7 |
| 84 | 38.6 | 47 | 39.9 | 103 | 44 | 36.5 | 95 | 6 |
| 88 | 38.3 | 47 | 39.6 | 103 | 44 | 36.0 | 94 | 4 |
| 92 | 38.9 | 46 | 40.4 | 104 | 44 | 34.3 | 88 | 3 |
| 96 | 38.3 | 45 | 39.6 | 103 | 38 | 34.3 | 90 | 3 |
| 100 | 38.1 | 44 | 40.0 | 105 | 35 | 35.3 | 93 | 3 |
| 104 | 37.3 | 42 | 39.0 | 105 | 31 | 36.5 | 98 | 2 |

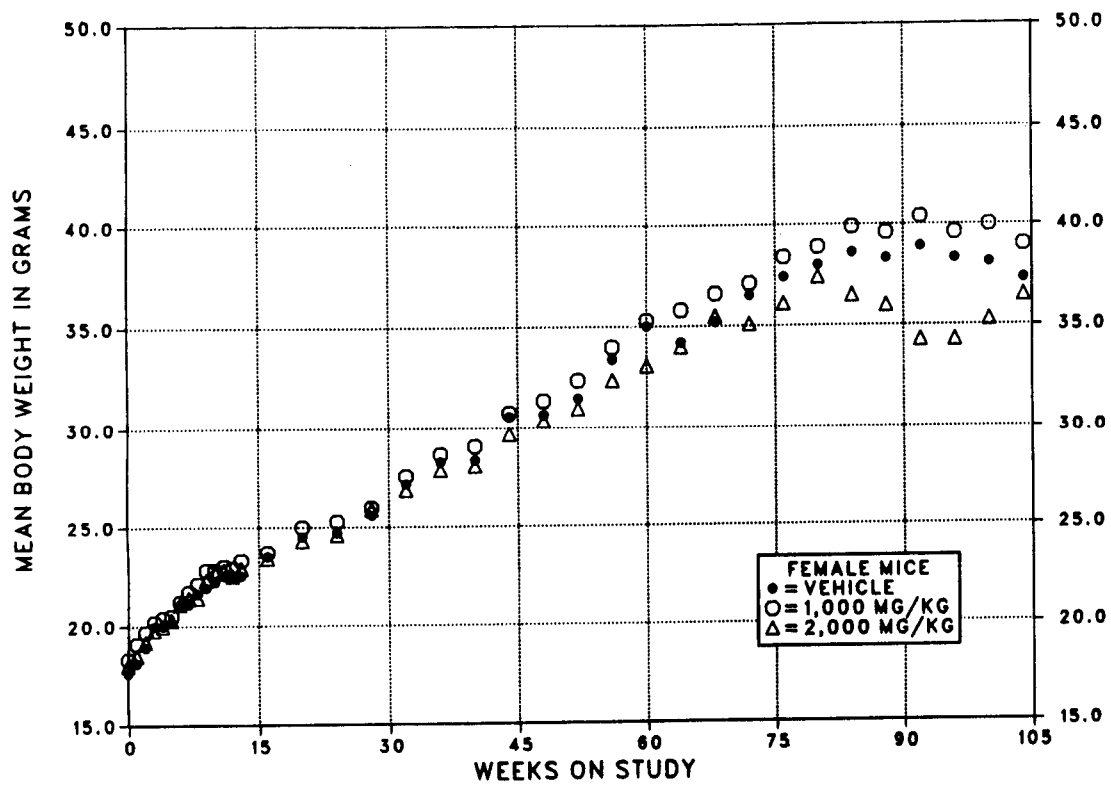
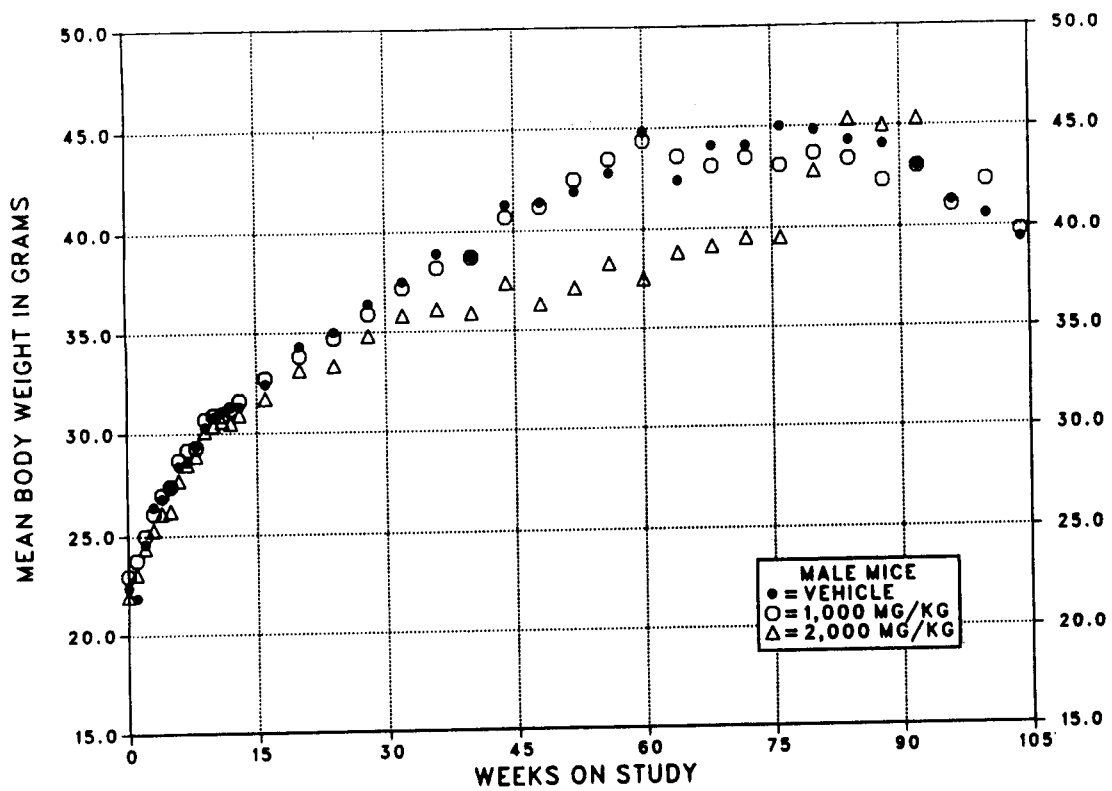


FIGURE 5. GROWTH CURVES FOR MICE ADMINISTERED DIMETHYL METHYLPHOSPHONATE IN CORN OIL BY GAVAGE FOR TWO YEARS

III. RESULTS: MICE

Survival

Estimates of the probabilities of survival for male and female mice administered dimethyl methylphosphonate at the doses used in these studies and for vehicle controls are shown in the Kaplan and Meier curves in Figure 6. Between weeks 23 and 44, there was a gradual decrease in survival in high dose male mice which was attributed to fighting; lesions around the penis were noted in many of these animals. Aggressive animals were housed separately to prevent animal attrition. At week 45, 17 high dose male mice and 22 high dose female mice were found dead; chemical analysis of the dose preparation found the dose to be 134% of the targeted

amount. For purposes of survival analysis, these deaths were censored. Eleven low dose male mice died at week 77.

The survival of both the low dose (after week 95) and the high dose (after week 23) groups of male mice was significantly lower than that of the vehicle controls (Table 27). The survival of the high dose group of female mice was significantly lower than that of the vehicle controls after week 52. The survival of the low dose female mice was comparable to that of the vehicle controls. Unadjusted survival curves (with accidental deaths not censored) for male and female mice are shown in Figure 7.

TABLE 27. SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 1,000 mg/kg | 2,000 mg/kg |
|---|-----------------|-------------|-------------|
| MALE (a) | | | |
| Animals initially in study | 50 | 50 | 50 |
| Nonaccidental deaths before termination (b) | 17 | 33 | 33 |
| Accidentally killed | 4 | 5 | 17 |
| Killed at termination | 28 | 12 | 0 |
| Died during termination period | 1 | 0 | 0 |
| Survival P values (c) | <0.001 | 0.004 | <0.001 |
| FEMALE (a) | | | |
| Animals initially in study | 50 | 50 | 50 |
| Nonaccidental deaths before termination (b) | 7 | 14 | 24 |
| Accidentally killed | 0 | 6 | 23 |
| Animals missing | 2 | 0 | 1 |
| Killed at termination | 41 | 30 | 2 |
| Survival P values (c) | <0.001 | 0.109 | <0.001 |

(a) Terminal-kill period: week 105

(b) Includes animals killed in a moribund condition

(c) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.

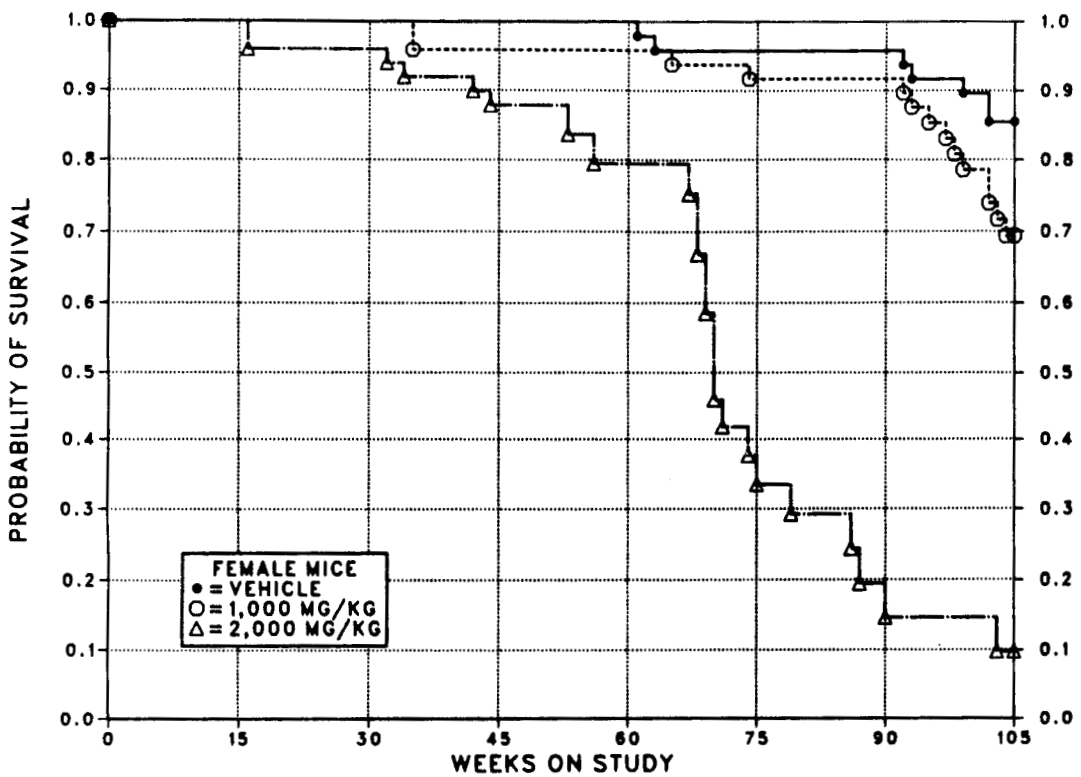
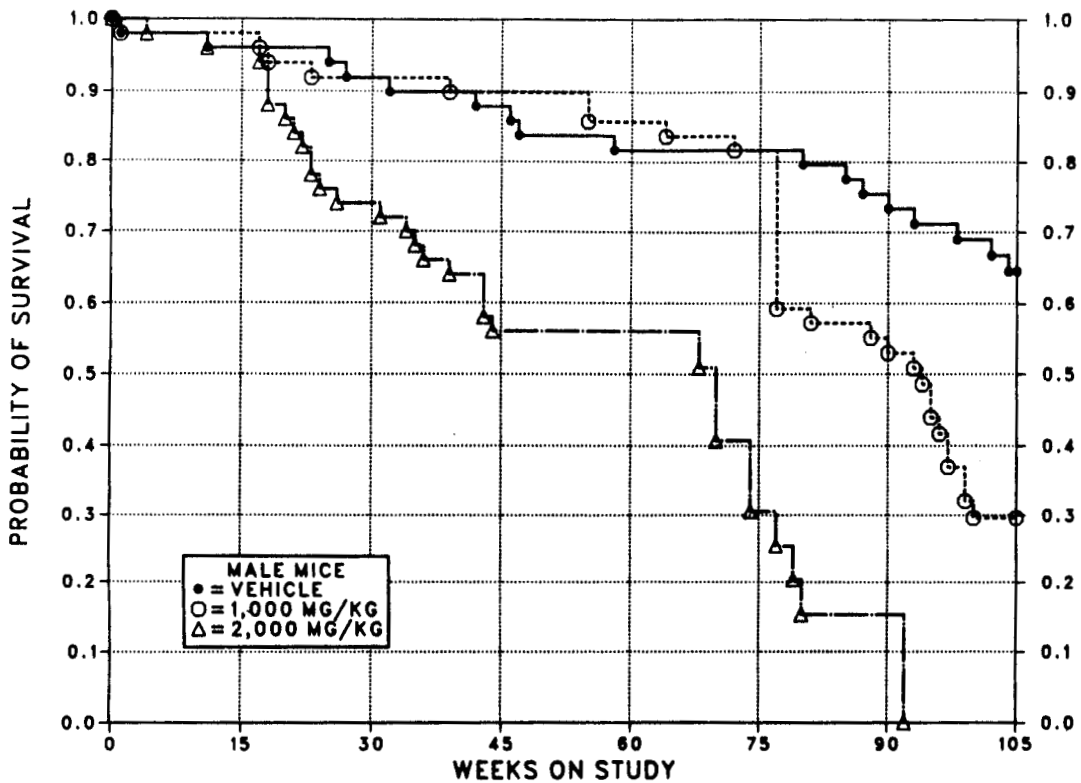


FIGURE 6. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED DIMETHYL METHYLPHOSPHONATE IN CORN OIL BY GAVAGE FOR TWO YEARS

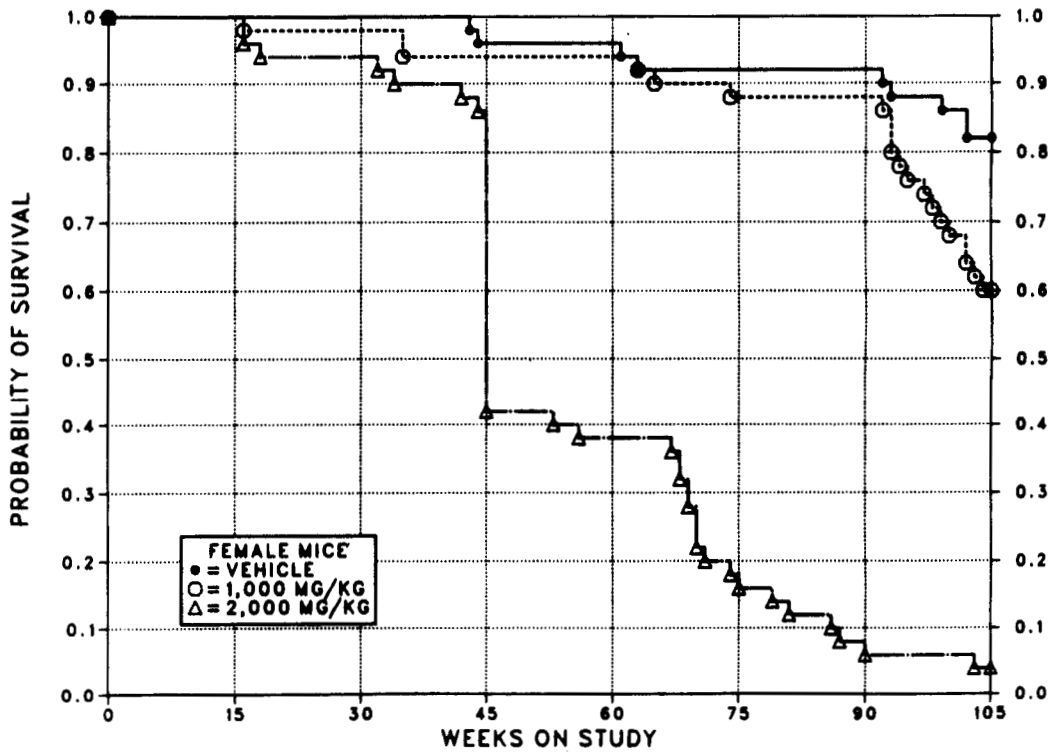
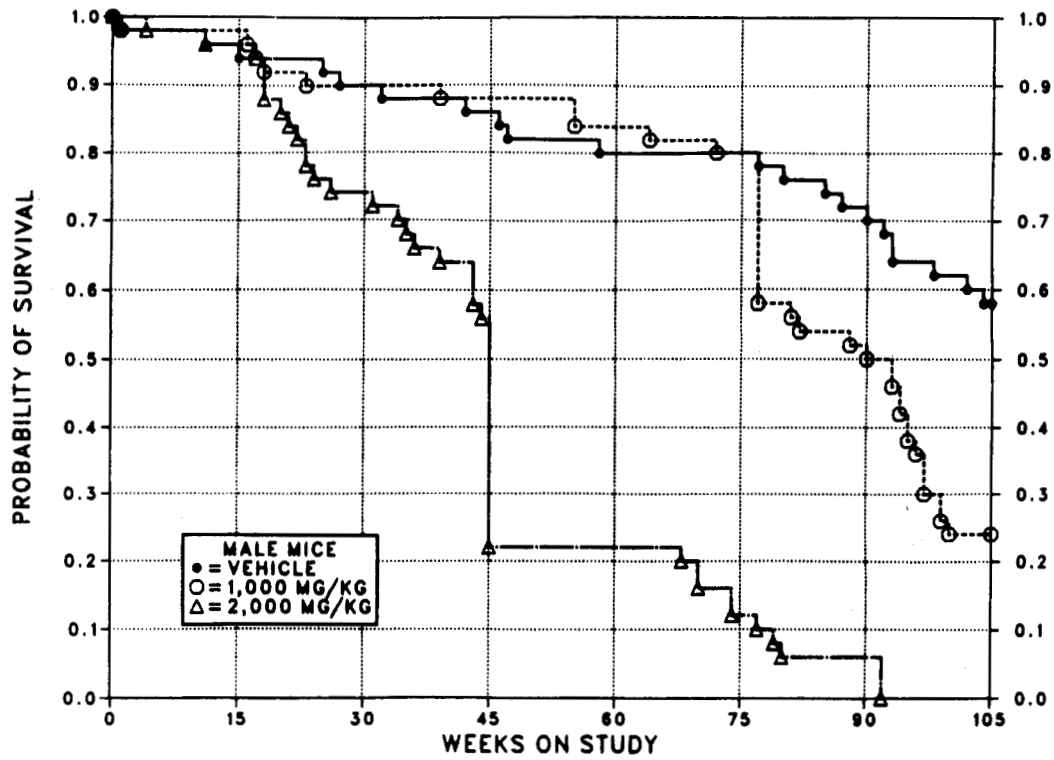


FIGURE 7. UNADJUSTED SURVIVAL CURVES FOR MICE ADMINISTERED DIMETHYL METHYLPHOSPHONATE IN CORN OIL BY GAVAGE FOR TWO YEARS

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the liver, hematopoietic system, and lung.

Lesions in male mice are summarized in Appendix C. Histopathologic findings on neoplasms are summarized in Table C1. Table C2 gives the survival and tumor status for individual male mice. Table C3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table C3 (footnotes). Historical incidences of tumors in corn oil vehicle control male mice are listed in Table C4. Findings on nonneoplastic lesions are summarized in Table C5.

Lesions in female mice are summarized in Appendix D. Histopathologic findings on neoplasms are summarized in Table D1. Table D2 gives the survival and tumor status for individual female mice. Table D3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table D3 (footnotes). Findings on nonneoplastic lesions are summarized in Table D4. The increased mortality in the high dose groups resulted in little overlap of survival times; thus, the sensitivity of the incidental tumor test for detecting carcinogenic effects was reduced.

Liver: Hepatocellular adenomas and hepatocellular adenomas or carcinomas (combined) in male mice occurred with significant positive trends by the life table test ($P < 0.001$) but not by the incidental tumor test ($P = 0.156$), the more appropriate test for nonfatal tumors such as these (vehicle control, 17/50; low dose, 21/50; high dose, 4/46). Hepatocytomegaly was observed at increased incidences in dosed male mice (5/50; 17/50; 10/46). No increase in neoplastic or nonneoplastic liver lesions was seen in dosed female mice.

Hematopoietic System: Malignant lymphomas in male mice occurred with a significant positive trend by the life table test (vehicle control, 2/50; low dose, 3/50; high dose, 1/50; $P = 0.026$). One vehicle control male mouse had granulocytic leukemia. Because final survival in high dose animals was lower than that in the other groups, there were fewer high dose animals at risk for leukemia. Female mice showed no significant increases in the incidence of either malignant lymphomas or leukemia.

Lung: Congestion was observed at increased incidences in dosed male mice (vehicle control, 2/50; low dose, 8/49; high dose, 16/45) and dosed female mice (0/48; 2/50; 14/49). The lung congestion was associated with early death, dosing errors, or gavage accidents. All animals (with the exception of one low dose female) with lung congestion died early in the studies; 14/16 high dose male mice and 11/14 high dose female mice with lung congestion died at week 45 when a dosing error was observed. Six of eight low dose male mice with lung congestion died at week 77, the week of an unexplained clustering of deaths.

IV. DISCUSSION AND CONCLUSIONS

Study Design

Short-Term Studies

Two-Year Studies in Rats

Two-Year Studies in Mice

Genetic Toxicology

Other NTP Studies

Data Audit

Conclusions

IV. DISCUSSION AND CONCLUSIONS

Study Design

Dimethyl methylphosphonate was nominated for study in 1976 by the U.S. Army because it was a candidate nerve gas simulant. Three other candidate simulants were nominated for study at the same time, and it was recommended that all chemicals be studied in the same way so that toxicity data could be compared. In the short-term and 2-year studies, the chemicals were administered by gavage in corn oil to F344/N rats and B6C3F₁ mice to mimic potential oral exposure. Water was used as the vehicle in the studies of reproductive effects in male rodents.

Short-Term Studies

In the single-administration studies, dimethyl methylphosphonate was given to rats and mice at doses up to 6,810 mg/kg. No compound-related deaths were seen in rats or in male mice; two high dose female mice died. In the 15-day studies, rats and mice received doses up to 15,000 mg/kg per day. Compound-related deaths occurred at 5,000, 10,000, and 15,000 mg/kg in rats and at 10,000 and 15,000 mg/kg in mice. No compound-related lesions were seen in rats in these studies, but stomach lesions were seen in mice at 5,000, 10,000, and 15,000 mg/kg in the 15-day studies.

In the 13-week studies, dimethyl methylphosphonate was given at doses up to 8,000 mg/kg. Compound-related deaths occurred at 2,000, 4,000, and 8,000 mg/kg in rats and at 4,000 and 8,000 mg/kg in mice. During the single-administration and 15-day studies, clinical signs reported in rats and mice after dosing included inactivity, unsteady gait, and prostration; these clinical signs were not observed in the 13-week studies. No weight effects were observed in rats at 1,000 mg/kg or in mice at 2,000 mg/kg; reduced body weights and deaths were seen at higher doses. Minimal to mild renal and testicular lesions were seen in dosed male rats; no target tissues were identified in female rats or male and female mice. Doses selected for the 2-year studies were based on body weight effects and deaths observed in the short-term studies. The severity of the kidney lesions, which were observed in male rats at all doses, did not increase

with increasing dose of the chemical. These lesions were not considered to be life threatening.

Like the kidney lesions, the testicular lesions in male rats were seen at all doses, and the severity was not increased as the dose increased. The reproductive effects of the chemical in male rats and mice were studied to further assess the effects of the chemical. In these studies, male F344/N rats and B6C3F₁ mice were administered dimethyl methylphosphonate by gavage in water at doses up to 2,000 mg/kg for 13 weeks and were mated to undosed females; necropsies were performed, and the testis, kidney, and sperm were examined. An increase in the number of fetal resorptions was seen when dosed male rats and mice were mated to undosed females. Lesions of the kidney and testis were seen in male rats but not in male mice, and decreased sperm count and motility were seen in male rats but not in male mice. Toxic effects to the reproductive system were seen in both male rats and mice. These effects were more severe in rats but were reversible after a 13- to 14-week recovery period without dosing. No clinical signs were observed after dosing (Dunnick et al., 1984a,b; Chapin et al., 1984).

Two-Year Studies in Rats

In the 2-year studies, dimethyl methylphosphonate was administered to rats at doses of 0, 500, or 1,000 mg/kg. Survival of dosed male rats was greater than 50% in all groups until week 80 but was reduced in both dosed groups in the last weeks of the study (final survival: vehicle control, 27/50; low dose, 17/50; high dose, 4/50). This decreased survival in dosed male rats was due in part to chemically related kidney toxicity. Final survival in high dose female rats was reduced (30/50; 33/50; 23/50). Mean body weights of high dose male rats were 5%-10% lower than those of the vehicle controls between weeks 28 and 76 and were 10%-24% lower between weeks 80 and 104. Mean body weights of high dose female rats were 8%-12% lower than those of the vehicle controls after week 80. No compound-related clinical signs were observed.

Dimethyl methylphosphonate administration was associated with increased severity of

IV. DISCUSSION AND CONCLUSIONS

nephropathy and increased incidence of mineralization (calcification) in the kidney of dosed male rats. Renal tubular cell adenocarcinomas (vehicle control, 0/50; low dose, 2/50; high dose, 3/49) and transitional cell papillomas or carcinomas (combined) of the pelvic epithelium (0/50; 8/50; 3/49) were seen in the kidney of dosed male rats. These are uncommon tumors in F344/N rats and are believed to be related to the administration of dimethyl methylphosphonate. This is supported by the incidences of tubular cell hyperplasia in dosed male rats. The incidence of tubular cell hyperplasia is often increased in association with the induction of tubular cell neoplasms, and there appears to be a morphologic spectrum suggesting progression from hyperplasia to adenoma to adenocarcinoma. Similarly, there is a morphologic spectrum from hyperplasia of the transitional epithelium to papilloma and carcinoma of the renal pelvis. The reduced survival of the high dose male rats may account for the smaller number of these neoplasms in this group.

The spectrum of renal toxicity in male rats given dimethyl methylphosphonate by gavage for 13 weeks and 2 years is similar to that in male rats exposed to gasoline vapors, related petroleum naphthas, or light hydrocarbons consisting of paraffins or cycloparaffins or to alkyl aromatic hydrocarbons, decalin (Mehlman et al., 1984), or 1,4-dichlorobenzene (NTP, 1987). Degeneration and regeneration of epithelium in proximal convoluted tubules and dilated tubules filled with granular proteinaceous material were observed in male rats exposed to light hydrocarbon compounds in short-term inhalation studies (Mehlman et al., 1984); similar lesions were seen in the short-term studies of 1,4-dichlorobenzene (NTP, 1987) and dimethyl methylphosphonate. Early lesions consisting of the accumulation of hyaline droplets in epithelial cells in the kidney of male rats have been demonstrated to be phagolysosomes filled with amorphous electron-dense material. Hyaline droplets formed within renal tubular cells of male rats after exposure to hydrocarbons were reported to be primarily α -2-microglobulin, a protein that is produced in the liver of male rats under the influence of testosterone (Phillips and Cockrell, 1984). Whether this protein plays a role in the pathogenesis of lesions associated with long-term

administration of dimethyl methylphosphonate in male rats is unknown.

In contrast to the kidney lesions seen in male rats after exposure to dimethyl methylphosphonate, the major nonneoplastic lesions seen in the kidney of both rats and mice after exposure to halogenated hydrocarbons such as trichloroethylene (NTP, unpublished), 2-chloroethanol (NTP, 1985b), and tetrachloroethylene (NTP, 1986b) are cytomegaly, karyomegaly, and toxic nephrosis.

The incidence of mononuclear cell leukemia was increased in high dose male rats (vehicle control, 10/50; low dose, 11/50; high dose, 17/50), and the incidence exceeded the highest incidence seen in historical vehicle control groups in the NTP studies. Staging of the leukemia indicated that most of the tumors were stage 3.

Dosed male rats had marginally increased incidences of pheochromocytomas of the adrenal gland (vehicle control, 12/50; low dose, 14/50; high dose, 18/49). Four malignant pheochromocytomas also were seen in low dose male rats. The incidences in the dosed male rats slightly exceeded the highest incidence recorded for historical vehicle controls at the study laboratory (Appendix A, Table A4c), but the results of pairwise comparisons of low and high dose incidence to vehicle control incidence were not significant by the incidental tumor test, the test more appropriate for analysis of this nonfatal tumor.

Squamous cell papillomas or carcinomas (combined) of the oral cavity (mouth, palate, or tongue) in female rats occurred with a marginally significant ($P=0.045$) positive trend by the life table test (vehicle control, 1/50; low dose, 0/50; high dose, 4/50). The incidence in the high dose group was not significantly greater than that in the vehicle controls, and this lesion was not considered to be clearly compound related.

Incidences of nonfatal neoplasms in the thyroid gland of high dose male rats were significantly increased by the life table test but not by the incidental tumor test. The latter test is considered more appropriate, and thus, these incidences were considered not to be clearly compound related. C-Cell adenomas or carcinomas (combined)

IV. DISCUSSION AND CONCLUSIONS

in the thyroid gland of male rats were seen in 4/49 vehicle control, 4/50 low dose, and 5/49 high dose animals, and follicular cell adenomas or carcinomas (combined) in the thyroid gland were seen in 0/40 vehicle control, 2/50 low dose, and 3/49 high dose animals. Mesotheliomas in the tunica vaginalis, nonfatal tumors, were increased in dosed male rats, but the combined incidence of mesotheliomas at all sites was not significant by the incidental tumor test.

Two-Year Studies in Mice

In the 2-year studies, dimethyl methylphosphonate was administered to mice at doses of 0, 1,000, or 2,000 mg/kg. Deaths in high dose male mice between weeks 23 and 45 were associated with fighting. At week 45, 17 high dose male and 22 high dose female mice died from an apparent overdose that appeared to be due in part to improper resuspension of the dose mixture. Eleven low dose male mice died on the same day during week 77, and although the cause of death was not determined, it might also have been due partly to improper handling of the dose mixtures in the animal room. Lung congestion was seen in mice that died at weeks 45 and 77 but not in mice surviving to the end of the studies. Final survival in dosed groups of male mice was reduced, and the number of animals surviving to the end of the study was considered inadequate for carcinogenicity determination (final survival: vehicle control, 29/50; low dose, 12/50; high dose, 0/50). The number of high dose female mice surviving to the end of the study also was inadequate for a determination of carcinogenic activity (final survival: 41/50; 30/50; 2/50).

Mean body weights of high dose male mice were 7%-17% lower than those of vehicle control males between weeks 36 and 76, and those of high dose female mice were 6%-12% lower between weeks 88 and 103.

No increases in neoplastic lesions were considered to be compound related in male or female mice. In male mice, the incidences of hepatocellular adenomas or carcinomas (combined), lesions considered to be nonfatal, were significantly increased when assessed by the life table test but not by the incidental tumor test (vehicle control, 17/50; low dose, 21/50; high dose, 4/46);

this finding was not considered to be compound related. The incidences of malignant lymphomas were increased in dosed male mice by the life table trend test (vehicle control, 7/50; low dose, 3/50; high dose 1/50); the low rate in the high dose group was due in part to decreased survival in this dose group. The incidences of lymphomas are considered not to be related to chemical administration. Dimethyl methylphosphonate caused increased incidences of hepatocytomegaly in dosed male mice (5/50; 17/50; 10/46).

Genetic Toxicology

Dimethyl methylphosphonate induced forward mutations in mouse lymphoma cells in the absence of metabolic activation, induced SCEs in CHO cells both with and without metabolic activation, gave limited evidence of clastogenicity in CHO cells in the absence of metabolic activation, and induced sex-linked recessive lethal mutations and translocations in *Drosophila* (Appendix E). Dimethyl methylphosphonate was negative in bacterial mutagenicity tests (Aerospace Medical Research Laboratory, 1983; Table E1). A dominant lethal effect was seen in male rats and mice (Dunnick et al., 1984a,b).

Other NTP Studies

The NTP has reported studies conducted on other nerve gas simulants nominated for study by the U.S. Army. Tris(2-ethylhexyl)phosphate was found to have equivocal evidence of carcinogenicity for male rats because of increased incidence of pheochromocytomas of the adrenal glands (NTP, 1984). There was no evidence of carcinogenicity for female F344/N rats or male B6C3F₁ mice. There was some evidence of carcinogenicity for female B6C3F₁ mice as shown by an increased incidence of hepatocellular carcinomas.

In the 2-year studies of dimethyl morpholinophosphoramidate, there was some evidence of carcinogenicity for male and female F344/N rats as indicated by increased incidences of mononuclear cell leukemia (NTP, 1986a). There was no evidence of carcinogenicity for male and female B6C3F₁ mice.

IV. DISCUSSION AND CONCLUSIONS

There was clear evidence of carcinogenicity for dimethyl hydrogen phosphite for male F344/N rats, as shown by increased incidences of lung and forestomach neoplasms (NTP, 1985a). There was equivocal evidence of carcinogenicity for female F344/N rats as shown by marginally increased incidences of lung and forestomach neoplasms. There was no evidence of carcinogenicity for male and female B6C3F₁ mice.

Data Audit

The experimental and tabulated data for the NTP Technical report on dimethyl methylphosphonate were examined for accuracy, consistency, and compliance with Good Laboratory Practice requirements (Appendix H). No discrepancies were found that influenced the final interpretation of the results of these studies.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence*

*of carcinogenic activity** of dimethyl methylphosphonate for male F344/N rats as shown by increased incidences of tubular cell hyperplasia, tubular cell adenocarcinomas, hyperplasia of the transitional cell epithelium, and transitional cell papillomas of the kidney. There was an increased incidence of mononuclear cell leukemia in male rats at 1,000 mg/kg. Renal toxicity and decreased survival occurred in dosed male rats. There was *no evidence of carcinogenic activity* of dimethyl methylphosphonate for female F344/N rats given doses of 500 or 1,000 mg/kg. The study in male B6C3F₁ mice was an *inadequate study of carcinogenic activity* because of decreased survival in both dosed groups. There was *no evidence of carcinogenic activity* for female B6C3F₁ mice receiving dimethyl methylphosphonate at 1,000 mg/kg; decreased survival of female mice at 2,000 mg/kg made this group inadequate for determination of carcinogenic activity.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 6.

A summary of the Peer Review comments and the public discussion on this Technical Report appears on page 10.

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V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

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TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Squamous cell papilloma | 2 (4%) | | 1 (2%) |
| Squamous cell carcinoma | | | 1 (2%) |
| Keratoacanthoma | | 3 (6%) | |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Neoplasm, benign, NOS | 1 (2%) | | |
| Sarcoma, NOS | 1 (2%) | 1 (2%) | |
| Fibroma | 4 (8%) | 3 (6%) | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| *Nasal cavity | (50) | (50) | (50) |
| Squamous cell carcinoma | 1 (2%) | | |
| Chondroma | | 1 (2%) | |
| #Lung | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | | 1 (2%) | |
| Alveolar/bronchiolar carcinoma | | | 2 (4%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Leukemia, mononuclear cell | 10 (20%) | 10 (20%) | 17 (34%) |
| #Spleen | (50) | (49) | (48) |
| Fibrosarcoma | | 1 (2%) | |
| Leukemia, mononuclear cell | | 1 (2%) | |
| #Lymph node | (49) | (49) | (48) |
| Pheochromocytoma, metastatic | | 1 (2%) | |
| #Thymus | (42) | (43) | (41) |
| Thymoma, benign | | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| #Heart | (50) | (50) | (50) |
| Neurilemoma | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| *Palate | (50) | (50) | (50) |
| Squamous cell papilloma | 1 (2%) | 1 (2%) | |
| *Tongue | (50) | (50) | (50) |
| Carcinoma, NOS | | | 1 (2%) |
| #Salivary gland | (49) | (50) | (49) |
| Neurilemoma | 1 (2%) | | |
| #Liver | (50) | (49) | (49) |
| Neoplastic nodule | 1 (2%) | 4 (8%) | 1 (2%) |
| #Pancreas | (49) | (49) | (49) |
| Adenocarcinoma, NOS | 1 (2%) | | |
| Acinar cell adenoma | 2 (4%) | 1 (2%) | 2 (4%) |
| Pheochromocytoma, metastatic | | 1 (2%) | |
| #Forestomach | (50) | (48) | (47) |
| Papilloma, NOS | | 1 (2%) | |
| #Jejunum | (45) | (48) | (42) |
| Adenocarcinoma, NOS | | 1 (2%) | |

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|------------------------------|-----------------|----------|-----------|
| URINARY SYSTEM | | | |
| #Kidney | (50) | (50) | (49) |
| Tubular cell adenocarcinoma | | 2 (4%) | 3 (6%) |
| Lipoma | 1 (2%) | | |
| #Kidney/pelvis | (50) | (50) | (49) |
| Transitional cell papilloma | | 7 (14%) | 3 (6%) |
| Transitional cell carcinoma | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #Pituitary intermedia | (50) | (50) | (46) |
| Adenoma, NOS | | 1 (2%) | 1 (2%) |
| #Anterior pituitary | (50) | (50) | (46) |
| Adenoma, NOS | 13 (26%) | 10 (20%) | 7 (15%) |
| #Pituitary posterior | (50) | (50) | (46) |
| Pheochromocytoma, metastatic | | 1 (2%) | |
| #Adrenal medulla | (50) | (50) | (49) |
| Pheochromocytoma | 12 (24%) | 14 (28%) | 18 (37%) |
| Pheochromocytoma, malignant | | 4 (8%) | |
| #Thyroid | (49) | (50) | (49) |
| Follicular cell adenoma | | | 2 (4%) |
| Follicular cell carcinoma | | 2 (4%) | 1 (2%) |
| C-cell adenoma | 3 (6%) | | 1 (2%) |
| C-cell carcinoma | 1 (2%) | 4 (8%) | 4 (8%) |
| #Parathyroid | (39) | (37) | (42) |
| Adenoma, NOS | | | 1 (2%) |
| #Pancreatic islets | (49) | (49) | (49) |
| Islet cell adenoma | 4 (8%) | 3 (6%) | 1 (2%) |
| Islet cell carcinoma | 2 (4%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Fibroadenoma | 1 (2%) | 2 (4%) | |
| *Preputial gland | (50) | (50) | (50) |
| Carcinoma, NOS | 2 (4%) | | |
| Adenoma, NOS | 1 (2%) | 2 (4%) | |
| #Prostate | (47) | (49) | (47) |
| Adenoma, NOS | 2 (4%) | 3 (6%) | 1 (2%) |
| #Testis | (50) | (50) | (49) |
| Interstitial cell tumor | 41 (82%) | 39 (78%) | 39 (80%) |
| *Epididymis | (50) | (50) | (50) |
| Mesothelioma, NOS | 1 (2%) | | |
| *Scrotum | (50) | (50) | (50) |
| Mesothelioma, NOS | | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #Brain | (50) | (50) | (50) |
| Astrocytoma | 2 (4%) | 1 (2%) | |
| #Cerebral cortex | (50) | (50) | (50) |
| Astrocytoma | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *Ear canal | (50) | (50) | (50) |
| Papilloma, NOS | | | 1 (2%) |
| *Zymbal gland | (50) | (50) | (50) |
| Carcinoma, NOS | | 1 (2%) | |
| Squamous cell carcinoma | | | 1 (2%) |
| Adenoma, NOS | 1 (2%) | | |

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|--|-----------------|----------|-----------|
| MUSCULOSKELETAL SYSTEM | | | |
| *Skull | (50) | (50) | (50) |
| Osteoma | 1 (2%) | | |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Pheochromocytoma, metastatic | | 1 (2%) | |
| *Mesentery | (50) | (50) | (50) |
| Lipoma | | 2 (4%) | |
| *Tunica vaginalis | (50) | (50) | (50) |
| Mesothelioma, NOS | | 4 (8%) | 6 (12%) |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (50) |
| Adenocarcinoma, NOS, metastatic | 1 (2%) | | |
| Mesothelioma, NOS | 1 (2%) | 1 (2%) | |
| Mesothelioma, malignant | 1 (2%) | | |
| Tail | | | |
| Squamous cell papilloma | 1 | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 18 | 24 | 27 |
| Moribund sacrifice | 5 | 9 | 18 |
| Terminal sacrifice | 26 | 17 | 4 |
| Dosing accident | 1 | | 1 |
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 46 | 45 | 43 |
| Total primary tumors | 117 | 133 | 118 |
| Total animals with benign tumors | 45 | 44 | 43 |
| Total benign tumors | 93 | 95 | 79 |
| Total animals with malignant tumors | 18 | 23 | 28 |
| Total malignant tumors | 21 | 29 | 31 |
| Total animals with secondary tumors## | 1 | 1 | |
| Total secondary tumors | 1 | 4 | |
| Total animals with tumors uncertain--benign or malignant | 3 | 7 | 7 |
| Total uncertain tumors | 3 | 9 | 8 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.

** Primary tumors: all tumors except secondary tumors

Number of animals examined microscopically at this site

Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: VEHICLE CONTROL
(Continued)

| ANIMAL NUMBER | 0 | | | | | | | | | | | | | | | | | | | | TOTAL TISSUES TUMORS | | |
|---------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------------------|---|-----|
| | 7 0 2 4 5 9 0 1 2 3 4 5 6 8 9 0 1 2 3 9 0 2 4 4 9 0 | | | | | | | | | | | | | | | | | | | | | | |
| WEEKS ON STUDY | 1 | | | | | | | | | | | | | | | | | | | | | | |
| | 5 | | | | | | | | | | | | | | | | | | | | | | |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | X | 2 |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Neoplasm, benign, NOS | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | X | 4 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Nasal cavity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | 1 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 42 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Neuilemoma | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Neuilemoma | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Neoplastic nodule | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Acinar cell adenoma | | | | | | | | | | | | | | | | | | | | | | X | 2 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lipoma | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | X | X | | | | | | | | | | | | | | | | | | | | | 13 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | | | | | | | | | | | | | | | | | | | | | | | 12 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | 3 |
| C-cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 39 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Islet cell adenoma | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Islet cell carcinoma | X | | | | | | | | | | | | | | | | | | | | | | 4 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | *50 |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Interstitial cell tumor | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | 41 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Preputial/clitoral gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Carcinoma, NOS | X | | | | | | | | | | | | | | | | | | | | | | 2 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Epididymis | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | | 1 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | X | 2 |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | | | | |
| Zymbal gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | 1 |
| MUSCULOSKELETAL SYSTEM | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Osteoma | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | *50 |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Mesothelioma, malignant | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia, mononuclear cell | | | | | | | | | | | | | | | | | | | | | | X | 10 |
| Tail | | | | | | | | | | | | | | | | | | | | | | | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | X | 1 |

* Animals necropsied

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|--|-----------------|-------------|-------------|
| Skin: Keratoacanthoma | | | |
| Overall Rates (a) | 0/50 (0%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted Rates (b) | 0.0% | 13.2% | 0.0% |
| Terminal Rates (c) | 0/27 (0%) | 1/17 (6%) | 0/4 (0%) |
| Week of First Observation | | 88 | |
| Life Table Tests (d) | P=0.322 | P=0.065 | (e) |
| Incidental Tumor Tests (d) | P=0.637 | P=0.129 | (e) |
| Cochran-Armitage Trend Test (d) | P=0.640 | | |
| Fisher Exact Test (d) | | P=0.121 | (e) |
| Subcutaneous Tissue: Fibroma | | | |
| Overall Rates (a) | 4/50 (8%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted Rates (b) | 13.5% | 16.4% | 8.3% |
| Terminal Rates (c) | 3/27 (11%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | 91 | 100 | 90 |
| Life Table Tests (d) | P=0.497 | P=0.565 | P=0.664 |
| Incidental Tumor Tests (d) | P=0.283N | P=0.622N | P=0.189N |
| Cochran-Armitage Trend Test (d) | P=0.133N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.181N |
| Subcutaneous Tissue: Fibroma or Sarcoma | | | |
| Overall Rates (a) | 5/50 (10%) | 4/50 (8%) | 1/50 (2%) |
| Adjusted Rates (b) | 17.1% | 20.6% | 8.3% |
| Terminal Rates (c) | 4/27 (15%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | 91 | 99 | 90 |
| Life Table Tests (d) | P=0.523 | P=0.491 | P=0.707 |
| Incidental Tumor Tests (d) | P=0.244N | P=0.626 | P=0.164N |
| Cochran-Armitage Trend Test (d) | P=0.080N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.102N |
| Hematopoietic System: Mononuclear Cell Leukemia | | | |
| Overall Rates (a) | 10/50 (20%) | 11/50 (22%) | 17/50 (34%) |
| Adjusted Rates (b) | 30.0% | 38.5% | 100.0% |
| Terminal Rates (c) | 4/27 (15%) | 4/17 (24%) | 4/4 (100%) |
| Week of First Observation | 91 | 63 | 76 |
| Life Table Tests (d) | P<0.001 | P=0.188 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.048 | P=0.493 | P=0.032 |
| Cochran-Armitage Trend Test (d) | P=0.068 | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.088 |
| Liver: Neoplastic Nodule | | | |
| Overall Rates (a) | 1/50 (2%) | 4/49 (8%) | 1/49 (2%) |
| Adjusted Rates (b) | 3.7% | 20.2% | 25.0% |
| Terminal Rates (c) | 1/27 (4%) | 3/17 (18%) | 1/4 (25%) |
| Week of First Observation | 105 | 82 | 105 |
| Life Table Tests (d) | P=0.104 | P=0.079 | P=0.302 |
| Incidental Tumor Tests (d) | P=0.188 | P=0.122 | P=0.302 |
| Cochran-Armitage Trend Test (d) | P=0.593 | | |
| Fisher Exact Test (d) | | P=0.175 | P=0.748 |
| Kidney: Transitional Cell Papilloma | | | |
| Overall Rates (a) | 0/50 (0%) | 7/50 (14%) | 3/49 (6%) |
| Adjusted Rates (b) | 0.0% | 38.5% | 17.5% |
| Terminal Rates (c) | 0/27 (0%) | 6/17 (35%) | 0/4 (0%) |
| Week of First Observation | | 99 | 85 |
| Life Table Tests (d) | P=0.001 | P<0.001 | P=0.031 |
| Incidental Tumor Tests (d) | P=0.014 | P=0.001 | P=0.301 |
| Cochran-Armitage Trend Test (d) | P=0.151 | | |
| Fisher Exact Test (d) | | P=0.006 | P=0.117 |

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|--|-----------------|-------------|-------------|
| Kidney: Transitional Cell Papilloma or Carcinoma | | | |
| Overall Rates (a) | 0/50 (0%) | 8/50 (16%) | 3/49 (6%) |
| Adjusted Rates (b) | 0.0% | 44.1% | 17.5% |
| Terminal Rates (c) | 0/27 (0%) | 7/17 (41%) | 0/4 (0%) |
| Week of First Observation | | 99 | 85 |
| Life Table Tests (d) | P<0.001 | P<0.001 | P=0.031 |
| Incidental Tumor Tests (d) | P=0.010 | P<0.001 | P=0.301 |
| Cochran-Armitage Trend Test (d) | P=0.161 | | |
| Fisher Exact Test (d) | | P=0.003 | P=0.117 |
| Kidney: Tubular Cell Adenocarcinoma | | | |
| Overall Rates (a) | 0/50 (0%) | 2/50 (4%) | 3/49 (6%) |
| Adjusted Rates (b) | 0.0% | 9.2% | 19.4% |
| Terminal Rates (c) | 0/27 (0%) | 1/17 (6%) | 0/4 (0%) |
| Week of First Observation | | 88 | 77 |
| Life Table Tests (d) | P=0.014 | P=0.160 | P=0.043 |
| Incidental Tumor Tests (d) | P=0.091 | P=0.288 | P=0.167 |
| Cochran-Armitage Trend Test (d) | P=0.079 | | |
| Fisher Exact Test (d) | | P=0.247 | P=0.117 |
| Pituitary Gland: Adenoma | | | |
| Overall Rates (a) | 13/50 (26%) | 10/50 (20%) | 7/46 (15%) |
| Adjusted Rates (b) | 39.6% | 38.5% | 46.9% |
| Terminal Rates (c) | 8/27 (30%) | 3/17 (18%) | 1/4 (25%) |
| Week of First Observation | 96 | 63 | 65 |
| Life Table Tests (d) | P=0.126 | P=0.426 | P=0.101 |
| Incidental Tumor Tests (d) | P=0.319N | P=0.539N | P=0.516N |
| Cochran-Armitage Trend Test (d) | P=0.119N | | |
| Fisher Exact Test (d) | | P=0.318N | P=0.148N |
| Adrenal Gland: Pheochromocytoma | | | |
| Overall Rates (a) | 12/50 (24%) | 14/50 (28%) | 18/49 (37%) |
| Adjusted Rates (b) | 35.5% | 57.8% | 91.3% |
| Terminal Rates (c) | 7/27 (26%) | 8/17 (47%) | 3/4 (75%) |
| Week of First Observation | 55 | 70 | 65 |
| Life Table Tests (d) | P<0.001 | P=0.092 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.022 | P=0.284 | P=0.071 |
| Cochran-Armitage Trend Test (d) | P=0.101 | | |
| Fisher Exact Test (d) | | P=0.410 | P=0.123 |
| Adrenal Gland: Malignant Pheochromocytoma | | | |
| Overall Rates (a) | 0/50 (0%) | 4/50 (8%) | 0/49 (0%) |
| Adjusted Rates (b) | 0.0% | 18.5% | 0.0% |
| Terminal Rates (c) | 0/27 (0%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | | 86 | |
| Life Table Tests (d) | P=0.236 | P=0.026 | (e) |
| Incidental Tumor Tests (d) | P=0.486 | P=0.052 | (e) |
| Cochran-Armitage Trend Test (d) | P=0.616 | | |
| Fisher Exact Test (d) | | P=0.059 | (e) |
| Adrenal Gland: Pheochromocytoma or Malignant Pheochromocytoma | | | |
| Overall Rates (a) | 12/50 (24%) | 18/50 (36%) | 18/49 (37%) |
| Adjusted Rates (b) | 35.5% | 69.7% | 91.3% |
| Terminal Rates (c) | 7/27 (26%) | 10/17 (59%) | 3/4 (75%) |
| Week of First Observation | 55 | 70 | 65 |
| Life Table Tests (d) | P<0.001 | P=0.012 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.017 | P=0.069 | P=0.071 |
| Cochran-Armitage Trend Test (d) | P=0.105 | | |
| Fisher Exact Test (d) | | P=0.138 | P=0.123 |

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|--|-----------------|------------|-------------|
| Thyroid Gland: Follicular Cell Adenoma or Carcinoma | | | |
| Overall Rates (a) | 0/49 (0%) | 2/50 (4%) | 3/49 (6%) |
| Adjusted Rates (b) | 0.0% | 11.8% | 22.9% |
| Terminal Rates (c) | 0/27 (0%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | | 105 | 88 |
| Life Table Tests (d) | P=0.003 | P=0.143 | P=0.014 |
| Incidental Tumor Tests (d) | P=0.050 | P=0.143 | P=0.301 |
| Cochran-Armitage Trend Test (d) | P=0.081 | | |
| Fisher Exact Test (d) | | P=0.253 | P=0.121 |
| Thyroid Gland: C-Cell Adenoma | | | |
| Overall Rates (a) | 3/49 (6%) | 0/50 (0%) | 1/49 (2%) |
| Adjusted Rates (b) | 11.1% | 0.0% | 6.7% |
| Terminal Rates (c) | 3/27 (11%) | 0/17 (0%) | 0/4 (0%) |
| Week of First Observation | 105 | | 89 |
| Life Table Tests (d) | P=0.567N | P=0.212N | P=0.596 |
| Incidental Tumor Tests (d) | P=0.317N | P=0.212N | P=0.643N |
| Cochran-Armitage Trend Test (d) | P=0.175N | | |
| Fisher Exact Test (d) | | P=0.117N | P=0.309N |
| Thyroid Gland: C-Cell Carcinoma | | | |
| Overall Rates (a) | 1/49 (2%) | 4/50 (8%) | 4/49 (8%) |
| Adjusted Rates (b) | 3.7% | 20.6% | 54.3% |
| Terminal Rates (c) | 1/27 (4%) | 3/17 (18%) | 2/4 (50%) |
| Week of First Observation | 105 | 88 | 79 |
| Life Table Tests (d) | P=0.002 | P=0.075 | P=0.004 |
| Incidental Tumor Tests (d)* | P=0.022 | P=0.122 | P=0.030 |
| Cochran-Armitage Trend Test (d) | P=0.145 | | |
| Fisher Exact Test (d) | | P=0.187 | P=0.181 |
| Thyroid Gland: C-Cell Adenoma or Carcinoma | | | |
| Overall Rates (a) | 4/49 (8%) | 4/50 (8%) | 5/49 (10%) |
| Adjusted Rates (b) | 14.8% | 20.6% | 57.3% |
| Terminal Rates (c) | 4/27 (15%) | 3/17 (18%) | 2/4 (50%) |
| Week of First Observation | 105 | 88 | 79 |
| Life Table Tests (d) | P=0.008 | P=0.387 | P=0.008 |
| Incidental Tumor Tests (d) | P=0.112 | P=0.486 | P=0.118 |
| Cochran-Armitage Trend Test (d) | P=0.429 | | |
| Fisher Exact Test (d) | | P=0.631N | P=0.500 |
| Pancreatic Islets: Islet Cell Adenoma | | | |
| Overall Rates (a) | 4/49 (8%) | 3/49 (6%) | 1/49 (2%) |
| Adjusted Rates (b) | 12.6% | 7.3% | 12.5% |
| Terminal Rates (c) | 2/27 (7%) | 0/17 (0%) | 0/4 (0%) |
| Week of First Observation | 91 | 58 | 100 |
| Life Table Tests (d) | P=0.434N | P=0.647N | P=0.678 |
| Incidental Tumor Tests (d) | P=0.112N | P=0.400N | P=0.289N |
| Cochran-Armitage Trend Test (d) | P=0.133N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.181N |
| Pancreatic Islets: Islet Cell Adenoma or Carcinoma | | | |
| Overall Rates (a) | 6/49 (12%) | 3/49 (6%) | 1/49 (2%) |
| Adjusted Rates (b) | 18.8% | 7.3% | 12.5% |
| Terminal Rates (c) | 3/27 (11%) | 0/17 (0%) | 0/4 (0%) |
| Week of First Observation | 91 | 58 | 100 |
| Life Table Tests (d) | P=0.259N | P=0.425N | P=0.606N |
| Incidental Tumor Tests (d) | P=0.042N | P=0.203N | P=0.153N |
| Cochran-Armitage Trend Test (d) | P=0.036N | | |
| Fisher Exact Test (d) | | P=0.243N | P=0.056N |

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|--|-----------------|-------------|-------------|
| Preputial Gland: Adenoma or Carcinoma | | | |
| Overall Rates (a) | 3/50 (6%) | 2/50 (4%) | 0/50 (0%) |
| Adjusted Rates (b) | 9.4% | 11.8% | 0.0% |
| Terminal Rates (c) | 2/27 (7%) | 2/17 (12%) | 0/4 (0%) |
| Week of First Observation | 57 | 105 | |
| Life Table Tests (d) | P=0.350N | P=0.669N | P=0.356N |
| Incidental Tumor Tests (d) | P=0.293N | P=0.622N | P=0.261N |
| Cochran-Armitage Trend Test (d) | P=0.082N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.121N |
| Prostate: Adenoma | | | |
| Overall Rates (a) | 2/47 (4%) | 3/49 (6%) | 1/47 (2%) |
| Adjusted Rates (b) | 7.4% | 13.9% | 4.2% |
| Terminal Rates (c) | 2/27 (7%) | 1/17 (6%) | 0/4 (0%) |
| Week of First Observation | 105 | 82 | 85 |
| Life Table Tests (d) | P=0.360 | P=0.319 | P=0.585 |
| Incidental Tumor Tests (d) | P=0.465N | P=0.472 | P=0.730N |
| Cochran-Armitage Trend Test (d) | P=0.399N | | |
| Fisher Exact Test (d) | | P=0.520 | P=0.500N |
| Testis: Interstitial Cell Tumor | | | |
| Overall Rates (a) | 41/50 (82%) | 39/50 (78%) | 39/49 (80%) |
| Adjusted Rates (b) | 100.0% | 97.4% | 100.0% |
| Terminal Rates (c) | 27/27 (100%) | 16/17 (94%) | 4/4 (100%) |
| Week of First Observation | 58 | 49 | 65 |
| Life Table Tests (d) | P<0.001 | P=0.028 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.283 | P=0.512 | P=0.320 |
| Cochran-Armitage Trend Test (d) | P=0.430N | | |
| Fisher Exact Test (d) | | P=0.402N | P=0.480N |
| Tunica Vaginalis: Mesothelioma | | | |
| Overall Rates (a) | 0/50 (0%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted Rates (b) | 0.0% | 20.2% | 34.3% |
| Terminal Rates (c) | 0/27 (0%) | 3/17 (18%) | 0/4 (0%) |
| Week of First Observation | | 82 | 68 |
| Life Table Tests (d) | P<0.001 | P=0.025 | P=0.002 |
| Incidental Tumor Tests (d) | P=0.007 | P=0.044 | P=0.046 |
| Cochran-Armitage Trend Test (d) | P=0.014 | | |
| Fisher Exact Test (d) | | P=0.059 | P=0.013 |
| All Sites: Mesothelioma | | | |
| Overall Rates (a) | (f) 2/50 (4%) | 5/50 (10%) | 6/50 (12%) |
| Adjusted Rates (b) | 6.4% | 25.9% | 34.3% |
| Terminal Rates (c) | 1/27 (4%) | 4/17 (24%) | 0/4 (0%) |
| Week of First Observation | 96 | 82 | 68 |
| Life Table Tests (d) | P=0.002 | P=0.083 | P=0.009 |
| Incidental Tumor Tests (d) | P=0.034 | P=0.130 | P=0.141 |
| Cochran-Armitage Trend Test (d) | P=0.107 | | |
| Fisher Exact Test (d) | | P=0.218 | P=0.134 |

(a) Number of tumor-bearing animals/number of animals examined at the site

(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality

(c) Observed tumor incidence at terminal kill

(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

(e) No P value is reported because no tumors were observed in the 1,000 mg/kg and vehicle control groups.

(f) Includes one malignant mesothelioma

TABLE A4a. HISTORICAL INCIDENCE OF RENAL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| | No. Examined | No. of Tumors | Diagnosis |
|---|--------------|---------------|-----------------------------|
| Transitional Cell | | | |
| Historical Incidence at Litton Bionetics, Inc. | | | |
| No renal transitional cell tumors have been observed in 450 corn oil vehicle control male rats. | | | |
| Overall Historical Incidence | 1,448 | 1 (<0.1%) | Transitional cell papilloma |
| Tubular Cell | | | |
| Historical Incidence at Litton Bionetics, Inc. | | | |
| | 450 | (b) 2 | Tubular cell adenoma |
| | | (c) 1 | Adenocarcinoma, NOS |
| | Total | 3 (0.7%) | |
| Overall Historical Incidence | | 3 | Tubular cell adenoma |
| | | 2 | Adenocarcinoma, NOS |
| | | 3 | Tubular cell adenocarcinoma |
| | Total | 8 (0.6%) | |

(a) Data as of August 30, 1985, for studies of at least 104 weeks. No more than one tumor was observed in any vehicle control group.

(b) Observed in the dimethylvinyl chloride and 4-vinylcyclohexene studies

(c) Observed in the 2,4-toluene diisocyanate study

TABLE A4b. HISTORICAL INCIDENCE OF LEUKEMIA IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls |
|---|-------------------------------|
| Historical Incidence at Litton Bionetics, Inc. | |
| Diallyl phthalate | 13/50 |
| Dimethyl morpholinophosphoramidate | 14/50 |
| Tris(2-ethylhexyl)phosphate | 2/50 |
| Dimethyl hydrogen phosphite | 9/50 |
| Dimethylvinyl chloride | 3/50 |
| 3-Chloro-2-methylpropene | 9/50 |
| 4-Vinylcyclohexene | 14/50 |
| Dimethyl methylphosphonate | 10/50 |
| 2,4-Toluene diisocyanate | 11/50 |
| TOTAL | 85/450 (18.9%) |
| SD (b) | 8.78% |
| Range (c) | |
| High | 14/50 |
| Low | 2/50 |
| Overall Historical Incidence at All Laboratories | |
| TOTAL | 202/1,450 (13.9%) |
| SD (b) | 7.55% |
| Range (c) | |
| High | 14/50 |
| Low | 1/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks

(b) Standard deviation

(c) Range and SD are presented for groups of 35 or more animals.

TABLE A4c. HISTORICAL INCIDENCE OF ADRENAL GLAND TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls | | |
|---|-------------------------------|----------------------------|--|
| | Pheochromocytoma | Malignant Pheochromocytoma | Pheochromocytoma or Malignant Pheochromocytoma |
| Historical Incidence at Litton Bionetics, Inc. | | | |
| Diallyl phthalate | 13/50 | 0/50 | 13/50 |
| Dimethyl morpholinophosphoramidate | 9/49 | 0/49 | 9/49 |
| Tris(2-ethylhexyl)phosphate | 2/50 | 0/50 | 2/50 |
| Dimethyl hydrogen phosphite | 6/50 | 0/50 | 6/50 |
| Dimethylvinyl chloride | 11/50 | 2/50 | 13/50 |
| 3-Chloro-2-methylpropene | 14/50 | 0/50 | 14/50 |
| 4-Vinylcyclohexene | 17/50 | 0/50 | 17/50 |
| Dimethyl methylphosphonate | 12/50 | 0/50 | 12/50 |
| 2,4-Toluene diisocyanate | 12/50 | 0/50 | 12/50 |
| TOTAL | 96/449 (21.4%) | 2/449 (0.4%) | 98/449 (21.8%) |
| SD (b) | 8.93% | 1.33% | 9.06% |
| Range (c) | | | |
| High | 17/50 | 2/50 | 17/50 |
| Low | 2/50 | 0/50 | 2/50 |
| Overall Historical Incidence | | | |
| TOTAL | 338/1,442 (23.4%) | 13/1,442 (0.9%) | 347/1,442 (24.1%) |
| SD (b) | 8.72% | 1.27% | 8.66% |
| Range (c) | | | |
| High | 20/49 | 2/50 | 20/49 |
| Low | 2/50 | 0/50 | 2/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks

(b) Standard deviation

(c) Range and SD are presented for groups of 35 or more animals.

TABLE A4d. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls | | |
|---|-------------------------------|-----------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Litton Bionetics, Inc. | | | |
| Diallyl phthalate | 0/49 | 0/49 | 0/49 |
| Dimethyl morpholinophosphoramidate | 2/50 | 0/50 | 2/50 |
| Tris(2-ethylhexyl)phosphate | 0/46 | 1/46 | 1/46 |
| Dimethyl hydrogen phosphite | 0/50 | 2/50 | 2/50 |
| Dimethylvinyl chloride | 1/50 | 0/50 | 1/50 |
| 3-Chloro-2-methylpropene | 0/49 | 0/49 | 0/49 |
| 4-Vinylcyclohexene | 0/48 | 0/48 | 0/48 |
| Dimethyl methylphosphonate | 0/49 | 0/49 | 0/49 |
| 2,4-Toluene diisocyanate | 0/46 | 0/46 | 0/46 |
| TOTAL | 3/437 (0.7%) | 3/437 (0.7%) | 6/437 (1.4%) |
| SD (b) | 1.41% | 1.44% | 1.74% |
| Range (c) | | | |
| High | 2/50 | 2/50 | 2/50 |
| Low | 0/50 | 0/50 | 0/49 |
| Overall Historical Incidence | | | |
| TOTAL | (d) 15/1,417 (1.1%) | 20/1,417 (1.4%) | (d) 35/1,417 (2.5%) |
| SD (b) | 1.92% | 1.95% | 2.66% |
| Range (c) | | | |
| High | 4/49 | 4/50 | 5/50 |
| Low | 0/50 | 0/50 | 0/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks

(b) Standard deviation

(c) Range and SD are presented for groups of 35 or more animals.

(d) Includes one cystadenoma, NOS

TABLE A4e. HISTORICAL INCIDENCE OF MESOTHELIOMAS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls | |
|---|-------------------------------|--------------------|
| | Tunica Vaginalis | All Sites Combined |
| Historical Incidence at Litton Bionetics, Inc. | | |
| Diallyl phthalate | 0/50 | 1/50 |
| Dimethyl morpholinophosphoramidate | 0/50 | 1/50 |
| Tris(2-ethylhexyl)phosphate | 2/50 | 2/50 |
| Dimethyl hydrogen phosphite | 0/50 | 6/50 |
| Dimethylvinyl chloride | 0/50 | 2/50 |
| 3-Chloro-2-methylpropene | 1/50 | 1/50 |
| 4-Vinylcyclohexene | 2/50 | 3/50 |
| Dimethyl methylphosphonate | 0/50 | 2/50 |
| 2,4-Toluene diisocyanate | 0/50 | 2/50 |
| TOTAL | 5/450 (1.1%) | 20/450 (4.4%) |
| SD (b) | 1.76% | 3.13% |
| Range (c) | | |
| High | 2/50 | 6/50 |
| Low | 0/50 | 1/50 |
| Overall Historical Incidence | | |
| TOTAL | 24/1,450 (1.7%) | 55/1,450 (3.8%) |
| SD (b) | 2.27% | 2.74% |
| Range (c) | | |
| High | 3/50 | 6/50 |
| Low | 0/50 | 0/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks; includes mesothelioma, NOS, benign and malignant.

(b) Standard deviation

(c) Range and SD are presented for groups of 35 or more animals.

TABLE A4F. HISTORICAL INCIDENCE OF THYROID GLAND C-CELL TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls | | |
|---|-------------------------------|------------------------|--------------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Litton Bionetics, Inc. | | | |
| Diallyl phthalate | 2/49 | 0/49 | 2/49 |
| Dimethyl morpholinophosphoramidate | 4/50 | 0/50 | 4/50 |
| Tris(2-ethylhexyl)phosphate | 4/46 | 2/46 | 6/46 |
| Dimethyl hydrogen phosphite | 2/50 | 2/50 | 4/50 |
| Dimethylvinyl chloride | 3/50 | 2/50 | 5/50 |
| 3-Chloro-2-methylpropene | 3/49 | 4/49 | 7/49 |
| 4-Vinylcyclohexene | 4/48 | 0/48 | 4/48 |
| Dimethyl methylphosphonate | 3/49 | 1/49 | 4/49 |
| 2,4-Toluene diisocyanate | 2/46 | 1/46 | 3/46 |
| TOTAL | 27/437 (6.2%) | 12/437 (2.7%) | 39/437 (8.9%) |
| SD (b) | 1.83% | 2.70% | 3.14% |
| Range (c) | | | |
| High | 4/46 | 4/49 | 7/49 |
| Low | 2/50 | 0/50 | 2/49 |
| Overall Historical Incidence | | | |
| TOTAL | 125/1,417 (8.8%) | 59/1,417 (4.2%) | 181/1,417 (12.8%) |
| SD (b) | 5.55% | 3.24% | 6.36% |
| Range (c) | | | |
| High | 10/49 | 6/50 | 12/49 |
| Low | 0/50 | 0/50 | 2/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks

(b) Standard deviation

(c) Range and SD are presented for groups of 35 or more animals.

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Epidermal inclusion cyst | 1 (2%) | | |
| Hyperkeratosis | | | 2 (4%) |
| Acanthosis | | | 2 (4%) |
| RESPIRATORY SYSTEM | | | |
| *Nasal cavity | (50) | (50) | (50) |
| Foreign body, NOS | | | 1 (2%) |
| Inflammation, serous | | | 1 (2%) |
| Inflammation, suppurative | 7 (14%) | 9 (18%) | 7 (14%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, acute/chronic | | | 1 (2%) |
| Inflammation, chronic | | 1 (2%) | 1 (2%) |
| Inflammation, chronic focal | | | 1 (2%) |
| Reaction, foreign body | | 1 (2%) | 2 (4%) |
| Inflammation, pyogranulomatous | 3 (6%) | | 1 (2%) |
| Granuloma, pyogenic | | | 1 (2%) |
| Infection, fungal | | | 1 (2%) |
| Polyp, NOS | | 1 (2%) | |
| Metaplasia, squamous | | | 1 (2%) |
| #Trachea | (50) | (47) | (48) |
| Inflammation, chronic | | | 1 (2%) |
| Inflammation, chronic focal | | 1 (2%) | 2 (4%) |
| #Lung/bronchus | (50) | (50) | (50) |
| Lymphocytic inflammatory infiltrate | 1 (2%) | | 1 (2%) |
| Reaction, foreign body | | | 1 (2%) |
| #Lung | (50) | (50) | (50) |
| Congestion, NOS | 7 (14%) | 9 (18%) | 7 (14%) |
| Edema, NOS | | 1 (2%) | |
| Hemorrhage | 1 (2%) | | |
| Bronchopneumonia, NOS | | | 1 (2%) |
| Lymphocytic inflammatory infiltrate | 1 (2%) | 1 (2%) | 7 (14%) |
| Inflammation, interstitial | 2 (4%) | 1 (2%) | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) |
| Pneumonia, interstitial chronic | 1 (2%) | | |
| Inflammation, chronic focal | 7 (14%) | 2 (4%) | 4 (8%) |
| Inflammation, granulomatous focal | | 1 (2%) | |
| Granuloma, foreign body | | | 1 (2%) |
| Reaction, foreign body | | 1 (2%) | |
| Calcification, focal | | | 1 (2%) |
| Hyperplasia, adenomatous | | | 1 (2%) |
| Hyperplasia, alveolar epithelium | 2 (4%) | | 2 (4%) |
| #Lung/alveoli | (50) | (50) | (50) |
| Histiocytosis | 10 (20%) | 8 (16%) | 3 (6%) |
| HEMATOPOIETIC SYSTEM | | | |
| #Bone marrow | (49) | (49) | (49) |
| Hemorrhage | | 1 (2%) | |
| Inflammation, granulomatous focal | | | 1 (2%) |
| Fibrosis, focal | | 1 (2%) | |
| Necrosis, focal | | 1 (2%) | |
| Hypoplasia, NOS | | 1 (2%) | |
| Mastocytosis | | | 1 (2%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|---|-----------------|----------|-----------|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Spleen | (50) | (49) | (48) |
| Fibrosis | 1 (2%) | 2 (4%) | 1 (2%) |
| Fibrosis, focal | 1 (2%) | 1 (2%) | 1 (2%) |
| Necrosis, NOS | 1 (2%) | 1 (2%) | |
| Hemosiderosis | | 1 (2%) | |
| Depletion, lymphoid | | 1 (2%) | |
| Hyperplasia, stromal | 1 (2%) | 1 (2%) | |
| #Lymph node | (49) | (49) | (48) |
| Plasmacytosis | | 1 (2%) | |
| #Mandibular lymph node | (49) | (49) | (48) |
| Dilatation/sinus | | 1 (2%) | |
| Plasmacytosis | | | 1 (2%) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Mediastinal lymph node | (49) | (49) | (48) |
| Hemorrhage | | 1 (2%) | |
| Plasmacytosis | | 1 (2%) | |
| Mastocytosis | | 2 (4%) | |
| #Mesenteric lymph node | (49) | (49) | (48) |
| Congestion, NOS | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | |
| #Ileum | (45) | (48) | (42) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Adrenal | (50) | (50) | (49) |
| Hematopoiesis | 1 (2%) | | |
| #Thymus | (42) | (43) | (41) |
| Hemorrhage | | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| #Heart | (50) | (50) | (50) |
| Inflammation, chronic focal | | | 1 (2%) |
| #Heart/atrium | (50) | (50) | (50) |
| Thrombosis, NOS | 1 (2%) | 1 (2%) | 1 (2%) |
| Thrombus, organized | 1 (2%) | | |
| #Myocardium | (50) | (50) | (50) |
| Degeneration, NOS | 39 (78%) | 37 (74%) | 40 (80%) |
| Necrosis, focal | 1 (2%) | | 1 (2%) |
| *Blood vessel | (50) | (50) | (50) |
| Periarteritis | 1 (2%) | | |
| Calcification, NOS | 1 (2%) | | |
| *Pulmonary artery | (50) | (50) | (50) |
| Calcification, NOS | | | 1 (2%) |
| Calcification, focal | 9 (18%) | 9 (18%) | 7 (14%) |
| #Pancreas | (49) | (49) | (49) |
| Periarteritis | | 1 (2%) | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #Parotid gland | (49) | (50) | (49) |
| Inflammation, suppurative | | | 1 (2%) |
| Necrosis, focal | | 1 (2%) | |
| Atrophy, NOS | | | 1 (2%) |
| #Liver | (50) | (49) | (49) |
| Hernia, NOS | 2 (4%) | 2 (4%) | 4 (8%) |
| Dilatation/sinus | | 2 (4%) | |
| Congestion, NOS | | | 2 (4%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-----------------------------|-----------------|----------|-----------|
| DIGESTIVE SYSTEM | | | |
| #Liver (Continued) | (50) | (49) | (49) |
| Hemorrhage | | 2 (4%) | |
| Inflammation, chronic focal | 2 (4%) | 3 (6%) | 1 (2%) |
| Inflammation, granulomatous | | | 1 (2%) |
| Degeneration, lipid | 2 (4%) | | |
| Necrosis, focal | 2 (4%) | | 1 (2%) |
| Lipoidosis | 2 (4%) | 1 (2%) | |
| Basophilic cyto change | 2 (4%) | | |
| Ground glass cyto change | 4 (8%) | 3 (6%) | |
| Focal cellular change | 1 (2%) | | 1 (2%) |
| Eosinophilic cyto change | 1 (2%) | 4 (8%) | |
| Clear cell change | 5 (10%) | | |
| Regeneration, NOS | 4 (8%) | | |
| #Liver/caudate lobe | (50) | (49) | (49) |
| Infarct, NOS | | 1 (2%) | |
| #Liver/centrilobular | (50) | (49) | (49) |
| Dilatation/sinus | | 1 (2%) | |
| Necrosis, NOS | 2 (4%) | 1 (2%) | |
| Lipoidosis | 3 (6%) | | |
| #Liver/periportal | (50) | (49) | (49) |
| Inflammation, chronic | | | 1 (2%) |
| Degeneration, lipid | | 1 (2%) | 1 (2%) |
| Necrosis, NOS | | 1 (2%) | |
| Lipoidosis | 1 (2%) | 1 (2%) | |
| #Liver/hepatocytes | (50) | (49) | (49) |
| Hypertrophy, focal | | | 1 (2%) |
| Regeneration, NOS | | 1 (2%) | |
| #Bile duct | (50) | (49) | (49) |
| Retention of content | | 1 (2%) | |
| Cyst, NOS | | | 1 (2%) |
| Hyperplasia, NOS | 40 (80%) | 39 (80%) | 31 (63%) |
| #Pancreas | (49) | (49) | (49) |
| Inflammation, chronic focal | | | 1 (2%) |
| #Pancreatic duct | (49) | (49) | (49) |
| Hyperplasia, focal | | 1 (2%) | |
| Hyperplasia, cystic | 1 (2%) | | |
| #Pancreatic acinus | (49) | (49) | (49) |
| Necrosis, focal | | 1 (2%) | |
| Atrophy, NOS | 1 (2%) | 1 (2%) | 2 (4%) |
| Atrophy, focal | 6 (12%) | 9 (18%) | 4 (8%) |
| Hyperplasia, focal | 6 (12%) | 4 (8%) | 4 (8%) |
| #Stomach | (50) | (48) | (47) |
| Inflammation, acute/chronic | | | 1 (2%) |
| #Glandular stomach | (50) | (48) | (47) |
| Dilatation, NOS | | | 1 (2%) |
| Ulcer, NOS | 1 (2%) | | |
| Necrosis, focal | 1 (2%) | | |
| Calcification, focal | 1 (2%) | | |
| #Forestomach | (50) | (48) | (47) |
| Ulcer, NOS | | 1 (2%) | 2 (4%) |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, acute/chronic | | 1 (2%) | |
| Hyperplasia, epithelial | | 2 (4%) | 5 (11%) |
| Dysplasia, epithelial | | 1 (2%) | |
| #Duodenum | (45) | (48) | (42) |
| Ectopia | 1 (2%) | | |
| Inflammation, chronic | | | 1 (2%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| DIGESTIVE SYSTEM (Continued) | | | |
| #Duodenal mucosa | (45) | (48) | (42) |
| Inflammation, chronic | | 1 (2%) | |
| Fibrosis, focal | | | 1 (2%) |
| #Ileal mucosa | (45) | (48) | (42) |
| Inflammation, chronic focal | | | 1 (2%) |
| Calcification, NOS | 2 (4%) | | |
| #Colon | (47) | (47) | (42) |
| Parasitism | 5 (11%) | 2 (4%) | 2 (5%) |
| #Colonic mucosa | (47) | (47) | (42) |
| Calcification, NOS | 2 (4%) | | |
| #Cecum | (47) | (47) | (42) |
| Calcification, NOS | 1 (2%) | 3 (6%) | |
| *Rectum | (50) | (50) | (50) |
| Parasitism | 2 (4%) | 2 (4%) | |
| URINARY SYSTEM | | | |
| #Kidney | (50) | (50) | (49) |
| Hydronephrosis | 1 (2%) | | |
| Pyelonephritis, focal | 2 (4%) | 4 (8%) | 1 (2%) |
| Nephropathy | 36 (72%) | 43 (86%) | 40 (82%) |
| Calcification, focal | 1 (2%) | | 1 (2%) |
| Hyperplasia, tubular cell | | 1 (2%) | |
| #Kidney/cortex | (50) | (50) | (49) |
| Cyst, NOS | | 2 (4%) | |
| Abscess, NOS | | | 1 (2%) |
| Inflammation, chronic focal | | 1 (2%) | |
| Hyperplasia, tubular cell | | 8 (16%) | 9 (18%) |
| #Renal papilla | (50) | (50) | (49) |
| Congestion, NOS | | 1 (2%) | |
| Degeneration, NOS | | 1 (2%) | |
| Necrosis, NOS | 1 (2%) | | |
| Calcification, NOS | 12 (24%) | 41 (82%) | 36 (73%) |
| #Kidney/tubule | (50) | (50) | (49) |
| Pigmentation, NOS | 3 (6%) | 1 (2%) | |
| #Kidney/pelvis | (50) | (50) | (49) |
| Calcification, focal | 3 (6%) | | 3 (6%) |
| Hyperplasia, epithelial | | 23 (46%) | 21 (43%) |
| #Urinary bladder | (46) | (43) | (41) |
| Hemorrhage | | 1 (2%) | |
| Lymphocytic inflammatory infiltrate | | 1 (2%) | |
| Inflammation, chronic focal | | | 1 (2%) |
| Granuloma, NOS | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #Pituitary | (50) | (50) | (46) |
| Hemorrhage | 1 (2%) | 1 (2%) | |
| #Pituitary intermedia | (50) | (50) | (46) |
| Cyst, NOS | | 1 (2%) | |
| #Anterior pituitary | (50) | (50) | (46) |
| Cyst, NOS | 2 (4%) | 5 (10%) | 3 (7%) |
| Hyperplasia, focal | 16 (32%) | 14 (28%) | 16 (35%) |
| Angiectasis | 1 (2%) | 1 (2%) | 4 (9%) |
| #Pituitary posterior | (50) | (50) | (46) |
| Metaplasia, osseous | | 1 (2%) | |
| #Adrenal | (50) | (50) | (49) |
| Necrosis, NOS | | 1 (2%) | |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| ENDOCRINE SYSTEM (Continued) | | | |
| #Adrenal cortex | (50) | (50) | (49) |
| Degeneration, lipoid | 9 (18%) | 6 (12%) | 8 (16%) |
| Lipoidosis | 2 (4%) | 1 (2%) | |
| Cytoplasmic vacuolization | 1 (2%) | | |
| Hyperplasia, focal | 9 (18%) | 2 (4%) | 4 (8%) |
| #Adrenal medulla | (50) | (50) | (49) |
| Fibrosis, focal | | 1 (2%) | |
| Hyperplasia, focal | 12 (24%) | 8 (16%) | 10 (20%) |
| Angiectasis | | 1 (2%) | |
| #Thyroid | (49) | (50) | (49) |
| Ultimobranchial cyst | 1 (2%) | | |
| Hyperplasia, C-cell | 6 (12%) | 6 (12%) | 3 (6%) |
| Hyperplasia, follicular cell | | 1 (2%) | |
| #Parathyroid | (39) | (37) | (42) |
| Hyperplasia, focal | | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Dilatation/ducts | | 1 (2%) | |
| Galactocele | 1 (2%) | | |
| Lactation | 10 (20%) | 5 (10%) | 1 (2%) |
| *Mammary lobule | (50) | (50) | (50) |
| Hyperplasia, NOS | 1 (2%) | 1 (2%) | |
| *Preputial gland | (50) | (50) | (50) |
| Dilatation/ducts | | 1 (2%) | 3 (6%) |
| Abscess, NOS | | 1 (2%) | |
| Inflammation, acute/chronic | | 2 (4%) | |
| Inflammation, chronic focal | | 2 (4%) | |
| Hyperplasia, NOS | | 1 (2%) | |
| Hyperplasia, epithelial | | 1 (2%) | |
| #Prostate | (47) | (49) | (47) |
| Inflammation, suppurative | 1 (2%) | | |
| Abscess, NOS | | | 1 (2%) |
| Inflammation, chronic | | | 2 (4%) |
| Inflammation, chronic focal | 4 (9%) | 1 (2%) | 2 (4%) |
| Necrosis, focal | | 1 (2%) | |
| Hyperplasia, focal | 12 (26%) | 9 (18%) | 9 (19%) |
| #Testis | (50) | (50) | (49) |
| Atrophy, NOS | | 2 (4%) | 2 (4%) |
| Hyperplasia, interstitial cell | 5 (10%) | 3 (6%) | 3 (6%) |
| #Spermatid | (50) | (50) | (49) |
| Cytomegaly | 1 (2%) | | |
| *Epididymis | (50) | (50) | (50) |
| Inflammation, chronic | | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | | |
| Fibrosis, focal | | | 1 (2%) |
| Atrophy, NOS | | 1 (2%) | |
| Hyperplasia, mesothelial | | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #Brain | (50) | (50) | (50) |
| Hemorrhage | 2 (4%) | | |
| Infarct, NOS | 1 (2%) | | |
| #Cerebral cortex | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| #Medulla oblongata | (50) | (50) | (50) |
| Demyelination | | | 1 (2%) |

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-----------------------------------|-----------------|----------|-----------|
| SPECIAL SENSE ORGANS | | | |
| *Eye | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Cataract | 4 (8%) | | 2 (4%) |
| *Eye/sclera | (50) | (50) | (50) |
| Metaplasia, osseous | 3 (6%) | 4 (8%) | 5 (10%) |
| *Eye/retina | (50) | (50) | (50) |
| Atrophy, NOS | 4 (8%) | 1 (2%) | 4 (8%) |
| *Nasolacrimal duct | (50) | (50) | (50) |
| Inflammation, suppurative | | | 2 (4%) |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 8 (16%) |
| *Ear canal | (50) | (50) | (50) |
| Inflammation, suppurative | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *Sternum | (50) | (50) | (50) |
| Traumatic abnormality | 1 (2%) | | |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | |
| Abscess, NOS | | 1 (2%) | |
| *Abdominal cavity | (50) | (50) | (50) |
| Necrosis, fat | | 1 (2%) | 3 (6%) |
| *Mesentery | (50) | (50) | (50) |
| Necrosis, NOS | | 1 (2%) | |
| Necrosis, fat | 1 (2%) | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (50) |
| Congestion, NOS | 1 (2%) | | |
| Periorbital region | | | |
| Hemorrhage | 1 | 2 | |
| Inflammation, suppurative | | 1 | |
| Adipose tissue | | | |
| Necrosis, fat | 3 | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| No lesion reported | | | 1 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
 # Number of animals examined microscopically at this site

APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|---------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Fibroma | 3 (6%) | 3 (6%) | 2 (4%) |
| Fibrosarcoma | | | 1 (2%) |
| Myxosarcoma | | 1 (2%) | |
| Rhabdomyosarcoma | | | 1 (2%) |
| Osteosarcoma | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #Lung | (50) | (50) | (49) |
| Squamous cell carcinoma, metastatic | | | 1 (2%) |
| Alveolar/bronchiolar adenoma | 1 (2%) | | 1 (2%) |
| Alveolar/bronchiolar carcinoma | | | 1 (2%) |
| Fibrosarcoma, metastatic | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Malignant lymphoma, NOS | | | 1 (2%) |
| Leukemia, mononuclear cell | 10 (20%) | 9 (18%) | 12 (24%) |
| #Spleen | (50) | (50) | (49) |
| Leukemia, mononuclear cell | | | 1 (2%) |
| #Mandibular lymph node | (50) | (50) | (46) |
| Squamous cell carcinoma, metastatic | | | 1 (2%) |
| #Thymus | (44) | (49) | (46) |
| Malignant lymphoma, lymphocytic type | | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| None | | | |
| DIGESTIVE SYSTEM | | | |
| *Mouth | (50) | (50) | (50) |
| Squamous cell carcinoma | | | 1 (2%) |
| *Palate | (50) | (50) | (50) |
| Squamous cell papilloma | | | 2 (4%) |
| *Tongue | (50) | (50) | (50) |
| Squamous cell papilloma | 1 (2%) | | |
| Squamous cell carcinoma | | | 1 (2%) |
| #Salivary gland | (50) | (50) | (49) |
| Adenocarcinoma, NOS | | 1 (2%) | |
| Fibrosarcoma | | 1 (2%) | |
| #Liver | (50) | (50) | (49) |
| Neoplastic nodule | | 2 (4%) | |
| #Jejunum | (45) | (50) | (47) |
| Adenomatous polyp, NOS | | 1 (2%) | |
| *Rectum | (50) | (50) | (50) |
| Endometrial stromal sarcoma, invasive | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #Urinary bladder | (42) | (45) | (44) |
| Endometrial stromal sarcoma, invasive | | 1 (2%) | |

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------|-----------------|----------|-----------|
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (50) | (50) | (48) |
| Carcinoma, NOS | 1 (2%) | 1 (2%) | 2 (4%) |
| Adenoma, NOS | 17 (34%) | 21 (42%) | 10 (21%) |
| #Adrenal | (50) | (50) | (49) |
| Cortical adenoma | 1 (2%) | 1 (2%) | 2 (4%) |
| Cortical carcinoma | | | 1 (2%) |
| #Adrenal medulla | (50) | (50) | (49) |
| Pheochromocytoma | 5 (10%) | 5 (10%) | 1 (2%) |
| Pheochromocytoma, malignant | | 3 (6%) | |
| #Thyroid | (49) | (50) | (48) |
| Follicular cell adenoma | 1 (2%) | | 1 (2%) |
| Follicular cell carcinoma | | | 1 (2%) |
| C-cell adenoma | 2 (4%) | 3 (6%) | 1 (2%) |
| C-cell carcinoma | 3 (6%) | 4 (8%) | 2 (4%) |
| #Pancreatic islets | (49) | (50) | (49) |
| Islet cell adenoma | 1 (2%) | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Adenoma, NOS | 2 (4%) | 2 (4%) | 1 (2%) |
| Adenocarcinoma, NOS | 2 (4%) | 2 (4%) | |
| Fibroadenoma | 12 (24%) | 12 (24%) | 10 (20%) |
| *Clitoral gland | (50) | (50) | (50) |
| Carcinoma, NOS | 1 (2%) | 1 (2%) | |
| Adenoma, NOS | 4 (8%) | 2 (4%) | 2 (4%) |
| *Vagina | (50) | (50) | (50) |
| Sarcoma, NOS | | | 1 (2%) |
| #Uterus | (50) | (50) | (49) |
| Adenocarcinoma, NOS | | 2 (4%) | |
| Sarcoma, NOS | 1 (2%) | | |
| Leiomyosarcoma | | 2 (4%) | |
| Endometrial stromal polyp | 5 (10%) | 7 (14%) | 6 (12%) |
| Endometrial stromal sarcoma | | 2 (4%) | 1 (2%) |
| #Cervix uteri | (50) | (50) | (49) |
| Fibroma | 1 (2%) | | 1 (2%) |
| #Ovary | (50) | (50) | (49) |
| Sertoli cell tumor | | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| #Brain | (50) | (50) | (49) |
| Carcinoma, NOS, invasive | 1 (2%) | 1 (2%) | 2 (4%) |
| Granular cell tumor, NOS | | | 1 (2%) |
| Glioma, NOS | | 1 (2%) | |
| Astrocytoma | 1 (2%) | | |
| SPECIAL SENSE ORGANS | | | |
| *Ear | (50) | (50) | (50) |
| Fibrosarcoma | | 1 (2%) | |
| Neurilemoma | 1 (2%) | | 1 (2%) |
| *Zymbal gland | (50) | (50) | (50) |
| Carcinoma, NOS | | 1 (2%) | |
| Squamous cell carcinoma | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| None | | | |

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|--|-----------------|----------|-----------|
| BODY CAVITIES | | | |
| None | | | |
| ALL OTHER SYSTEMS | | | |
| Head | | | |
| Squamous cell papilloma | 1 | | |
| Lumbar region | | | |
| Chordoma | | | 1 |
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 10 | 5 | 13 |
| Moribund sacrifice | 10 | 13 | 14 |
| Terminal sacrifice | 30 | 32 | 23 |
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 40 | 42 | 40 |
| Total primary tumors | 78 | 93 | 72 |
| Total animals with benign tumors | 34 | 36 | 29 |
| Total benign tumors | 58 | 58 | 42 |
| Total animals with malignant tumors | 19 | 23 | 26 |
| Total malignant tumors | 20 | 33 | 29 |
| Total animals with secondary tumors## | 1 | 2 | 3 |
| Total secondary tumors | 1 | 4 | 4 |
| Total animals with tumors uncertain-- benign or malignant | | 2 | 1 |
| Total uncertain tumors | | 2 | 1 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.

** Primary tumors: all tumors except secondary tumors

Number of animals examined microscopically at this site

Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE
(Continued)

| ANIMAL NUMBER | WEEKS ON STUDY | | | | | | | | | | | | | | | | | | | | TOTAL TISSUES TUMORS |
|---------------------------------------|----------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------------------|
| | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibroma | | | | X | | | | | | | | | | | | | | | | | |
| Myxosarcoma | | | | | | | | | | | | | | | | | | | | X | |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibrosarcoma, metastatic | | | | | | | | | | | | | | | | | | | | X | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | | | | | | | | X |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | |
| Fibrosarcoma | | | | | | | | | | | | | | | | | | | | X | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Neoplastic nodule | | | | | | | | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenomatous polyp, NOS | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Rectum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endometrial stromal sarcoma, invasive | | | | | | | N | | N | | N | | | | | | | | | | N |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endometrial stromal sarcoma, invasive | | | | | | | | | | | | | | | | | | | | | - |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | X | |
| Adenoma, NOS | X | | | | X | X | | | X | | X | X | | X | X | | | | X | X | X |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Cortical adenoma | | | | | | | | | | | | | | | | | | | | | |
| Pheochromocytoma | | | | | | | X | | X | X | | | | | | | | | X | | |
| Pheochromocytoma, malignant | | | | | | | | | X | X | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | | | X | | | | | | | | | | | | | | |
| C-cell carcinoma | | | | | | | | | X | X | X | | | | | | | | | | X |
| Parathyroid | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | |
| Preputial/clitoral gland | X | X | | X | | X | | | | | | | | | | | | | | | X |
| Carcinoma, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | |
| Leiomyosarcoma | | | | | | | | | | | | | | | | | | | | | |
| Endometrial stromal polyp | X | | | | | | | | | | | | | | | | | | | | |
| Endometrial stromal sarcoma | | | | | | | | | | | | | | | | | | | | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sertoli cell tumor | | | | | | | | | | | | | | | | | | | | | |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS, invasive | | | | | | | | | | | | | | | | | | | | | X |
| Glioma, NOS | | | | | | | | | | | | | | | | | | | | | |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | | |
| Ear | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Fibrosarcoma | | | | | | | | | | | | | | | | | | | | | |
| Zymbal gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Leukemia, mononuclear cell | X | | | | | | | | | | | | | | | | | | | | X |

* Animals necropsied

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|--|-----------------|-------------|---------------|
| Subcutaneous Tissue: Fibroma | | | |
| Overall Rates (a) | 3/50 (6%) | 3/50 (6%) | (b) 2/50 (4%) |
| Adjusted Rates (c) | 10.0% | 9.1% | 8.3% |
| Terminal Rates (d) | 3/30 (10%) | 3/33 (9%) | 1/23 (4%) |
| Week of First Observation | 105 | 105 | 102 |
| Life Table Tests (e) | P = 0.533N | P = 0.620N | P = 0.627N |
| Incidental Tumor Tests (e) | P = 0.573N | P = 0.620N | P = 0.672 |
| Cochran-Armitage Trend Test (e) | P = 0.412N | | |
| Fisher Exact Test (e) | | P = 0.661 | P = 0.500N |
| Hematopoietic System: Mononuclear Cell Leukemia | | | |
| Overall Rates (a) | 10/50 (20%) | 9/50 (18%) | 13/50 (26%) |
| Adjusted Rates (c) | 27.8% | 22.8% | 45.0% |
| Terminal Rates (d) | 6/30 (20%) | 4/33 (12%) | 8/23 (35%) |
| Week of First Observation | 84 | 91 | 76 |
| Life Table Tests (e) | P = 0.083 | P = 0.448N | P = 0.098 |
| Incidental Tumor Tests (e) | P = 0.090 | P = 0.480 | P = 0.125 |
| Cochran-Armitage Trend Test (e) | P = 0.271 | | |
| Fisher Exact Test (e) | | P = 0.500N | P = 0.317 |
| Oral Cavity: Squamous Cell Papilloma or Carcinoma | | | |
| Overall Rates (a) | 1/50 (2%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted Rates (c) | 2.9% | 0.0% | 13.9% |
| Terminal Rates (d) | 0/30 (0%) | 0/33 (0%) | 2/23 (9%) |
| Week of First Observation | 102 | | 77 |
| Life Table Tests (e) | P = 0.045 | P = 0.494N | P = 0.104 |
| Incidental Tumor Tests (e) | P = 0.083 | P = 0.521N | P = 0.163 |
| Cochran-Armitage Trend Test (e) | P = 0.082 | | |
| Fisher Exact Test (e) | | P = 0.500N | P = 0.181 |
| Pituitary Gland: Adenoma | | | |
| Overall Rates (a) | 17/50 (34%) | 21/50 (42%) | 10/48 (21%) |
| Adjusted Rates (c) | 45.7% | 52.9% | 35.1% |
| Terminal Rates (d) | 11/30 (37%) | 15/33 (45%) | 6/23 (26%) |
| Week of First Observation | 92 | 65 | 77 |
| Life Table Tests (e) | P = 0.334N | P = 0.372 | P = 0.354N |
| Incidental Tumor Tests (e) | P = 0.257N | P = 0.299 | P = 0.306N |
| Cochran-Armitage Trend Test (e) | P = 0.103N | | |
| Fisher Exact Test (e) | | P = 0.268 | P = 0.109N |
| Pituitary Gland: Adenoma or Carcinoma | | | |
| Overall Rates (a) | 18/50 (36%) | 22/50 (44%) | 12/48 (25%) |
| Adjusted Rates (c) | 46.9% | 55.5% | 41.2% |
| Terminal Rates (d) | 11/30 (37%) | 16/33 (48%) | 7/23 (30%) |
| Week of First Observation | 86 | 65 | 77 |
| Life Table Tests (e) | P = 0.455N | P = 0.376 | P = 0.494N |
| Incidental Tumor Tests (e) | P = 0.362N | P = 0.261 | P = 0.420N |
| Cochran-Armitage Trend Test (e) | P = 0.155N | | |
| Fisher Exact Test (e) | | P = 0.270 | P = 0.168N |
| Adrenal Gland: Cortical Adenoma or Carcinoma | | | |
| Overall Rates (a) | 1/50 (2%) | 1/50 (2%) | 3/49 (6%) |
| Adjusted Rates (c) | 3.3% | 3.0% | 12.2% |
| Terminal Rates (d) | 1/30 (3%) | 1/33 (3%) | 2/23 (9%) |
| Week of First Observation | 105 | 105 | 97 |
| Life Table Tests (e) | P = 0.130 | P = 0.741N | P = 0.210 |
| Incidental Tumor Tests (e) | P = 0.102 | P = 0.741N | P = 0.152 |
| Cochran-Armitage Trend Test (e) | P = 0.196 | | |
| Fisher Exact Test (e) | | P = 0.753 | P = 0.301 |

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|--|-----------------|------------|-------------|
| Adrenal Gland: Pheochromocytoma | | | |
| Overall Rates (a) | 5/50 (10%) | 5/50 (10%) | 1/49 (2%) |
| Adjusted Rates (c) | 16.7% | 14.2% | 4.3% |
| Terminal Rates (d) | 5/30 (17%) | 4/33 (12%) | 1/23 (4%) |
| Week of First Observation | 105 | 92 | 105 |
| Life Table Tests (e) | P=0.152N | P=0.578N | P=0.169N |
| Incidental Tumor Tests (e) | P=0.143N | P=0.612 | P=0.169N |
| Cochran-Armitage Trend Test (e) | P=0.094N | | |
| Fisher Exact Test (e) | | P=0.630 | P=0.107N |
| Adrenal Gland: Malignant Pheochromocytoma | | | |
| Overall Rates (a) | 0/50 (0%) | 3/50 (6%) | 0/49 (0%) |
| Adjusted Rates (c) | 0.0% | 8.5% | 0.0% |
| Terminal Rates (d) | 0/30 (0%) | 2/33 (6%) | 0/23 (0%) |
| Week of First Observation | | 97 | |
| Life Table Tests (e) | P=0.568 | P=0.138 | (f) |
| Incidental Tumor Tests (e) | P=0.506 | P=0.128 | (f) |
| Cochran-Armitage Trend Test (e) | P=0.634 | | |
| Fisher Exact Test (e) | | P=0.121 | (f) |
| Adrenal Gland: Pheochromocytoma or Malignant Pheochromocytoma | | | |
| Overall Rates (a) | 5/50 (10%) | 7/50 (14%) | 1/49 (2%) |
| Adjusted Rates (c) | 16.7% | 19.2% | 4.3% |
| Terminal Rates (d) | 5/30 (17%) | 5/33 (15%) | 1/23 (4%) |
| Week of First Observation | 105 | 92 | 105 |
| Life Table Tests (e) | P=0.190N | P=0.438 | P=0.169N |
| Incidental Tumor Tests (e) | P=0.199N | P=0.356 | P=0.169N |
| Cochran-Armitage Trend Test (e) | P=0.112N | | |
| Fisher Exact Test (e) | | P=0.380 | P=0.107N |
| Thyroid Gland: C-Cell Adenoma | | | |
| Overall Rates (a) | 2/49 (4%) | 3/50 (6%) | 1/48 (2%) |
| Adjusted Rates (c) | 6.7% | 8.2% | 4.3% |
| Terminal Rates (d) | 2/30 (7%) | 2/33 (6%) | 1/23 (4%) |
| Week of First Observation | 105 | 91 | 105 |
| Life Table Tests (e) | P=0.514N | P=0.535 | P=0.593N |
| Incidental Tumor Tests (e) | P=0.481N | P=0.423 | P=0.593N |
| Cochran-Armitage Trend Test (e) | P=0.407N | | |
| Fisher Exact Test (e) | | P=0.510 | P=0.508N |
| Thyroid Gland: C-Cell Carcinoma | | | |
| Overall Rates (a) | 3/49 (6%) | 4/50 (8%) | 2/48 (4%) |
| Adjusted Rates (c) | 8.8% | 12.1% | 8.7% |
| Terminal Rates (d) | 2/30 (7%) | 4/33 (12%) | 2/23 (9%) |
| Week of First Observation | 92 | 105 | 105 |
| Life Table Tests (e) | P=0.555N | P=0.543 | P=0.642N |
| Incidental Tumor Tests (e) | P=0.526N | P=0.450 | P=0.596N |
| Cochran-Armitage Trend Test (e) | P=0.426N | | |
| Fisher Exact Test (e) | | P=0.511 | P=0.510N |
| Thyroid Gland: C-Cell Adenoma or Carcinoma | | | |
| Overall Rates (a) | 5/49 (10%) | 7/50 (14%) | 3/48 (6%) |
| Adjusted Rates (c) | 15.3% | 20.0% | 13.0% |
| Terminal Rates (d) | 4/30 (13%) | 6/33 (18%) | 3/23 (13%) |
| Week of First Observation | 92 | 91 | 105 |
| Life Table Tests (e) | P=0.482N | P=0.434 | P=0.527N |
| Incidental Tumor Tests (e) | P=0.440N | P=0.290 | P=0.489N |
| Cochran-Armitage Trend Test (e) | P=0.320N | | |
| Fisher Exact Test (e) | | P=0.394 | P=0.369N |

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---|-----------------|-------------|-------------|
| Mammary Gland: Fibroadenoma | | | |
| Overall Rates (a) | 12/50 (24%) | 12/50 (24%) | 10/50 (20%) |
| Adjusted Rates (c) | 36.8% | 35.1% | 35.9% |
| Terminal Rates (d) | 10/30 (33%) | 11/33 (33%) | 7/23 (30%) |
| Week of First Observation | 92 | 98 | 73 |
| Life Table Tests (e) | P=0.459 | P=0.497N | P=0.496 |
| Incidental Tumor Tests (e) | P=0.454N | P=0.568N | P=0.413N |
| Cochran-Armitage Trend Test (e) | P=0.360N | | |
| Fisher Exact Test (e) | | P=0.592 | P=0.405N |
| Mammary Gland: Adenoma or Fibroadenoma | | | |
| Overall Rates (a) | 14/50 (28%) | 14/50 (28%) | 11/50 (22%) |
| Adjusted Rates (c) | 41.2% | 41.0% | 39.9% |
| Terminal Rates (d) | 11/30 (37%) | 13/33 (39%) | 8/23 (35%) |
| Week of First Observation | 86 | 98 | 73 |
| Life Table Tests (e) | P=0.510 | P=0.486N | P=0.541 |
| Incidental Tumor Tests (e) | P=0.390N | P=0.578 | P=0.346N |
| Cochran-Armitage Trend Test (e) | P=0.284N | | |
| Fisher Exact Test (e) | | P=0.588 | P=0.322N |
| Mammary Gland: Adenoma or Adenocarcinoma | | | |
| Overall Rates (a) | 4/50 (8%) | 4/50 (8%) | 1/50 (2%) |
| Adjusted Rates (c) | 12.0% | 12.1% | 4.3% |
| Terminal Rates (d) | 3/30 (10%) | 4/33 (12%) | 1/23 (4%) |
| Week of First Observation | 86 | 105 | 105 |
| Life Table Tests (e) | P=0.227N | P=0.601N | P=0.287N |
| Incidental Tumor Tests (e) | P=0.212N | P=0.610 | P=0.249N |
| Cochran-Armitage Trend Test (e) | P=0.146N | | |
| Fisher Exact Test (e) | | P=0.643 | P=0.181N |
| Clitoral Gland: Adenoma | | | |
| Overall Rates (a) | 4/50 (8%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted Rates (c) | 11.7% | 6.1% | 7.9% |
| Terminal Rates (d) | 1/30 (3%) | 2/33 (6%) | 1/23 (4%) |
| Week of First Observation | 98 | 105 | 89 |
| Life Table Tests (e) | P=0.373N | P=0.308N | P=0.504N |
| Incidental Tumor Tests (e) | P=0.459N | P=0.338N | P=0.657N |
| Cochran-Armitage Trend Test (e) | P=0.252N | | |
| Fisher Exact Test (e) | | P=0.339N | P=0.339N |
| Clitoral Gland: Adenoma or Carcinoma | | | |
| Overall Rates (a) | 5/50 (10%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted Rates (c) | 14.8% | 9.1% | 7.9% |
| Terminal Rates (d) | 2/30 (7%) | 3/33 (9%) | 1/23 (4%) |
| Week of First Observation | 98 | 105 | 89 |
| Life Table Tests (e) | P=0.262N | P=0.318N | P=0.372N |
| Incidental Tumor Tests (e) | P=0.328N | P=0.345N | P=0.502N |
| Cochran-Armitage Trend Test (e) | P=0.158N | | |
| Fisher Exact Test (e) | | P=0.357N | P=0.218N |
| Uterus: Endometrial Stromal Polyp | | | |
| Overall Rates (a) | 5/50 (10%) | 7/50 (14%) | 6/49 (12%) |
| Adjusted Rates (c) | 16.7% | 20.0% | 16.0% |
| Terminal Rates (d) | 5/30 (17%) | 6/33 (18%) | 1/23 (4%) |
| Week of First Observation | 105 | 91 | 57 |
| Life Table Tests (e) | P=0.268 | P=0.441 | P=0.340 |
| Incidental Tumor Tests (e) | P=0.510N | P=0.366 | P=0.406N |
| Cochran-Armitage Trend Test (e) | P=0.424 | | |
| Fisher Exact Test (e) | | P=0.380 | P=0.486 |

TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 500 mg/kg | 1,000 mg/kg |
|---|-----------------|------------|-------------|
| Uterus: Endometrial Stromal Polyp or Sarcoma | | | |
| Overall Rates (a) | 5/50 (10%) | 9/50 (18%) | 7/49 (14%) |
| Adjusted Rates (c) | 16.7% | 24.9% | 18.5% |
| Terminal Rates (d) | 5/30 (17%) | 7/33 (21%) | 1/23 (4%) |
| Week of First Observation | 105 | 91 | 57 |
| Life Table Tests (e) | P=0.171 | P=0.244 | P=0.229 |
| Incidental Tumor Tests (e) | P=0.442 | P=0.182 | P=0.551N |
| Cochran-Armitage Trend Test (e) | P=0.318 | | |
| Fisher Exact Test (e) | | P=0.194 | P=0.365 |

(a) Number of tumor-bearing animals/number of animals examined at the site

(b) A fibrosarcoma was also observed in an animal bearing a fibroma.

(c) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality

(d) Observed tumor incidence at terminal kill

(e) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

(f) No P value is reported because no tumors were observed in the 1,000 mg/kg and vehicle control groups.

**TABLE B4. HISTORICAL INCIDENCE OF ORAL CAVITY TUMORS IN FEMALE F344/N RATS
ADMINISTERED CORN OIL BY GAVAGE (a)**

| | No. of Animals Examined | No. of Tumors in Vehicle Controls | Site | Diagnosis |
|---|----------------------------|--------------------------------------|-----------------|-------------------------|
| Historical Incidence at Litton Bionetics, Inc. | | | | |
| No tumors observed in 450 animals | | | | |
| Overall Historical Incidence | | | | |
| | 1,450 | 3 | Tongue | Squamous cell papilloma |
| | | 1 | Tongue (dorsum) | Squamous cell papilloma |
| | | 1 | Palate | Squamous cell papilloma |
| TOTAL | | 5 (0.3%) | | |

(a) Data as of August 30, 1985, for studies of at least 104 weeks

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Epidermal inclusion cyst | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| *Nasal cavity | (50) | (50) | (50) |
| Inflammation, suppurative | 1 (2%) | 3 (6%) | 3 (6%) |
| Inflammation, chronic focal | | | 1 (2%) |
| Reaction, foreign body | 3 (6%) | | |
| #Trachea | (49) | (50) | (47) |
| Inflammation, chronic focal | | | 1 (2%) |
| #Lung/bronchus | (50) | (50) | (49) |
| Lymphocytic inflammatory infiltrate | | | 1 (2%) |
| #Lung | (50) | (50) | (49) |
| Atelectasis | | 1 (2%) | |
| Congestion, NOS | 1 (2%) | 3 (6%) | 2 (4%) |
| Hemorrhage | 1 (2%) | 1 (2%) | 1 (2%) |
| Lymphocytic inflammatory infiltrate | 1 (2%) | | 1 (2%) |
| Inflammation, interstitial | 1 (2%) | 1 (2%) | |
| Pneumonia, interstitial chronic | 1 (2%) | 1 (2%) | |
| Inflammation, chronic focal | 3 (6%) | 4 (8%) | 1 (2%) |
| Inflammation, granulomatous focal | | | 1 (2%) |
| Reaction, foreign body | 1 (2%) | | |
| Calcification, focal | | | 2 (4%) |
| Hyperplasia, alveolar epithelium | 1 (2%) | | 1 (2%) |
| #Lung/alveoli | (50) | (50) | (49) |
| Histiocytosis | 16 (32%) | 19 (38%) | 18 (37%) |
| HEMATOPOIETIC SYSTEM | | | |
| #Bone marrow | (49) | (50) | (48) |
| Inflammation, granulomatous focal | | | 2 (4%) |
| Fibrosis, focal | | | 1 (2%) |
| Hypoplasia, NOS | 2 (4%) | | |
| Atrophy, focal | | 1 (2%) | |
| Hyperplasia, granulocytic | | 1 (2%) | |
| #Spleen | (50) | (50) | (49) |
| Congestion, NOS | | | 1 (2%) |
| Granuloma, NOS | | 1 (2%) | |
| Hemosiderosis | 6 (12%) | | 5 (10%) |
| Metaplasia, osseous | | 1 (2%) | |
| Hematopoiesis | 4 (8%) | 2 (4%) | 3 (6%) |
| #Splenic capsule | (50) | (50) | (49) |
| Hyperplasia, focal | 1 (2%) | | |
| #Lymph node | (50) | (50) | (46) |
| Plasmacytosis | | 1 (2%) | |
| #Lung/bronchus | (50) | (50) | (49) |
| Hyperplasia, lymphoid | | | 1 (2%) |
| #Ileum | (45) | (50) | (47) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Colon | (44) | (49) | (47) |
| Hyperplasia, lymphoid | | 1 (2%) | |

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|---|-----------------|----------|-----------|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Thymus | (44) | (49) | (46) |
| Cyst, NOS | | 3 (6%) | 1 (2%) |
| Hemorrhage | | | 2 (4%) |
| Atrophy, NOS | 1 (2%) | | |
| Hyperplasia, epithelial | 2 (5%) | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Periarteritis | 1 (2%) | | 1 (2%) |
| #Mediastinal lymph node | (50) | (50) | (46) |
| Lymphangiectasis | | | 1 (2%) |
| #Myocardium | (50) | (50) | (49) |
| Fibrosis, focal | | | 1 (2%) |
| Degeneration, NOS | 30 (60%) | 31 (62%) | 27 (55%) |
| #Endocardium | (50) | (50) | (49) |
| Inflammation, chronic | | 1 (2%) | |
| *Blood vessel | (50) | (50) | (50) |
| Aneurysm | 1 (2%) | | |
| Inflammation, chronic | 1 (2%) | | |
| *Pulmonary artery | (50) | (50) | (50) |
| Thrombosis, NOS | | | 1 (2%) |
| Calcification, focal | 9 (18%) | 11 (22%) | 8 (16%) |
| #Kidney | (50) | (50) | (49) |
| Periarteritis | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| *Tongue | (50) | (50) | (50) |
| Hyperplasia, epithelial | | | 1 (2%) |
| *Tooth | (50) | (50) | (50) |
| Inflammation, acute/chronic | | 1 (2%) | |
| #Parotid duct | (50) | (50) | (49) |
| Necrosis, NOS | 1 (2%) | | |
| #Major sublingual duct | (50) | (50) | (49) |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Liver | (50) | (50) | (49) |
| Congenital malformation, NOS | 3 (6%) | 1 (2%) | 1 (2%) |
| Hernia, NOS | 5 (10%) | 1 (2%) | 4 (8%) |
| Deformity, NOS | | 1 (2%) | |
| Bile stasis | | | 1 (2%) |
| Congestion, NOS | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | 1 (2%) |
| Inflammation, acute necrotizing | | | 1 (2%) |
| Inflammation, chronic focal | 16 (32%) | 18 (36%) | 17 (35%) |
| Inflammation, granulomatous | | | 1 (2%) |
| Inflammation, granulomatous focal | | 1 (2%) | |
| Necrosis, focal | 1 (2%) | 2 (4%) | 1 (2%) |
| Lipoidosis | 1 (2%) | 2 (4%) | 1 (2%) |
| Basophilic cyto change | 17 (34%) | 7 (14%) | 5 (10%) |
| Ground glass cyto change | 3 (6%) | 5 (10%) | 5 (10%) |
| Focal cellular change | 1 (2%) | 1 (2%) | 1 (2%) |
| Eosinophilic cyto change | 1 (2%) | 1 (2%) | |
| Clear cell change | 3 (6%) | 1 (2%) | 1 (2%) |
| Hyperplasia, nodular | | 1 (2%) | |
| Angiectasis | | 2 (4%) | |
| Regeneration, NOS | 3 (6%) | 3 (6%) | |
| #Liver/centrilobular | (50) | (50) | (49) |
| Degeneration, NOS | | 1 (2%) | |
| Necrosis, NOS | 1 (2%) | | |
| Lipoidosis | 1 (2%) | 1 (2%) | |

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| DIGESTIVE SYSTEM (Continued) | | | |
| #Liver/perportal | (50) | (50) | (49) |
| Inflammation, chronic | | | 2 (4%) |
| Metamorphosis, fatty | | 1 (2%) | |
| Lipoidosis | 2 (4%) | 1 (2%) | 2 (4%) |
| #Liver/hepatocytes | (50) | (50) | (49) |
| Hyperplasia, diffuse | | 1 (2%) | |
| #Bile duct | (50) | (50) | (49) |
| Cyst | | 1 (2%) | |
| Hyperplasia, NOS | 30 (60%) | 29 (58%) | 28 (57%) |
| #Pancreas | (49) | (50) | (49) |
| Dilatation/ducts | | | 1 (2%) |
| Inflammation, chronic focal | | | 1 (2%) |
| Atrophy, NOS | 1 (2%) | | |
| #Pancreatic acinus | (49) | (50) | (49) |
| Atrophy, NOS | 2 (4%) | 2 (4%) | 1 (2%) |
| Atrophy, focal | 8 (16%) | 10 (20%) | 8 (16%) |
| Hyperplasia, focal | | 1 (2%) | |
| #Periesophageal tissue | (50) | (50) | (49) |
| Hemorrhage | | 1 (2%) | |
| #Glandular stomach | (48) | (50) | (49) |
| Dilatation, NOS | 1 (2%) | | |
| Inflammation, serous | | 1 (2%) | |
| #Forestomach | (48) | (50) | (49) |
| Ulcer, NOS | 1 (2%) | | |
| Inflammation, acute | 1 (2%) | | |
| Inflammation, chronic focal | | 1 (2%) | |
| Hyperplasia, epithelial | 2 (4%) | 1 (2%) | |
| #Duodenum | (45) | (50) | (47) |
| Ulcer, NOS | 1 (2%) | | |
| Inflammation, chronic | | 1 (2%) | |
| #Colon | (44) | (49) | (47) |
| Parasitism | 1 (2%) | 2 (4%) | 1 (2%) |
| #Colonic mucosa | (44) | (49) | (47) |
| Calcification, NOS | | 1 (2%) | 1 (2%) |
| #Cecum | (44) | (49) | (47) |
| Parasitism | | | 1 (2%) |
| Calcification, NOS | | 1 (2%) | 3 (6%) |
| *Rectum | (50) | (50) | (50) |
| Parasitism | 2 (4%) | 3 (6%) | |
| URINARY SYSTEM | | | |
| #Kidney | (50) | (50) | (49) |
| Nephropathy | 9 (18%) | 17 (34%) | 14 (29%) |
| Calcification, focal | 2 (4%) | 3 (6%) | 1 (2%) |
| #Kidney/cortex | (50) | (50) | (49) |
| Cyst, NOS | | 1 (2%) | |
| Fibrosis, focal | | 1 (2%) | |
| #Renal papilla | (50) | (50) | (49) |
| Calcification, NOS | 22 (44%) | 12 (24%) | 7 (14%) |
| #Kidney/pelvis | (50) | (50) | (49) |
| Dilatation, NOS | | | 1 (2%) |
| Calcification, focal | 22 (44%) | 27 (54%) | 13 (27%) |
| Hyperplasia, epithelial | | 1 (2%) | 2 (4%) |
| ENDOCRINE SYSTEM | | | |
| #Pituitary intermedia | (50) | (50) | (48) |
| Hemorrhage | | | 1 (2%) |

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| ENDOCRINE SYSTEM (Continued) | | | |
| #Anterior pituitary | (50) | (50) | (48) |
| Cyst, NOS | 13 (26%) | 17 (34%) | 19 (40%) |
| Hemorrhage | 3 (6%) | | |
| Hyperplasia, focal | 16 (32%) | 13 (26%) | 12 (25%) |
| Angiectasis | 6 (12%) | 5 (10%) | 6 (13%) |
| #Pituitary posterior | (50) | (50) | (48) |
| Gliosis | 1 (2%) | | |
| Pigmentation, NOS | 1 (2%) | | |
| Metaplasia, osseous | | 1 (2%) | |
| #Adrenal cortex | (50) | (50) | (49) |
| Cyst, NOS | 2 (4%) | | |
| Degeneration, lipoid | 12 (24%) | 11 (22%) | 10 (20%) |
| Necrosis, NOS | | 1 (2%) | |
| Necrosis, focal | 1 (2%) | 1 (2%) | |
| Lipoidosis | 2 (4%) | 2 (4%) | |
| Cytomegaly | | | 1 (2%) |
| Hyperplasia, focal | 8 (16%) | 8 (16%) | 7 (14%) |
| #Adrenal medulla | (50) | (50) | (49) |
| Hyperplasia, focal | 5 (10%) | 5 (10%) | 4 (8%) |
| #Thyroid | (49) | (50) | (48) |
| Ultimobranchial cyst | | 1 (2%) | |
| Hyperplasia, cystic | | | 1 (2%) |
| Hyperplasia, C-cell | 14 (29%) | 10 (20%) | 7 (15%) |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (50) | (50) | (50) |
| Galactocele | 2 (4%) | | |
| Inflammation, chronic | | | 1 (2%) |
| Lactation | 37 (74%) | 35 (70%) | 26 (52%) |
| *Mammary duct | (50) | (50) | (50) |
| Hyperplasia, NOS | 1 (2%) | | |
| Hyperplasia, focal | | | 1 (2%) |
| *Mammary lobule | (50) | (50) | (50) |
| Hyperplasia, NOS | 1 (2%) | | 1 (2%) |
| *Clitoral gland | (50) | (50) | (50) |
| Dilatation/ducts | 1 (2%) | 2 (4%) | 2 (4%) |
| Retention of content | 2 (4%) | 1 (2%) | 2 (4%) |
| Inflammation, acute | 2 (4%) | | |
| Inflammation, acute/chronic | 1 (2%) | | |
| Hyperplasia, NOS | 1 (2%) | | |
| #Uterus | (50) | (50) | (49) |
| Abscess, NOS | | 1 (2%) | |
| Decidual alteration, NOS | | | 1 (2%) |
| #Cervix uteri | (50) | (50) | (49) |
| Abscess, NOS | | 1 (2%) | |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Uterus/endometrium | (50) | (50) | (49) |
| Cyst, NOS | 2 (4%) | 1 (2%) | 1 (2%) |
| Inflammation, suppurative | | | 1 (2%) |
| Hyperplasia, cystic | 2 (4%) | 3 (6%) | 2 (4%) |
| Hyperplasia, stromal | 1 (2%) | 1 (2%) | |
| #Endometrial gland | (50) | (50) | (49) |
| Cyst, NOS | 1 (2%) | | |
| Hyperplasia, NOS | 1 (2%) | | |
| #Ovary | (50) | (50) | (49) |
| Cyst, NOS | 2 (4%) | 3 (6%) | 2 (4%) |
| Corpus luteum cyst | 1 (2%) | | |
| Atrophy, NOS | | | 1 (2%) |

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-----------------------------------|-----------------|----------|-----------|
| NERVOUS SYSTEM | | | |
| #Lateral ventricle | (50) | (50) | (49) |
| Dilatation, NOS | | | 1 (2%) |
| #Aqueduct of Sylvius | (50) | (50) | (49) |
| Dilatation, NOS | | 1 (2%) | |
| *Choroid plexus | (50) | (50) | (50) |
| Hyperplasia, NOS | | 1 (2%) | |
| #Brain | (50) | (50) | (49) |
| Scar | | 1 (2%) | |
| Infarct, hemorrhagic | 1 (2%) | | |
| SPECIAL SENSE ORGANS | | | |
| *Eye | (50) | (50) | (50) |
| Cataract | 7 (14%) | 7 (14%) | 3 (6%) |
| *Eye/sclera | (50) | (50) | (50) |
| Inflammation, acute/chronic | | | 1 (2%) |
| Metaplasia, osseous | 1 (2%) | 3 (6%) | 2 (4%) |
| *Eye/retina | (50) | (50) | (50) |
| Degeneration, NOS | 1 (2%) | | |
| Atrophy, NOS | 5 (10%) | 9 (18%) | 5 (10%) |
| *Nasolacrimal duct | (50) | (50) | (50) |
| Inflammation, suppurative | 1 (2%) | | |
| Inflammation, chronic | | | 2 (4%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *Cartilage, NOS | (50) | (50) | (50) |
| Necrosis, NOS | 1 (2%) | | |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | | |
| Inflammation, suppurative | 1 (2%) | | |
| Inflammation, fibrinous | | 1 (2%) | |
| Abscess, NOS | | | 1 (2%) |
| Hemosiderosis | | 1 (2%) | |
| *Pericardium | (50) | (50) | (50) |
| Inflammation, fibrinous | | 1 (2%) | |
| *Mesentery | (50) | (50) | (50) |
| Necrosis, fat | | 1 (2%) | 1 (2%) |
| ALL OTHER SYSTEMS | | | |
| *Multiple organs | (50) | (50) | (50) |
| Congestion, NOS | | | 1 (2%) |
| Adipose tissue | | | |
| Necrosis, NOS | | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| No lesion reported | | | 1 |
| Auto/necropsy/no histocytic | | | 1 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
 # Number of animals examined microscopically at this site

APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

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TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 47 |
| INTEGUMENTARY SYSTEM | | | |
| *Subcutaneous tissue | (50) | (50) | (50) |
| Sarcoma, NOS | 1 (2%) | 3 (6%) | |
| Fibroma | 2 (4%) | 1 (2%) | |
| Fibrosarcoma | 4 (8%) | 3 (6%) | |
| Rhabdomyosarcoma | 2 (4%) | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #Lung | (50) | (49) | (45) |
| Squamous cell carcinoma | 1 (2%) | | |
| Bile duct carcinoma, metastatic | | 1 (2%) | |
| Hepatocellular carcinoma, metastatic | 2 (4%) | | 1 (2%) |
| Alveolar/bronchiolar adenoma | 4 (8%) | | 2 (4%) |
| Alveolar/bronchiolar carcinoma | 2 (4%) | | 1 (2%) |
| Carcinosarcoma, metastatic | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Malignant lymphoma, lymphocytic type | 1 (2%) | 1 (2%) | 1 (2%) |
| Malignant lymphoma, mixed type | 1 (2%) | 2 (4%) | |
| Granulocytic leukemia | 1 (2%) | | |
| #Lymph node | (46) | (43) | (27) |
| Squamous cell carcinoma, metastatic | 1 (2%) | | |
| Fibrosarcoma, metastatic | 1 (2%) | 1 (2%) | |
| #Hepatic lymph node | (46) | (43) | (27) |
| Carcinosarcoma, metastatic | 1 (2%) | | |
| #Inguinal lymph node | (46) | (43) | (27) |
| Fibrosarcoma, metastatic | 1 (2%) | | |
| #Thymus | (38) | (41) | (41) |
| Bile duct carcinoma, metastatic | | 1 (2%) | |
| Carcinosarcoma, metastatic | 1 (3%) | | |
| CIRCULATORY SYSTEM | | | |
| *Multiple organs | (50) | (50) | (50) |
| Hemangiosarcoma | 1 (2%) | 1 (2%) | |
| #Spleen | (50) | (49) | (46) |
| Hemangiosarcoma | 2 (4%) | | |
| #Heart | (50) | (49) | (45) |
| Bile duct carcinoma, metastatic | | 1 (2%) | |
| Sarcoma, NOS | 1 (2%) | | |
| #Liver | (50) | (50) | (46) |
| Hemangiosarcoma | 3 (6%) | | 1 (2%) |
| #Pancreas | (49) | (49) | (46) |
| Hemangioma | 1 (2%) | | |
| #Urinary bladder | (46) | (45) | (38) |
| Hemangioma | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #Liver | (50) | (50) | (46) |
| Bile duct carcinoma | | 1 (2%) | |
| Hepatocellular adenoma | 12 (24%) | 15 (30%) | 3 (7%) |
| Hepatocellular carcinoma | 6 (12%) | 6 (12%) | 1 (2%) |
| Carcinosarcoma, metastatic | 1 (2%) | | |

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| DIGESTIVE SYSTEM (Continued) | | | |
| #Pancreas | (49) | (49) | (46) |
| Bile duct carcinoma, metastatic | | 1 (2%) | |
| Carcinosarcoma | 1 (2%) | | |
| #Fore stomach | (46) | (48) | (43) |
| Papilloma, NOS | 1 (2%) | | |
| Squamous cell carcinoma | 1 (2%) | | |
| URINARY SYSTEM | | | |
| None | | | |
| ENDOCRINE SYSTEM | | | |
| #Adrenal/capsule | (50) | (49) | (44) |
| Adenoma, NOS | 1 (2%) | | |
| #Adrenal medulla | (50) | (49) | (44) |
| Pheochromocytoma | 4 (8%) | | |
| #Thyroid | (49) | (46) | (43) |
| Follicular cell adenoma | 2 (4%) | | |
| Follicular cell carcinoma | 1 (2%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *Preputial gland | (50) | (50) | (50) |
| Carcinoma, NOS | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| None | | | |
| SPECIAL SENSE ORGANS | | | |
| *Harderian gland | (50) | (50) | (50) |
| Adenoma, NOS | 2 (4%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| None | | | |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Bile duct carcinoma, metastatic | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| Adipose tissue | | | |
| Carcinosarcoma, metastatic | 1 | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 15 | 26 | 25 |
| Moribund sacrifice | 3 | 7 | 8 |
| Terminal sacrifice | 28 | 12 | |
| Dosing accident | 4 | 5 | 17 |

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|---------------------------------------|-----------------|----------|-----------|
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 34 | 27 | 10 |
| Total primary tumors | 60 | 33 | 10 |
| Total animals with benign tumors | 24 | 16 | 5 |
| Total benign tumors | 30 | 16 | 5 |
| Total animals with malignant tumors | 23 | 14 | 5 |
| Total malignant tumors | 30 | 17 | 5 |
| Total animals with secondary tumors## | 5 | 2 | 1 |
| Total secondary tumors | 10 | 6 | 1 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.

** Primary tumors: all tumors except secondary tumors

Number of animals examined microscopically at this site

Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE: LOW DOSE

| ANIMAL NUMBER | 03 | 02 | 02 | 02 | 02 | 03 | 01 | 03 | 04 | 02 | 00 | 00 | 00 | 00 | 00 | 00 | 01 | 01 | 01 | 01 | 04 | 04 | 01 | 04 | 00 | 03 | | | |
|--------------------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|
| WEEKS ON STUDY | 01 | 01 | 01 | 01 | 02 | 03 | 05 | 05 | 06 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 07 | 08 | 08 | 08 | 09 | |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | X | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibrosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | - | + | + | + | + | + | + | + | + | + |
| Fibrosarcoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thymus | + | + | + | - | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | N | N | + | N | + | + | + | + | + | N | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | - | + | + | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thyroid | + | + | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Parathyroid | - | - | + | - | - | - | + | + | + | + | - | - | + | + | + | - | - | + | + | - | + | + | - | + | + | + | + | - | - |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Prostate | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mediastinum | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Bile duct carcinoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Hamangiosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 1,000 mg/kg | 2,000 mg/kg |
|---|-----------------|-------------|-------------|
| Subcutaneous Tissue: Sarcoma | | | |
| Overall Rates (a) | 1/50 (2%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted Rates (b) | 3.3% | 13.8% | 0.0% |
| Terminal Rates (c) | 0/29 (0%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 104 | 64 | |
| Life Table Tests (d) | P=0.220 | P=0.149 | (e) |
| Incidental Tumor Tests (d) | P=0.569N | P=0.714N | (e) |
| Cochran-Armitage Trend Test (d) | P=0.378N | | |
| Fisher Exact Test (d) | | P=0.309 | P=0.500N |
| Subcutaneous Tissue: Fibrosarcoma | | | |
| Overall Rates (a) | 4/50 (8%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted Rates (b) | 12.6% | 17.5% | 0.0% |
| Terminal Rates (c) | 3/29 (10%) | 1/12 (8%) | 0/0 |
| Week of First Observation | 80 | 95 | |
| Life Table Tests (d) | P=0.481 | P=0.452 | P=0.919N |
| Incidental Tumor Tests (d) | P=0.466N | P=0.646N | P=0.500N |
| Cochran-Armitage Trend Test (d) | P=0.049N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.059N |
| Subcutaneous Tissue: Sarcoma or Fibrosarcoma | | | |
| Overall Rates (a) | 5/50 (10%) | 6/50 (12%) | 0/50 (0%) |
| Adjusted Rates (b) | 15.6% | 28.9% | 0.0% |
| Terminal Rates (c) | 3/29 (10%) | 1/12 (8%) | 0/0 |
| Week of First Observation | 80 | 64 | |
| Life Table Tests (d) | P=0.202 | P=0.145 | P=0.919N |
| Incidental Tumor Tests (d) | P=0.388N | P=0.612N | P=0.500N |
| Cochran-Armitage Trend Test (d) | P=0.042N | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.028N |
| Subcutaneous Tissue: Fibroma or Fibrosarcoma | | | |
| Overall Rates (a) | 6/50 (12%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted Rates (b) | 19.4% | 17.5% | 0.0% |
| Terminal Rates (c) | 5/29 (17%) | 1/12 (8%) | 0/0 |
| Week of First Observation | 80 | 95 | |
| Life Table Tests (d) | P=0.634N | P=0.628 | P=0.919N |
| Incidental Tumor Tests (d) | P=0.318N | P=0.456N | P=0.500N |
| Cochran-Armitage Trend Test (d) | P=0.010N | | |
| Fisher Exact Test (d) | | P=0.243N | P=0.013N |
| Subcutaneous Tissue: Fibroma, Sarcoma, or Fibrosarcoma | | | |
| Overall Rates (a) | 7/50 (14%) | 6/50 (12%) | 0/50 (0%) |
| Adjusted Rates (b) | 22.1% | 28.9% | 0.0% |
| Terminal Rates (c) | 5/29 (17%) | 1/12 (8%) | 0/0 |
| Week of First Observation | 80 | 64 | |
| Life Table Tests (d) | P=0.326 | P=0.262 | P=0.919N |
| Incidental Tumor Tests (d) | P=0.270N | P=0.448N | P=0.500N |
| Cochran-Armitage Trend Test (d) | P=0.010N | | |
| Fisher Exact Test (d) | | P=0.500N | P=0.006N |
| Lung: Alveolar/Bronchiolar Adenoma | | | |
| Overall Rates (a) | 4/50 (8%) | 0/49 (0%) | 2/45 (4%) |
| Adjusted Rates (b) | 12.8% | 0.0% | 35.8% |
| Terminal Rates (c) | 3/29 (10%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 90 | | 45 |
| Life Table Tests (d) | P=0.285 | P=0.191N | P=0.060 |
| Incidental Tumor Tests (d) | P=0.523N | P=0.184N | P=0.650 |
| Cochran-Armitage Trend Test (d) | P=0.253N | | |
| Fisher Exact Test (d) | | P=0.061N | P=0.390N |

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 1,000 mg/kg | 2,000 mg/kg |
|--|-----------------|-------------|-------------|
| Lung: Alveolar/Bronchiolar Adenoma or Carcinoma | | | |
| Overall Rates (a) | 6/50 (12%) | 0/49 (0%) | 3/45 (7%) |
| Adjusted Rates (b) | 19.5% | 0.0% | 43.8% |
| Terminal Rates (c) | 5/29 (17%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 90 | | 45 |
| Life Table Tests (d) | P=0.168 | P=0.101N | P=0.006 |
| Incidental Tumor Tests (d) | P=0.592N | P=0.097N | P=0.485 |
| Cochran-Armitage Trend Test (d) | P=0.177N | | |
| Fisher Exact Test (d) | | P=0.014N | P=0.299N |
| Hematopoietic System: Lymphoma, All Malignant | | | |
| Overall Rates (a) | 2/50 (4%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted Rates (b) | 6.6% | 19.9% | 33.3% |
| Terminal Rates (c) | 1/29 (3%) | 2/12 (17%) | 0/0 |
| Week of First Observation | 102 | 90 | 92 |
| Life Table Tests (d) | P=0.026 | P=0.182 | P=0.059 |
| Incidental Tumor Tests (d) | P=0.268 | P=0.327 | P=0.500 |
| Cochran-Armitage Trend Test (d) | P=0.399N | | |
| Fisher Exact Test (d) | | P=0.500 | P=0.500N |
| Hematopoietic System: Lymphoma or Leukemia | | | |
| Overall Rates (a) | 3/50 (6%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted Rates (b) | 9.9% | 19.9% | 33.3% |
| Terminal Rates (c) | 2/29 (7%) | 2/12 (17%) | 0/0 |
| Week of First Observation | 102 | 90 | 92 |
| Life Table Tests (d) | P=0.054 | P=0.283 | P=0.059 |
| Incidental Tumor Tests (d) | P=0.344 | P=0.441 | P=0.500 |
| Cochran-Armitage Trend Test (d) | P=0.238N | | |
| Fisher Exact Test (d) | | P=0.661 | P=0.309N |
| Circulatory System: Hemangiosarcoma | | | |
| Overall Rates (a) | 6/50 (12%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted Rates (b) | 18.6% | 3.7% | 3.6% |
| Terminal Rates (c) | 4/29 (14%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 87 | 88 | 45 |
| Life Table Tests (d) | P=0.511N | P=0.220N | P=0.478 |
| Incidental Tumor Tests (d) | P=0.125N | P=0.139N | P=0.616N |
| Cochran-Armitage Trend Test (d) | P=0.023N | | |
| Fisher Exact Test (d) | | P=0.056N | P=0.056N |
| Circulatory System: Hemangioma or Hemangiosarcoma | | | |
| Overall Rates (a) | 8/50 (16%) | 1/50 (2%) | 1/50 (2%) |
| Adjusted Rates (b) | 25.1% | 3.7% | 3.6% |
| Terminal Rates (c) | 6/29 (21%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 87 | 88 | 45 |
| Life Table Tests (d) | P=0.369N | P=0.128N | P=0.478 |
| Incidental Tumor Tests (d) | P=0.078N | P=0.077N | P=0.616N |
| Cochran-Armitage Trend Test (d) | P=0.005N | | |
| Fisher Exact Test (d) | | P=0.016N | P=0.016N |
| Liver: Hepatocellular Adenoma | | | |
| Overall Rates (a) | 12/50 (24%) | 15/50 (30%) | 3/46 (7%) |
| Adjusted Rates (b) | 38.5% | 70.1% | 51.4% |
| Terminal Rates (c) | 10/29 (34%) | 7/12 (58%) | 0/0 |
| Week of First Observation | 93 | 55 | 74 |
| Life Table Tests (d) | P<0.001 | P=0.006 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.051 | P=0.180 | P=0.196 |
| Cochran-Armitage Trend Test (d) | P=0.026N | | |
| Fisher Exact Test (d) | | P=0.326 | P=0.018N |

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 1,000 mg/kg | 2,000 mg/kg |
|--|-----------------|-------------|-------------|
| Liver: Hepatocellular Carcinoma | | | |
| Overall Rates (a) | 6/50 (12%) | 6/50 (12%) | 1/46 (2%) |
| Adjusted Rates (b) | 16.8% | 24.8% | 20.0% |
| Terminal Rates (c) | 2/29 (7%) | 1/12 (8%) | 0/0 |
| Week of First Observation | 58 | 77 | 79 |
| Life Table Tests (d) | P=0.175 | P=0.319 | P=0.430 |
| Incidental Tumor Tests (d) | P=0.245N | P=0.215N | P=0.419N |
| Cochran-Armitage Trend Test (d) | P=0.069N | | |
| Fisher Exact Test (d) | | P=0.620N | P=0.070N |
| Liver: Hepatocellular Adenoma or Carcinoma | | | |
| Overall Rates (a) | 17/50 (34%) | 21/50 (42%) | 4/46 (9%) |
| Adjusted Rates (b) | 49.4% | 80.5% | 61.1% |
| Terminal Rates (c) | 12/29 (41%) | 8/12 (67%) | 0/0 |
| Week of First Observation | 58 | 55 | 74 |
| Life Table Tests (d) | P<0.001 | P=0.003 | P<0.001 |
| Incidental Tumor Tests (d) | P=0.156 | P=0.341 | P=0.500 |
| Cochran-Armitage Trend Test (d) | P=0.005N | | |
| Fisher Exact Test (d) | | P=0.268 | P=0.003N |
| Adrenal Gland: Pheochromocytoma | | | |
| Overall Rates (a) | 4/50 (8%) | 0/49 (0%) | 0/44 (0%) |
| Adjusted Rates (b) | 12.7% | 0.0% | 0.0% |
| Terminal Rates (c) | 3/29 (10%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 85 | | |
| Life Table Tests (d) | P=0.185N | P=0.192N | P=0.949N |
| Incidental Tumor Tests (d) | P=0.114N | P=0.184N | P=0.500N |
| Cochran-Armitage Trend Test (d) | P=0.018N | | |
| Fisher Exact Test (d) | | P=0.061N | P=0.076N |
| Thyroid Gland: Follicular Cell Adenoma or Carcinoma | | | |
| Overall Rates (a) | 3/49 (6%) | 0/46 (0%) | 0/43 (0%) |
| Adjusted Rates (b) | 9.0% | 0.0% | 0.0% |
| Terminal Rates (c) | 2/29 (7%) | 0/12 (0%) | 0/0 |
| Week of First Observation | 42 | | |
| Life Table Tests (d) | P=0.165N | P=0.235N | P=0.564N |
| Incidental Tumor Tests (d) | P=0.049N | P=0.298N | P=0.215N |
| Cochran-Armitage Trend Test (d) | P=0.045N | | |
| Fisher Exact Test (d) | | P=0.133N | P=0.147N |

(a) Number of tumor-bearing animals/number of animals examined at the site

(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality

(c) Observed tumor incidence at terminal kill

(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

(e) No P value is reported because all high dose animals died before the vehicle control tumor was observed.

TABLE C4a. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN MALE B6C3F₁ MICE ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls | |
|---|-------------------------------|----------------------|
| | Lymphoma | Lymphoma or Leukemia |
| Historical Incidence at Litton Bionetics, Inc. | | |
| Dimethyl hydrogen phosphite | 3/50 | 5/50 |
| Dimethyl methylphosphonate | 2/50 | 3/50 |
| 2,4-Toluene diisocyanate | 6/50 | 6/50 |
| Diallyl phthalate | 6/50 | 6/50 |
| Dimethyl morpholinophosphoramidate | 3/50 | 3/50 |
| Tris(2-ethylhexyl)phosphate | 7/50 | 7/50 |
| 3-Chloro-2-methylpropene | 4/50 | 4/50 |
| 4-Vinylcyclohexene | 4/50 | 4/50 |
| Dimethylvinyl chloride | 6/50 | 6/50 |
| TOTAL | 41/450 (9.1%) | 44/450 (9.8%) |
| SD (b) | 3.48% | 2.91% |
| Range (c) | | |
| High | 7/50 | 7/50 |
| Low | 2/50 | 3/50 |
| Overall Historical Incidence | | |
| TOTAL | 181/1,497 (12.1%) | 185/1,497 (12.4%) |
| SD (b) | 4.41% | 4.21% |
| Range (c) | | |
| High | 11/50 | 11/50 |
| Low | 2/50 | 3/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks
 (b) Standard deviation
 (c) Range and SD are presented for groups of 35 or more animals.

TABLE C4b. HISTORICAL INCIDENCE OF HEPATOCELLULAR TUMORS IN MALE B6C3F₁ MICE ADMINISTERED CORN OIL BY GAVAGE (a)

| Study | Incidence in Vehicle Controls | | |
|---|-------------------------------|-------------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence at Litton Bionetics, Inc. | | | |
| Dimethyl hydrogen phosphite | 12/50 | 9/50 | 19/50 |
| Dimethyl methylphosphonate | 10/50 | 6/50 | 15/50 |
| 2,4-Toluene diisocyanate | 5/49 | 6/49 | 11/49 |
| Diallyl phthalate | 0/50 | 7/50 | 7/50 |
| Dimethyl morpholinophosphoramidate | 6/50 | 6/50 | 11/50 |
| Tris(2-ethylhexyl)phosphate | 7/50 | 9/50 | 15/50 |
| 3-Chloro-2-methylpropene | 4/50 | 19/50 | 22/50 |
| 4-Vinylcyclohexene | 7/49 | 11/49 | 18/49 |
| Dimethylvinyl chloride | 8/49 | 3/49 | 11/49 |
| TOTAL | 59/447 (13.2%) | 76/447 (17.0%) | 129/447 (28.9%) |
| SD (b) | 6.95% | 9.17% | 9.54% |
| Range (c) | | | |
| High | 12/50 | 19/50 | 22/50 |
| Low | 0/50 | 3/49 | 7/50 |
| Overall Historical Incidence | | | |
| TOTAL | 201/1,490 (13.5%) | 306/1,490 (20.5%) | 477/1,490 (32.0%) |
| SD (b) | 6.45% | 7.70% | 8.99% |
| Range (c) | | | |
| High | 14/50 | 19/50 | 25/50 |
| Low | 0/50 | 3/49 | 7/50 |

(a) Data as of August 30, 1985, for studies of at least 104 weeks

(b) Standard deviation

(c) Range and SD are presented for groups of 35 or more animals.

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 47 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (50) | (50) | (50) |
| Epidermal inclusion cyst | | 1 (2%) | |
| Ulcer, NOS | 1 (2%) | 5 (10%) | 5 (10%) |
| Inflammation, acute | | 2 (4%) | |
| Inflammation, acute/chronic | | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 2 (4%) |
| Fibrosis | 1 (2%) | | |
| Fibrosis, focal | 1 (2%) | | |
| Exfoliative dermatitis | 1 (2%) | | |
| Hyperplasia, NOS | | 1 (2%) | |
| Acanthosis | | | 2 (4%) |
| RESPIRATORY SYSTEM | | | |
| *Nasal cavity | (50) | (50) | (50) |
| Congenital malformation, NOS | | 1 (2%) | |
| Ectopia | 1 (2%) | | |
| #Lung | (50) | (49) | (45) |
| Bronchiectasis | 1 (2%) | | |
| Atelectasis | | 1 (2%) | 2 (4%) |
| Congestion, NOS | 2 (4%) | 8 (16%) | 16 (36%) |
| Hemorrhage | 2 (4%) | 1 (2%) | |
| Bronchopneumonia, focal | | | 1 (2%) |
| Lymphocytic inflammatory infiltrate | 1 (2%) | 1 (2%) | |
| Inflammation, interstitial | 1 (2%) | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | | |
| Fibrosis, focal | 1 (2%) | | |
| Hyperplasia, adenomatous | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| #Bone marrow | (46) | (47) | (45) |
| Hyperplasia, NOS | 1 (2%) | | 1 (2%) |
| Hyperplasia, granulocytic | 2 (4%) | 5 (11%) | |
| #Spleen | (50) | (49) | (46) |
| Hemosiderosis | | | 1 (2%) |
| Hyperplasia, lymphoid | 1 (2%) | 1 (2%) | 1 (2%) |
| Hematopoiesis | 42 (84%) | 42 (86%) | 42 (91%) |
| #Splenic follicles | (50) | (49) | (46) |
| Necrosis, NOS | | | 1 (2%) |
| #Lymph node | (46) | (43) | (27) |
| Plasmacytosis | | | 1 (4%) |
| Mastocytosis | 1 (2%) | | |
| #Mandibular lymph node | (46) | (43) | (27) |
| Necrosis, NOS | | | 1 (4%) |
| Plasmacytosis | 1 (2%) | 1 (2%) | |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Pancreatic lymph node | (46) | (43) | (27) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Mesenteric lymph node | (46) | (43) | (27) |
| Congestion, NOS | | 1 (2%) | 1 (4%) |
| Hemorrhage | 5 (11%) | 2 (5%) | |
| Hyperplasia, lymphoid | 1 (2%) | | |
| Hematopoiesis | 1 (2%) | 3 (7%) | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|---|-----------------|----------|-----------|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Renal lymph node | (46) | (43) | (27) |
| Hyperplasia, NOS | 1 (2%) | | |
| Plasmacytosis | | 1 (2%) | |
| #Axillary lymph node | (46) | (43) | (27) |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Inguinal lymph node | (46) | (43) | (27) |
| Hyperplasia, NOS | 1 (2%) | | 1 (4%) |
| Plasmacytosis | | | 1 (4%) |
| #Femoral lymph node | (46) | (43) | (27) |
| Plasmacytosis | | 1 (2%) | |
| #Lung | (50) | (49) | (45) |
| Leukocytosis, NOS | | | 1 (2%) |
| Leukemoid reaction | 1 (2%) | | |
| #Liver | (50) | (50) | (46) |
| Hematopoiesis | | 1 (2%) | |
| #Pancreas | (49) | (49) | (46) |
| Hematopoiesis | 1 (2%) | | |
| #Thymus | (38) | (41) | (41) |
| Cyst, NOS | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | |
| Atrophy, NOS | 1 (3%) | 1 (2%) | 1 (2%) |
| #Thymic lymphocytes | (38) | (41) | (41) |
| Necrosis, NOS | | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| #Inguinal lymph node | (46) | (43) | (27) |
| Lymphangiectasis | | | 1 (4%) |
| *Lymphatics of lung | (50) | (50) | (50) |
| Sequestration | | | 1 (2%) |
| #Lung | (50) | (49) | (45) |
| Thrombus, fibrin | | | 1 (2%) |
| #Heart | (50) | (49) | (45) |
| Inflammation, suppurative | | | 1 (2%) |
| Periarteritis | | 1 (2%) | |
| #Myocardium | (50) | (49) | (45) |
| Inflammation, chronic focal | 1 (2%) | | |
| Necrosis, focal | | 1 (2%) | |
| Calcification, NOS | 1 (2%) | | |
| #Aortic valve | (50) | (49) | (45) |
| Pigmentation, NOS | | 1 (2%) | |
| #Pancreas | (49) | (49) | (46) |
| Periarteritis | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| *Tooth | (50) | (50) | (50) |
| Congenital malformation, NOS | 1 (2%) | | |
| *Pulp of tooth | (50) | (50) | (50) |
| Abscess, NOS | 1 (2%) | | |
| *Alveolus dentalis | (50) | (50) | (50) |
| Inflammation, acute/chronic | | 1 (2%) | |
| #Sublingual gland | (49) | (49) | (46) |
| Atrophy, focal | 1 (2%) | | |
| #Liver | (50) | (50) | (46) |
| Cyst, NOS | 1 (2%) | | |
| Hemorrhagic cyst | | 1 (2%) | |
| Inflammation, chronic focal | 2 (4%) | | |
| Necrosis, NOS | 2 (4%) | | |
| Necrosis, focal | 5 (10%) | 2 (4%) | 1 (2%) |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| DIGESTIVE SYSTEM | | | |
| #Liver (Continued) | (50) | (50) | (46) |
| Infarct, NOS | 3 (6%) | 1 (2%) | |
| Lipoidosis | 2 (4%) | 2 (4%) | |
| Ground glass cyto change | 2 (4%) | 2 (4%) | |
| Focal cellular change | 2 (4%) | 1 (2%) | 1 (2%) |
| Regeneration, NOS | | 1 (2%) | |
| #Portal tract | (50) | (50) | (46) |
| Atrophy, NOS | | | 1 (2%) |
| #Liver/centrilobular | (50) | (50) | (46) |
| Necrosis, NOS | 1 (2%) | | |
| Lipoidosis | 2 (4%) | 1 (2%) | |
| Hepatocytomegaly | 5 (10%) | 17 (34%) | 10 (22%) |
| #Liver/hepatocytes | (50) | (50) | (46) |
| Hyperplasia, focal | 1 (2%) | | |
| *Gallbladder | (50) | (50) | (50) |
| Inflammation, acute/chronic | | 1 (2%) | |
| #Bile duct | (50) | (50) | (46) |
| Hyperplasia, NOS | | 1 (2%) | |
| #Pancreas | (49) | (49) | (46) |
| Inflammation, acute/chronic | | 1 (2%) | |
| Inflammation, chronic focal | 1 (2%) | | |
| #Pancreatic acinus | (49) | (49) | (46) |
| Atrophy, focal | | 2 (4%) | |
| Atrophy, diffuse | | 1 (2%) | |
| Hyperplasia, NOS | | | 1 (2%) |
| #Esophagus/muscularis | (48) | (48) | (42) |
| Degeneration, NOS | | 1 (2%) | |
| #Esophageal adventitia | (48) | (48) | (42) |
| Hemorrhage | 1 (2%) | | |
| #Gastric mucosa | (46) | (48) | (43) |
| Necrosis, focal | | 1 (2%) | |
| #Glandular stomach | (46) | (48) | (43) |
| Dilatation, NOS | 1 (2%) | | |
| Ulcer, NOS | | 1 (2%) | |
| Calcification, focal | | | 1 (2%) |
| Pigmentation, NOS | 1 (2%) | 1 (2%) | |
| #Gastric submucosa | (46) | (48) | (43) |
| Edema, NOS | | 1 (2%) | |
| Eosinophilic leukocytic infiltrate | 1 (2%) | | |
| #Forestomach | (46) | (48) | (43) |
| Ulcer, NOS | | 1 (2%) | |
| Inflammation, acute focal | 1 (2%) | | |
| Inflammation, chronic focal | | | 1 (2%) |
| Necrosis, focal | 1 (2%) | | |
| Hyperplasia, epithelial | | 1 (2%) | |
| #Colonic serosa | (44) | (46) | (41) |
| Hemorrhage | | 1 (2%) | |
| #Cecum | (44) | (46) | (41) |
| Ulcer, NOS | | 1 (2%) | |
| *Anus | (50) | (50) | (50) |
| Ulcer, NOS | | | 1 (2%) |
| URINARY SYSTEM | | | |
| #Kidney | (50) | (49) | (46) |
| Calculus, gross observation only | | 1 (2%) | |
| Mineralization | 4 (8%) | 1 (2%) | 1 (2%) |
| Cast, NOS | 1 (2%) | | |
| Hydronephrosis | 1 (2%) | | 1 (2%) |
| Pyelonephritis, NOS | | 3 (6%) | 1 (2%) |
| Lymphocytic inflammatory infiltrate | 2 (4%) | 3 (6%) | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|------------------------------|-----------------|----------|-----------|
| URINARY SYSTEM | | | |
| #Kidney (Continued) | (50) | (49) | (46) |
| Plasma cell infiltrate | 1 (2%) | | |
| Metaplasia, osseous | 2 (4%) | 1 (2%) | |
| #Kidney/cortex | (50) | (49) | (46) |
| Cyst, NOS | 1 (2%) | | 1 (2%) |
| Atrophy, focal | | 1 (2%) | |
| #Kidney/tubule | (50) | (49) | (46) |
| Regeneration, NOS | | | 1 (2%) |
| #Kidney/pelvis | (50) | (49) | (46) |
| Inflammation, suppurative | | 1 (2%) | 1 (2%) |
| #Urinary bladder | (46) | (45) | (38) |
| Distention | | | 2 (5%) |
| Hemorrhage | | 1 (2%) | |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | | |
| *Urethra | (50) | (50) | (50) |
| Retention of content | 1 (2%) | | |
| Inflammation, necrotizing | | | 1 (2%) |
| *Prostatic urethra | (50) | (50) | (50) |
| Hyperplasia, epithelial | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (43) | (45) | (45) |
| Cyst, NOS | | 1 (2%) | |
| Hyperplasia, focal | | 2 (4%) | 1 (2%) |
| #Adrenal/capsule | (50) | (49) | (44) |
| Hyperplasia, NOS | 38 (76%) | 34 (69%) | 7 (16%) |
| #Adrenal cortex | (50) | (49) | (44) |
| Pigmentation, NOS | | 1 (2%) | |
| Hypertrophy, focal | 3 (6%) | 1 (2%) | |
| Hyperplasia, focal | | 2 (4%) | |
| #Adrenal medulla | (50) | (49) | (44) |
| Inflammation, suppurative | 1 (2%) | | |
| Hyperplasia, focal | | 4 (8%) | |
| #Periadrenal tissue | (50) | (49) | (44) |
| Inflammation, granulomatous | | 1 (2%) | |
| #Thyroid | (49) | (46) | (43) |
| Follicular cyst, NOS | 1 (2%) | | |
| Degeneration, NOS | | 1 (2%) | |
| Atrophy, focal | | 1 (2%) | |
| Hyperplasia, follicular cell | | 1 (2%) | |
| #Pancreatic islets | (49) | (49) | (46) |
| Hypertrophy, NOS | 1 (2%) | | |
| Hyperplasia, NOS | 1 (2%) | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| *Penis | (50) | (50) | (50) |
| Retention of content | 1 (2%) | | 1 (2%) |
| Obstruction, NOS | | | 1 (2%) |
| Inflammation, NOS | 1 (2%) | 2 (4%) | 3 (6%) |
| Hyperplasia, epithelial | 1 (2%) | | |
| *Prepuce | (50) | (50) | (50) |
| Retention of content | 1 (2%) | | |
| Obstruction, NOS | | | 1 (2%) |
| Ulcer, NOS | 1 (2%) | | 2 (4%) |
| Inflammation, acute | 1 (2%) | | 2 (4%) |
| Abscess, NOS | | | 1 (2%) |
| Necrosis, focal | 1 (2%) | | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|--|-----------------|----------|-----------|
| REPRODUCTIVE SYSTEM (Continued) | | | |
| *Preputial gland | (50) | (50) | (50) |
| Retention of content | 2 (4%) | | 1 (2%) |
| Cystic ducts | | 1 (2%) | 1 (2%) |
| Inflammation, NOS | 2 (4%) | 4 (8%) | 2 (4%) |
| Inflammation, suppurative | | 1 (2%) | 1 (2%) |
| Abscess, NOS | 8 (16%) | 3 (6%) | 4 (8%) |
| Hyperplasia, NOS | | | 1 (2%) |
| Hyperplasia, epithelial | 1 (2%) | | |
| #Prostate | (48) | (46) | (43) |
| Inflammation, suppurative | | 2 (4%) | 3 (7%) |
| Abscess, NOS | 1 (2%) | | |
| Inflammation, chronic | 1 (2%) | 1 (2%) | |
| Hyperplasia, NOS | 1 (2%) | | |
| Hyperplasia, focal | | | 1 (2%) |
| *Seminal vesicle | (50) | (50) | (50) |
| Dilatation, NOS | 4 (8%) | 5 (10%) | |
| #Testis | (49) | (49) | (46) |
| Calcification, focal | 16 (33%) | 14 (29%) | 10 (22%) |
| Atrophy, NOS | 1 (2%) | 1 (2%) | |
| Hyperplasia, interstitial cell | 1 (2%) | | |
| #Testis/tubule | (49) | (49) | (46) |
| Cytomegaly | | 1 (2%) | |
| *Epididymis | (50) | (50) | (50) |
| Granuloma, spermatic | | 2 (4%) | |
| NERVOUS SYSTEM | | | |
| #Brain | (50) | (49) | (47) |
| Calcification, focal | 30 (60%) | 21 (43%) | 9 (19%) |
| SPECIAL SENSE ORGANS | | | |
| None | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| *Sternum | (50) | (50) | (50) |
| Necrosis, NOS | 2 (4%) | | |
| *Skeletal muscle | (50) | (50) | (50) |
| Degeneration, NOS | | | 1 (2%) |
| BODY CAVITIES | | | |
| *Mediastinum | (50) | (50) | (50) |
| Hemorrhage | 1 (2%) | 1 (2%) | |
| Inflammation, suppurative | 2 (4%) | 4 (8%) | |
| Abscess, NOS | 1 (2%) | | |
| Inflammation, acute/chronic | 1 (2%) | | |
| Foreign material, NOS | 2 (4%) | 3 (6%) | |
| *Peritoneum | (50) | (50) | (50) |
| Inflammation, suppurative | | 1 (2%) | |
| *Pleura | (50) | (50) | (50) |
| Inflammation, suppurative | 1 (2%) | 2 (4%) | |
| Inflammation, acute/chronic | 1 (2%) | 1 (2%) | |
| *Epicardium | (50) | (50) | (50) |
| Inflammation, acute/chronic | | 1 (2%) | |
| *Mesentery | (50) | (50) | (50) |
| Necrosis, fat | 2 (4%) | | |

TABLE C5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|----------------------------|-----------------|----------|-----------|
| ALL OTHER SYSTEMS | | | |
| Tail | | | |
| Granuloma, NOS | | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| Auto/necropsy/no histo | | | 3 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.
 # Number of animals examined microscopically at this site

APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

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TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS MISSING | 2 | | 1 |
| ANIMALS NECROPSIED | 48 | 50 | 49 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 48 | 50 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (48) | (50) | (49) |
| Basal cell carcinoma | | 1 (2%) | |
| *Subcutaneous tissue | (48) | (50) | (49) |
| Fibrosarcoma | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #Lung | (48) | (50) | (49) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 5 (10%) | 1 (2%) |
| Alveolar/bronchiolar carcinoma | 2 (4%) | | |
| Adenosquamous carcinoma, metastatic | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *Multiple organs | (48) | (50) | (49) |
| Malignant lymphoma, lymphocytic type | 4 (8%) | 3 (6%) | 1 (2%) |
| Malignant lymphoma, histiocytic type | 3 (6%) | 3 (6%) | 1 (2%) |
| Malignant lymphoma, mixed type | 6 (13%) | 7 (14%) | |
| Lymphocytic leukemia | | 1 (2%) | |
| Granulocytic leukemia | 1 (2%) | | |
| #Mesenteric lymph node | (43) | (49) | (39) |
| Malignant lymphoma, NOS | | | 1 (3%) |
| Malignant lymphoma, mixed type | 1 (2%) | | |
| #Duodenum | (46) | (47) | (34) |
| Malignant lymphoma, histiocytic type | | 1 (2%) | |
| #Thymus | (46) | (44) | (49) |
| Sarcoma, NOS | | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| *Multiple organs | (48) | (50) | (49) |
| Hemangiosarcoma | 1 (2%) | | |
| *Mediastinum | (48) | (50) | (49) |
| Hemangioma | 1 (2%) | | |
| #Ovary | (44) | (48) | (49) |
| Hemangioma | | 1 (2%) | |
| #Adrenal | (48) | (50) | (49) |
| Hemangioma | | | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #Liver | (48) | (50) | (49) |
| Hepatocellular adenoma | 3 (6%) | 5 (10%) | |
| #Forestomach | (47) | (49) | (46) |
| Papilloma, NOS | 1 (2%) | | |
| URINARY SYSTEM | | | |
| None | | | |

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------|-----------------|----------|-----------|
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (46) | (50) | (45) |
| Carcinoma, NOS | 1 (2%) | | |
| Adenoma, NOS | 5 (11%) | 3 (6%) | |
| #Adrenal | (48) | (50) | (49) |
| Cortical adenoma | | 1 (2%) | |
| #Adrenal/capsule | (48) | (50) | (49) |
| Adenoma, NOS | 1 (2%) | | |
| #Adrenal medulla | (48) | (50) | (49) |
| Pheochromocytoma | | 1 (2%) | |
| #Thyroid | (47) | (49) | (40) |
| Follicular cell adenoma | | | 1 (3%) |
| #Pancreatic islets | (47) | (50) | (49) |
| Islet cell adenoma | 1 (2%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (48) | (50) | (49) |
| Adenoma, NOS | 1 (2%) | | |
| Adenocarcinoma, NOS | | 2 (4%) | 1 (2%) |
| Adenosquamous carcinoma | | | 1 (2%) |
| #Uterus/endometrium | (48) | (50) | (49) |
| Adenocarcinoma, NOS | | 2 (4%) | |
| #Ovary | (44) | (48) | (49) |
| Granulosa cell tumor | 1 (2%) | 2 (4%) | |
| Tubular adenoma | | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| #Brain | (48) | (50) | (49) |
| Carcinoma, NOS, invasive | 1 (2%) | | |
| #Cerebellum | (48) | (50) | (49) |
| Neoplasm, NOS | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *Harderian gland | (48) | (50) | (49) |
| Adenoma, NOS | 1 (2%) | 1 (2%) | |
| MUSCULOSKELETAL SYSTEM | | | |
| None | | | |
| BODY CAVITIES | | | |
| *Abdominal wall | (48) | (50) | (49) |
| Sarcoma, NOS | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| None | | | |

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|--|-----------------|----------|-----------|
| ANIMAL DISPOSITION SUMMARY | | | |
| Animals initially in study | 50 | 50 | 50 |
| Natural death | 4 | 5 | 17 |
| Moribund sacrifice | 3 | 9 | 7 |
| Terminal sacrifice | 41 | 30 | 2 |
| Dosing accident | | 4 | 22 |
| Accidentally killed, nda | | 2 | 1 |
| Animal missing | 2 | | 1 |
| TUMOR SUMMARY | | | |
| Total animals with primary tumors** | 27 | 31 | 8 |
| Total primary tumors | 36 | 42 | 9 |
| Total animals with benign tumors | 14 | 14 | 3 |
| Total benign tumors | 15 | 18 | 3 |
| Total animals with malignant tumors | 18 | 20 | 5 |
| Total malignant tumors | 20 | 22 | 5 |
| Total animals with secondary tumors## | 1 | | 1 |
| Total secondary tumors | 1 | | 1 |
| Total animals with tumors uncertain-- benign or malignant | 1 | 2 | 1 |
| Total uncertain tumors | 1 | 2 | 1 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.

** Primary tumors: all tumors except secondary tumors

Number of animals examined microscopically at this site

Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE: VEHICLE CONTROL

| ANIMAL NUMBER | 0 | | | | | | | | | | | | | | | | | | | |
|--------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 3 4 6 0 6 6 7 6 1 2 3 4 5 7 8 9 1 2 3 4 5 8 9 0 | | | | | | | | | | | | | | | | | | | |
| WEEKS ON STUDY | 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 | | | | | | | | | | | | | | | | | | | |
| | 3 4 1 3 2 3 9 2 2 5 5 5 5 5 5 5 5 5 5 5 | | | | | | | | | | | | | | | | | | | |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibrosarcoma | | | | | | | | | | | X | | | | | | | | | |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | X | | | | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | |
| Trachea | M | M | X | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | M | M | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Malignant lymphoma, mixed type | | | | | | | | | | | | X | | | | | | | | |
| Thymus | M | M | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Heart | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | X |
| Bile duct | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pancreas | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | M | M | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | M | M | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Papilloma, NOS | | | | | | | | | | | | | | | | | | | | |
| Small intestine | M | M | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | M | M | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Kidney | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Pituitary | M | M | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | X | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | X | | | | | | | | |
| Adrenal | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | X | | | | | | | |
| Thyroid | M | M | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Parathyroid | M | M | - | - | - | + | - | + | + | + | + | + | - | - | - | - | - | - | - | - |
| Pancreatic islets | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Islet cell adenoma | | | | | | | | | | | X | | | | | | | | | |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | X | |
| Uterus | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Ovary | M | M | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Granulosa cell tumor | | | | | | | | | | | | | | | | | | | | |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | |
| Brain | M | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS, invasive | | | | | | | | | | | X | | | | | | | | | |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | M | M | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | |
| Mediastinum | M | M | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Hemangioma | | | | | | | | | | | | | | | | | | | | |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | |
| Multiple organs, NOS | M | M | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Hemangiosarcoma | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, lymphocytic type | | | | | | | | | | | | | | | | | | | | |
| Malignant lymphoma, histiocytic type | | | X | | | | X | | | | X | | | | | | | | | |
| Malignant lymphoma, mixed type | | | | | | | | | | | | | | | | | | | | |
| Granulocytic leukemia | | | | | | | | | | | | | | | | | | | X | X |

+: Tissue examined microscopically
 -: Required tissue not examined microscopically
 X: Tumor incidence
 N: Necropsy, no autolysis, no microscopic examination
 S: Animal missexed

: No tissue information submitted
 C: Necropsy, no histology due to protocol
 A: Autolysis
 M: Animal missing
 B: No necropsy performed

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | 1,000 mg/kg | 2,000 mg/kg |
|---|-----------------|-------------|-------------|
| Lung: Alveolar/Bronchiolar Adenoma | | | |
| Overall Rates (a) | 1/48 (2%) | 5/50 (10%) | 1/49 (2%) |
| Adjusted Rates (b) | 2.4% | 14.0% | 2.3% |
| Terminal Rates (c) | 1/41 (2%) | 2/30 (7%) | 0/2 (0%) |
| Week of First Observation | 105 | 93 | 45 |
| Life Table Tests (d) | P=0.051 | P=0.062 | P=0.514 |
| Incidental Tumor Tests (d) | P=0.292 | P=0.194 | P=0.984N |
| Cochran-Armitage Trend Test (d) | P=0.585N | | |
| Fisher Exact Test (d) | | P=0.112 | P=0.747N |
| Lung: Alveolar/Bronchiolar Adenoma or Carcinoma | | | |
| Overall Rates (a) | 3/48 (6%) | 5/50 (10%) | 1/49 (2%) |
| Adjusted Rates (b) | 6.9% | 14.0% | 2.3% |
| Terminal Rates (c) | 2/41 (5%) | 2/30 (7%) | 0/2 (0%) |
| Week of First Observation | 61 | 93 | 45 |
| Life Table Tests (d) | P=0.224 | P=0.250 | P=0.683 |
| Incidental Tumor Tests (d) | P=0.403N | P=0.526 | P=0.153N |
| Cochran-Armitage Trend Test (d) | P=0.254N | | |
| Fisher Exact Test (d) | | P=0.381 | P=0.301N |
| Hematopoietic System: Malignant Lymphoma, Lymphocytic Type | | | |
| Overall Rates (a) | 4/48 (8%) | 3/50 (6%) | 1/49 (2%) |
| Adjusted Rates (b) | 9.1% | 8.1% | 2.1% |
| Terminal Rates (c) | 2/41 (5%) | 1/30 (3%) | 0/2 (0%) |
| Week of First Observation | 92 | 35 | 32 |
| Life Table Tests (d) | P=0.585 | P=0.610N | P=0.619 |
| Incidental Tumor Tests (d) | P=0.020N | P=0.242N | P=0.157N |
| Cochran-Armitage Trend Test (d) | P=0.126N | | |
| Fisher Exact Test (d) | | P=0.477N | P=0.175N |
| Hematopoietic System: Malignant Lymphoma, Histiocytic Type | | | |
| Overall Rates (a) | 3/48 (6%) | 4/50 (8%) | 1/49 (2%) |
| Adjusted Rates (b) | 6.6% | 10.6% | 33.3% |
| Terminal Rates (c) | 1/41 (2%) | 1/30 (3%) | 0/2 (0%) |
| Week of First Observation | 61 | 93 | 103 |
| Life Table Tests (d) | P=0.224 | P=0.409 | P=0.475 |
| Incidental Tumor Tests (d) | P=0.441N | P=0.499N | P=0.616N |
| Cochran-Armitage Trend Test (d) | P=0.243N | | |
| Fisher Exact Test (d) | | P=0.523 | P=0.301N |
| Hematopoietic System: Malignant Lymphoma, Mixed Type | | | |
| Overall Rates (a) | 7/48 (15%) | 7/50 (14%) | 0/49 (0%) |
| Adjusted Rates (b) | 17.1% | 21.9% | 0.0% |
| Terminal Rates (c) | 7/41 (17%) | 6/30 (20%) | 0/2 (0%) |
| Week of First Observation | 105 | 93 | |
| Life Table Tests (d) | P=0.520 | P=0.380 | P=0.632N |
| Incidental Tumor Tests (d) | P=0.582 | P=0.443 | P=0.632N |
| Cochran-Armitage Trend Test (d) | P=0.011N | | |
| Fisher Exact Test (d) | | P=0.581N | P=0.006N |
| Hematopoietic System: Lymphoma, All Malignant | | | |
| Overall Rates (a) | 14/48 (29%) | 14/50 (28%) | 3/49 (6%) |
| Adjusted Rates (b) | 30.9% | 37.0% | 42.9% |
| Terminal Rates (c) | 10/41 (24%) | 8/30 (27%) | 0/2 (0%) |
| Week of First Observation | 61 | 35 | 32 |
| Life Table Tests (d) | P=0.150 | P=0.311 | P=0.213 |
| Incidental Tumor Tests (d) | P=0.118N | P=0.397N | P=0.127N |
| Cochran-Armitage Trend Test (d) | P=0.004N | | |
| Fisher Exact Test (d) | | P=0.538N | P=0.003N |

TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | 1,000 mg/kg | 2,000 mg/kg |
|---|-----------------|-------------|-------------|
| Hematopoietic System: Lymphoma or Leukemia | | | |
| Overall Rates (a) | 15/48 (31%) | 15/50 (30%) | 3/49 (6%) |
| Adjusted Rates (b) | 33.1% | 38.4% | 42.9% |
| Terminal Rates (c) | 11/41 (27%) | 8/30 (27%) | 0/2 (0%) |
| Week of First Observation | 61 | 35 | 32 |
| Life Table Tests (d) | P=0.165 | P=0.305 | P=0.228 |
| Incidental Tumor Tests (d) | P=0.062N | P=0.396N | P=0.120N |
| Cochran-Armitage Trend Test (d) | P=0.002N | | |
| Fisher Exact Test (d) | | P=0.534N | P=0.002N |
| Liver: Hepatocellular Adenoma | | | |
| Overall Rates (a) | 3/48 (6%) | 5/50 (10%) | 0/49 (0%) |
| Adjusted Rates (b) | 7.3% | 16.7% | 0.0% |
| Terminal Rates (c) | 3/41 (7%) | 5/30 (17%) | 0/2 (0%) |
| Week of First Observation | 105 | 105 | |
| Life Table Tests (d) | P=0.301 | P=0.199 | P=0.844N |
| Incidental Tumor Tests (d) | P=0.301 | P=0.199 | P=0.844N |
| Cochran-Armitage Trend Test (d) | P=0.126N | | |
| Fisher Exact Test (d) | | P=0.381 | P=0.118N |
| Pituitary Gland: Adenoma | | | |
| Overall Rates (a) | 5/46 (11%) | 3/50 (6%) | 0/45 (0%) |
| Adjusted Rates (b) | 12.5% | 10.0% | 0.0% |
| Terminal Rates (c) | 5/40 (13%) | 3/30 (10%) | 0/2 (0%) |
| Week of First Observation | 105 | 105 | |
| Life Table Tests (d) | P=0.426N | P=0.521N | P=0.719N |
| Incidental Tumor Tests (d) | P=0.426N | P=0.521N | P=0.719N |
| Cochran-Armitage Trend Test (d) | P=0.022N | | |
| Fisher Exact Test (d) | | P=0.311N | P=0.029N |
| Pituitary Gland: Adenoma or Carcinoma | | | |
| Overall Rates (a) | 6/46 (13%) | 3/50 (6%) | 0/45 (0%) |
| Adjusted Rates (b) | 14.5% | 10.0% | 0.0% |
| Terminal Rates (c) | 5/40 (13%) | 3/30 (10%) | 0/2 (0%) |
| Week of First Observation | 102 | 105 | |
| Life Table Tests (d) | P=0.312N | P=0.397N | P=0.649N |
| Incidental Tumor Tests (d) | P=0.239N | P=0.309N | P=0.541N |
| Cochran-Armitage Trend Test (d) | P=0.010N | | |
| Fisher Exact Test (d) | | P=0.203N | P=0.014N |
| Ovary: Granulosa Cell Tumor or Tubular Adenoma | | | |
| Overall Rates (a) | 1/44 (2%) | 3/48 (6%) | 0/49 (0%) |
| Adjusted Rates (b) | 2.6% | 10.0% | 0.0% |
| Terminal Rates (c) | 1/39 (3%) | 3/30 (10%) | 0/2 (0%) |
| Week of First Observation | 105 | 105 | |
| Life Table Tests (d) | P=0.295 | P=0.216 | P=0.982N |
| Incidental Tumor Tests (d) | P=0.295 | P=0.216 | P=0.982N |
| Cochran-Armitage Trend Test (d) | P=0.344N | | |
| Fisher Exact Test (d) | | P=0.342 | P=0.473N |

(a) Number of tumor-bearing animals/number of animals examined at the site

(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality

(c) Observed tumor incidence at terminal kill

(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE

| | Vehicle Control | Low Dose | High Dose |
|--------------------------------------|-----------------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS MISSING | 2 | | 1 |
| ANIMALS NECROPSIED | 48 | 50 | 49 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 48 | 50 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *Skin | (48) | (50) | (49) |
| Epidermal inclusion cyst | | 1 (2%) | |
| Ulcer, NOS | | 1 (2%) | |
| Inflammation, chronic focal | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| *Nasal cavity | (48) | (50) | (49) |
| Inflammation, suppurative | | 1 (2%) | |
| Inflammation, acute/chronic | | 1 (2%) | |
| *Nasal gland | (48) | (50) | (49) |
| Necrosis, focal | 1 (2%) | | |
| *Nasal turbinate | (48) | (50) | (49) |
| Reaction, foreign body | | | 1 (2%) |
| #Bronchial mucous gland | (48) | (50) | (49) |
| Dilatation, NOS | 1 (2%) | | |
| #Lung | (48) | (50) | (49) |
| Atelectasis | | | 1 (2%) |
| Congestion, NOS | | 2 (4%) | 14 (29%) |
| Hemorrhage | | 1 (2%) | |
| Lymphocytic inflammatory infiltrate | 2 (4%) | 1 (2%) | 3 (6%) |
| Inflammation, chronic focal | | | 1 (2%) |
| #Lung/alveoli | (48) | (50) | (49) |
| Histiocytosis | 2 (4%) | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| #Bone marrow | (48) | (49) | (49) |
| Granuloma, NOS | | | 1 (2%) |
| Myelofibrosis | 34 (71%) | 37 (76%) | 9 (18%) |
| Hyperplasia, granulocytic | 1 (2%) | | |
| #Spleen | (48) | (50) | (49) |
| Hemosiderosis | 2 (4%) | 6 (12%) | 3 (6%) |
| Hyperplasia, lymphoid | 6 (13%) | 2 (4%) | 3 (6%) |
| Hematopoiesis | 37 (77%) | 45 (90%) | 46 (94%) |
| #Splenic follicles | (48) | (50) | (49) |
| Necrosis, NOS | | 2 (4%) | |
| #Lymph node | (43) | (49) | (39) |
| Hemorrhage | | 1 (2%) | |
| Hyperplasia, NOS | | | 1 (3%) |
| Plasmacytosis | | 1 (2%) | |
| Hyperplasia, lymphoid | 1 (2%) | | |
| #Mandibular lymph node | (43) | (49) | (39) |
| Fibrosis | 1 (2%) | | |
| #Mediastinal lymph node | (43) | (49) | (39) |
| Hyperplasia, NOS | 1 (2%) | | |
| #Lumbar lymph node | (43) | (49) | (39) |
| Histiocytosis | | 1 (2%) | |
| Plasmacytosis | | 1 (2%) | 1 (3%) |
| #Mesenteric lymph node | (43) | (49) | (39) |
| Hemorrhage | 1 (2%) | | |
| Histiocytosis | | | 1 (3%) |
| Hyperplasia, lymphoid | | | 1 (3%) |
| #Lung | (48) | (50) | (49) |
| Hematopoiesis | | 1 (2%) | |

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|---|-----------------|----------|-----------|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #Liver | (48) | (50) | (49) |
| Hematopoiesis | | | 1 (2%) |
| #Ileum | (46) | (47) | (34) |
| Hyperplasia, lymphoid | | | 1 (3%) |
| #Thymus | (46) | (44) | (49) |
| Inflammation, suppurative | | 1 (2%) | |
| Atrophy, NOS | 1 (2%) | | |
| Hyperplasia, lymphoid | 1 (2%) | | |
| CIRCULATORY SYSTEM | | | |
| #Lung | (48) | (50) | (49) |
| Embolism, NOS | 1 (2%) | | |
| #Myocardium | (48) | (50) | (49) |
| Degeneration, NOS | | 1 (2%) | |
| #Cardiac valve | (48) | (50) | (49) |
| Pigmentation, NOS | | | 1 (2%) |
| *Aorta | (48) | (50) | (49) |
| Calcification, focal | | 1 (2%) | |
| #Liver | (48) | (50) | (49) |
| Thrombus, fibrin | | 1 (2%) | |
| #Pancreas | (47) | (50) | (49) |
| Periarteritis | | 1 (2%) | |
| DIGESTIVE SYSTEM | | | |
| *Intestinal tract | (48) | (50) | (49) |
| Infarct, NOS | 1 (2%) | | |
| #Salivary gland | (47) | (49) | (49) |
| Fibrosis | | 1 (2%) | |
| #Liver | (48) | (50) | (49) |
| Lymphocytic inflammatory infiltrate | 3 (6%) | | |
| Inflammation, chronic focal | 3 (6%) | | 2 (4%) |
| Necrosis, focal | 1 (2%) | 2 (4%) | |
| Lipoidosis | | 2 (4%) | |
| Cytoplasmic vacuolization | 1 (2%) | | |
| Basophilic cyto change | 1 (2%) | 2 (4%) | |
| Ground glass cyto change | 3 (6%) | 3 (6%) | |
| Focal cellular change | | | 1 (2%) |
| Angiectasis | 1 (2%) | | |
| #Liver/centrilobular | (48) | (50) | (49) |
| Necrosis, NOS | | 1 (2%) | |
| Hepatocytomegaly | 1 (2%) | | |
| #Liver/periportal | (48) | (50) | (49) |
| Necrosis, NOS | 1 (2%) | | |
| Atrophy, NOS | 1 (2%) | | |
| *Gallbladder | (48) | (50) | (49) |
| Inflammation, suppurative | 1 (2%) | | |
| #Pancreas | (47) | (50) | (49) |
| Dilatation/ducts | 2 (4%) | 2 (4%) | |
| Cyst, NOS | 1 (2%) | | |
| Inflammation, acute/chronic | 1 (2%) | | |
| Metamorphosis, fatty | | 2 (4%) | |
| #Pancreatic acinus | (47) | (50) | (49) |
| Necrosis, focal | | 1 (2%) | |
| Cytoplasmic vacuolization | 1 (2%) | | |
| Atrophy, NOS | 2 (4%) | | |
| Atrophy, focal | | 1 (2%) | |
| #Esophageal adventitia | (47) | (50) | (48) |
| Inflammation, suppurative | | 1 (2%) | |

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| DIGESTIVE SYSTEM (Continued) | | | |
| #Gastric fundal gland | (47) | (49) | (46) |
| Retention of content | | 1 (2%) | |
| #Glandular stomach | (47) | (49) | (46) |
| Dilatation, NOS | 1 (2%) | 2 (4%) | |
| Ulcer, NOS | | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | | |
| Calcification, focal | | | 1 (2%) |
| #Gastric submucosa | (47) | (49) | (46) |
| Cyst, NOS | | 1 (2%) | |
| #Forestomach | (47) | (49) | (46) |
| Ulcer, NOS | | 1 (2%) | |
| Inflammation, focal | 1 (2%) | | |
| Erosion | | | 1 (2%) |
| Hyperplasia, epithelial | | 1 (2%) | |
| #Peyer's patch | (46) | (47) | (34) |
| Ulcer, NOS | | 1 (2%) | |
| Inflammation, acute/chronic | | 1 (2%) | |
| #Duodenum | (46) | (47) | (34) |
| Polyp, NOS | | 1 (2%) | |
| *Rectum | (48) | (50) | (49) |
| Ulcer, NOS | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #Kidney | (48) | (50) | (49) |
| Hydronephrosis | 1 (2%) | | |
| Pyelonephritis, NOS | | 1 (2%) | |
| Lymphocytic inflammatory infiltrate | 2 (4%) | | 2 (4%) |
| Metaplasia, osseous | 2 (4%) | 2 (4%) | |
| #Kidney/cortex | (48) | (50) | (49) |
| Atrophy, focal | | 3 (6%) | 1 (2%) |
| #Renal papilla | (48) | (50) | (49) |
| Calcification, NOS | | | 1 (2%) |
| #Urinary bladder | (46) | (49) | (43) |
| Lymphocytic inflammatory infiltrate | 1 (2%) | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #Anterior pituitary | (46) | (50) | (45) |
| Hyperplasia, NOS | | 1 (2%) | |
| Hyperplasia, focal | 9 (20%) | 8 (16%) | 1 (2%) |
| Angiectasis | | 1 (2%) | |
| #Adrenal | (48) | (50) | (49) |
| Congestion, NOS | | | 2 (4%) |
| Hemorrhage | 1 (2%) | | |
| Atrophy, brown | 1 (2%) | | |
| #Adrenal/capsule | (48) | (50) | (49) |
| Hyperplasia, NOS | 44 (92%) | 43 (86%) | 18 (37%) |
| Hyperplasia, focal | | 1 (2%) | |
| #Adrenal cortex | (48) | (50) | (49) |
| Hemorrhage | | 1 (2%) | |
| Degeneration, NOS | 1 (2%) | | |
| Hypertrophy, focal | | 1 (2%) | |
| Hyperplasia, focal | | 2 (4%) | |
| Metaplasia, osseous | 1 (2%) | | |
| #Adrenal medulla | (48) | (50) | (49) |
| Hemorrhage | | 1 (2%) | |
| Hyperplasia, focal | 2 (4%) | 1 (2%) | |

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-------------------------------------|-----------------|----------|-----------|
| ENDOCRINE SYSTEM (Continued) | | | |
| #Thyroid | (47) | (49) | (40) |
| Atrophy, focal | 1 (2%) | 1 (2%) | |
| Hyperplasia, C-cell | | 1 (2%) | |
| Hyperplasia, follicular cell | 1 (2%) | 1 (2%) | |
| #Parathyroid | (27) | (32) | (19) |
| Hyperplasia, focal | 1 (4%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *Mammary gland | (48) | (50) | (49) |
| Lactation | | 1 (2%) | |
| *Mammary duct | (48) | (50) | (49) |
| Hyperplasia, NOS | | | 1 (2%) |
| *Mammary lobule | (48) | (50) | (49) |
| Hyperplasia, NOS | 2 (4%) | | |
| #Uterus | (48) | (50) | (49) |
| Dilatation, NOS | | 2 (4%) | |
| Inflammation, suppurative | | 1 (2%) | 1 (2%) |
| Abscess, NOS | 1 (2%) | | |
| Polyp, NOS | | | 1 (2%) |
| #Cervix uteri | (48) | (50) | (49) |
| Inflammation, suppurative | 1 (2%) | | 1 (2%) |
| Inflammation, chronic | 1 (2%) | | |
| Hyperplasia, epithelial | 3 (6%) | | 1 (2%) |
| #Uterus/endometrium | (48) | (50) | (49) |
| Hyperplasia, NOS | | 1 (2%) | |
| Hyperplasia, cystic | 45 (94%) | 43 (86%) | 35 (71%) |
| #Endometrial gland | (48) | (50) | (49) |
| Dilatation, NOS | | 1 (2%) | |
| #Uterus/myometrium | (48) | (50) | (49) |
| Degeneration, mucoid | | 1 (2%) | |
| #Ovary/parovarian | (44) | (48) | (49) |
| Fibrosis | 1 (2%) | | |
| #Ovary | (44) | (48) | (49) |
| Cyst, NOS | 8 (18%) | 11 (23%) | |
| Hemorrhagic cyst | 1 (2%) | | |
| Abscess, NOS | 3 (7%) | 1 (2%) | 2 (4%) |
| Inflammation, acute/chronic | | | 1 (2%) |
| Atrophy, NOS | | 1 (2%) | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #Brain/meninges | (48) | (50) | (49) |
| Perivascular cuffing | | | 1 (2%) |
| #Brain | (48) | (50) | (49) |
| Epidermal inclusion cyst | 1 (2%) | | |
| Hemorrhage | | | 1 (2%) |
| Calcification, focal | 24 (50%) | 24 (48%) | 13 (27%) |
| SPECIAL SENSE ORGANS | | | |
| *Eye | (48) | (50) | (49) |
| Cataract | | 1 (2%) | |
| *Eye/cornea | (48) | (50) | (49) |
| Inflammation, chronic focal | | 1 (2%) | |
| *Nasolacrimal duct | (48) | (50) | (49) |
| Inflammation, suppurative | | | 2 (4%) |
| *Ear | (48) | (50) | (49) |
| Inflammation, suppurative | | 1 (2%) | |

TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF DIMETHYL METHYLPHOSPHONATE (Continued)

| | Vehicle Control | Low Dose | High Dose |
|-----------------------------------|-----------------|----------|-----------|
| MUSCULOSKELETAL SYSTEM | | | |
| *Sternum | (48) | (50) | (49) |
| Necrosis, NOS | 1 (2%) | 1 (2%) | |
| BODY CAVITIES | | | |
| *Mediastinum | (48) | (50) | (49) |
| Inflammation, suppurative | | 3 (6%) | 1 (2%) |
| Foreign material, NOS | | 2 (4%) | |
| *Abdominal cavity | (48) | (50) | (49) |
| Inflammation, suppurative | | | 1 (2%) |
| *Peritoneum | (48) | (50) | (49) |
| Inflammation, suppurative | 1 (2%) | 1 (2%) | |
| *Pericardium | (48) | (50) | (49) |
| Inflammation, suppurative | | 2 (4%) | |
| Foreign material, NOS | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| Tail | | | |
| Necrosis, NOS | | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| Animal missing/no necropsy | 2 | | 1 |

* Number of animals receiving complete necropsy examination; all gross lesions including masses examined microscopically.

Number of animals examined microscopically at this site

APPENDIX E

GENETIC TOXICOLOGY OF

DIMETHYL METHYLPHOSPHONATE

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TABLE E1. MUTAGENICITY OF DIMETHYL METHYLPHOSPHONATE IN *SALMONELLA TYPHIMURIUM* (a)

| Strain | Dose ($\mu\text{g}/\text{plate}$) | Revertants/plate (b) | | | | | |
|--------|--|------------------------------|------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | | -S9 | | +S9 (hamster) | | +S9 (rat) | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| TA100 | 0 | 129 \pm 4.3 | 130 \pm 6.2 | 125 \pm 1.7 | 132 \pm 4.4 | 142 \pm 6.1 | 129 \pm 0.9 |
| | 100 | 138 \pm 3.8 | 135 \pm 8.7 | 141 \pm 9.4 | 118 \pm 5.0 | 145 \pm 8.3 | 133 \pm 4.7 |
| | 333 | 138 \pm 3.1 | 117 \pm 7.0 | 137 \pm 11.0 | 119 \pm 6.8 | 145 \pm 5.8 | 118 \pm 10.7 |
| | 1,000 | 140 \pm 9.3 | 133 \pm 2.7 | 143 \pm 11.6 | 132 \pm 4.3 | 151 \pm 6.9 | 140 \pm 6.7 |
| | 3,333 | 142 \pm 6.5 | 129 \pm 6.6 | 129 \pm 11.1 | 135 \pm 7.6 | 136 \pm 4.0 | 125 \pm 10.1 |
| | 10,000 | 149 \pm 9.3 | 138 \pm 2.8 | 131 \pm 2.6 | 132 \pm 3.5 | 135 \pm 3.4 | 127 \pm 4.6 |
| | Trial Summary Positive control (c) | Negative 1,066 \pm 24.3 | Negative 934 \pm 19.0 | Negative 891 \pm 18.7 | Negative 527 \pm 84.8 | Negative 784 \pm 10.7 | Negative 650 \pm 67.0 |
| TA1535 | 0 | 26 \pm 2.3 | 26 \pm 2.4 | 11 \pm 2.3 | 12 \pm 1.5 | 13 \pm 3.3 | 12 \pm 1.9 |
| | 100 | 25 \pm 3.8 | 25 \pm 2.6 | 8 \pm 0.7 | 8 \pm 0.9 | 13 \pm 2.1 | 10 \pm 0.3 |
| | 333 | 27 \pm 5.0 | 21 \pm 2.5 | 8 \pm 1.2 | 10 \pm 1.7 | 11 \pm 1.2 | 13 \pm 0.9 |
| | 1,000 | 26 \pm 2.6 | 26 \pm 3.2 | 9 \pm 1.3 | 9 \pm 1.9 | 13 \pm 1.2 | 13 \pm 1.7 |
| | 3,333 | 28 \pm 3.2 | 25 \pm 2.3 | 10 \pm 0.6 | 8 \pm 1.9 | 11 \pm 1.2 | 9 \pm 0.6 |
| | 10,000 | 27 \pm 4.7 | 22 \pm 1.5 | 12 \pm 0.7 | 12 \pm 1.9 | 14 \pm 2.6 | 10 \pm 2.1 |
| | Trial Summary Positive control (c) | Negative 823 \pm 9.9 | Negative 689 \pm 35.2 | Negative 59 \pm 6.8 | Negative 30 \pm 3.3 | Negative 62 \pm 8.5 | Negative 27 \pm 2.2 |
| TA1537 | 0 | 7 \pm 0.7 | 6 \pm 1.5 | 4 \pm 1.3 | 8 \pm 0.9 | 8 \pm 0.9 | 11 \pm 2.6 |
| | 100 | 8 \pm 1.2 | 5 \pm 2.3 | 8 \pm 0.6 | 9 \pm 1.8 | 5 \pm 0.7 | 9 \pm 2.1 |
| | 333 | 7 \pm 0.3 | 5 \pm 1.3 | 8 \pm 0.3 | 9 \pm 2.7 | 7 \pm 2.3 | 7 \pm 0.6 |
| | 1,000 | 4 \pm 0.6 | 5 \pm 1.0 | 7 \pm 1.2 | 9 \pm 3.5 | 7 \pm 0.3 | 8 \pm 1.2 |
| | 3,333 | 10 \pm 2.2 | 7 \pm 0.3 | 7 \pm 0.6 | 9 \pm 2.7 | 11 \pm 2.3 | 6 \pm 0.7 |
| | 10,000 | 6 \pm 1.5 | 8 \pm 0.0 | 5 \pm 1.2 | 9 \pm 1.7 | 8 \pm 0.7 | 10 \pm 0.3 |
| | Trial Summary Positive control (c) | Negative 231 \pm 57.8 | Negative 294 \pm 52.6 | Negative 68 \pm 8.2 | Negative 74 \pm 4.8 | Negative 52 \pm 4.7 | Negative 63 \pm 4.2 |
| TA98 | 0 | 21 \pm 0.3 | 19 \pm 3.4 | 31 \pm 2.6 | 30 \pm 2.6 | 31 \pm 3.2 | 28 \pm 1.7 |
| | 100 | 20 \pm 1.5 | 14 \pm 0.9 | 30 \pm 3.6 | 27 \pm 0.7 | 38 \pm 4.1 | 23 \pm 3.8 |
| | 333 | 22 \pm 3.1 | 15 \pm 3.8 | 26 \pm 3.2 | 27 \pm 1.9 | 35 \pm 2.9 | 21 \pm 4.4 |
| | 1,000 | 21 \pm 4.4 | 15 \pm 0.3 | 32 \pm 3.1 | 30 \pm 3.0 | 31 \pm 2.3 | 31 \pm 4.3 |
| | 3,333 | 24 \pm 4.1 | 17 \pm 3.5 | 33 \pm 2.3 | 23 \pm 2.2 | 25 \pm 2.0 | 27 \pm 2.0 |
| | 10,000 | 18 \pm 2.2 | 18 \pm 5.1 | 25 \pm 2.6 | 25 \pm 1.0 | 34 \pm 3.2 | 20 \pm 1.5 |
| | Trial Summary Positive control (c) | Negative 1,352 \pm 60.1 | Negative 1,129 \pm 34.0 | Negative 816 \pm 30.5 | Negative 396 \pm 61.9 | Negative 697 \pm 6.7 | Negative 447 \pm 41.3 |

(a) Study performed at EG&G Mason Research Institute. The detailed protocol is presented in Haworth et al. (1983). Cells and study compound or solvent (distilled water) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 $\mu\text{g}/\text{plate}$ dose is the solvent control.

(b) Revertants are presented as mean \pm standard error from three plates.

(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

TABLE E2. MUTAGENICITY OF DIMETHYL METHYLPHOSPHONATE IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

| Compound | Concentration (µl/ml) | Cloning Efficiency (percent) | Relative Total Growth | Mutant Count | Mutant Fraction (c) |
|----------------------------|-----------------------|------------------------------|-----------------------|--------------|---------------------|
| -S9 | | | | | |
| Trial 1 | | | | | |
| Distilled water | | 67.5 ± 5.0 | 100.0 ± 5.4 | 86.0 ± 1.9 | 43.3 ± 3.0 |
| Dimethyl methylphosphonate | | | | | |
| | (d) 0.25 | 62.0 ± 13.6 | 106.7 ± 14.1 | 62.3 ± 5.2 | 36.3 ± 6.2 |
| | 0.5 | 63.7 ± 2.0 | 87.7 ± 5.8 | 94.3 ± 5.6 | 49.3 ± 1.3 |
| | 1.0 | 64.7 ± 12.0 | 75.7 ± 12.8 | 108.7 ± 10.2 | 58.0 ± 5.9 |
| | 1.5 | 70.3 ± 11.3 | 61.0 ± 10.0 | 157.7 ± 22.4 | 75.3 ± 5.9 |
| | 3.0 | 74.7 ± 6.6 | 76.0 ± 7.4 | 185.0 ± 5.1 | (e) 83.3 ± 4.9 |
| | 5.0 | 78.7 ± 5.2 | 69.0 ± 6.7 | 209.7 ± 15.4 | (e) 89.7 ± 10.7 |
| Methyl methanesulfonate | | | | | |
| | 5.0 µg/ml | 54.7 ± 10.5 | 36.7 ± 10.9 | 592.0 ± 27.2 | (e) 387.0 ± 68.2 |
| Trial 2 | | | | | |
| Distilled water | | 85.8 ± 6.5 | 100.3 ± 10.7 | 58.8 ± 3.9 | 23.5 ± 3.1 |
| Dimethyl methylphosphonate | | | | | |
| | 0.25 | 76.3 ± 7.7 | 83.0 ± 7.2 | 87.7 ± 12.2 | (e) 40.7 ± 10.2 |
| | 0.5 | 58.3 ± 11.6 | 73.7 ± 7.9 | 83.3 ± 12.2 | (e) 48.7 ± 4.1 |
| | 1.0 | 59.0 ± 3.0 | 72.5 ± 10.5 | 87.5 ± 13.5 | (e) 50.5 ± 10.5 |
| | (d) 2.0 | 66.7 ± 4.2 | 67.3 ± 3.3 | 105.0 ± 5.0 | (e) 53.0 ± 2.5 |
| | 3.0 | 61.0 ± 5.5 | 62.3 ± 6.4 | 116.7 ± 19.2 | (e) 62.7 ± 5.8 |
| | 5.0 | 86.5 ± 6.5 | 48.5 ± 4.5 | 198.0 ± 12.0 | (e) 77.0 ± 10.0 |
| Methyl methanesulfonate | | | | | |
| | 5.0 µg/ml | 72.7 ± 5.9 | 50.3 ± 3.2 | 315.3 ± 22.1 | (e) 145.3 ± 2.8 |

(a) Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. Cells (6×10^5 /ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in non-selective medium and soft agar to determine the cloning efficiency.

(b) Mean ± standard error of replicate trials of approximately 3×10^6 cells each. All data are evaluated statistically for both trend and peak response. Both responses must be significantly ($P < 0.05$) positive for a chemical to be considered mutagenic. If only one of these responses is significant, the call is "questionable"; the absence of both trend and peak response results in a "negative" call.

(c) Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.

(d) Acidic pH shift at this and all higher doses for this trial

(e) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY DIMETHYL METHYLPHOSPHONATE (a)

| | Dose (µg/ml) | Total Cells | No. of Chromo- somes | No. of SCEs | SCEs/ Chromo- some | SCEs/ Cell | Hours in BrdU | Relative SCEs/cell (percent) (b) |
|--|-----------------|----------------|----------------------------|----------------|--------------------------|---------------|------------------|--|
| -S9 (c) | | | | | | | | |
| Study Performed at Environmental Health Research and Testing Laboratory | | | | | | | | |
| Trial No. 1--Summary: Positive | | | | | | | | |
| Medium | | 50 | 1,034 | 375 | 0.36 | 7.5 | 26.5 | |
| Dimethyl methyl- phosphonate | 160 | 50 | 1,032 | 370 | 0.36 | 7.4 | 26.5 | 98.7 |
| | 500 | 50 | 1,038 | 446 | 0.43 | 8.9 | 26.5 | 118.7 |
| | 1,600 | 50 | 1,039 | 580 | 0.56 | 11.6 | 26.5 | 154.7 |
| | 5,000 | 50 | 1,036 | 884 | 0.85 | 17.7 | 26.5 | 236.0 |
| Mitomycin C | 0.01 | 50 | 1,042 | 2,461 | 2.36 | 49.2 | 26.5 | 656.0 |
| Trial No. 2--Summary: Positive | | | | | | | | |
| Medium | | 50 | 1,033 | 378 | 0.37 | 7.6 | 26.0 | |
| Dimethyl methyl- phosphonate | 2,000 | 50 | 1,038 | 580 | 0.56 | 11.6 | 26.0 | 152.6 |
| | 3,000 | 50 | 1,031 | 679 | 0.66 | 13.6 | 26.0 | 178.9 |
| | 4,000 | 50 | 1,003 | 729 | 0.73 | 14.6 | 26.0 | 192.1 |
| | 5,000 | 50 | 1,023 | 845 | 0.83 | 16.9 | 26.0 | 222.4 |
| Mitomycin C | 0.005 | 50 | 1,039 | 1,301 | 1.25 | 26.0 | 26.0 | 342.1 |
| +S9 (d) | | | | | | | | |
| Study Performed at Environmental Health Research and Testing Laboratory | | | | | | | | |
| Trial No. 1--Summary: Negative | | | | | | | | |
| Medium | | 50 | 1,042 | 383 | 0.37 | 7.7 | 26.5 | |
| Dimethyl methyl- phosphonate | 160 | 50 | 1,049 | 376 | 0.36 | 7.5 | 26.5 | 97.4 |
| | 500 | 50 | 1,046 | 393 | 0.38 | 7.9 | 26.5 | 102.6 |
| | 1,600 | 50 | 1,052 | 415 | 0.39 | 8.3 | 26.5 | 107.8 |
| | 5,000 | 50 | 1,048 | 401 | 0.38 | 8.0 | 26.5 | 103.9 |
| Cyclophosphamide | 1.5 | 50 | 1,052 | 1,123 | 1.07 | 22.5 | 26.5 | 292.2 |
| Trial No. 2--Summary: Negative | | | | | | | | |
| Medium | | 50 | 1,044 | 437 | 0.42 | 8.7 | 26.0 | |
| Dimethyl methyl- phosphonate | 2,000 | 50 | 1,029 | 447 | 0.43 | 8.9 | 26.0 | 102.3 |
| | 3,000 | 50 | 1,027 | 409 | 0.40 | 8.2 | 26.0 | 94.3 |
| | 4,000 | 50 | 1,045 | 397 | 0.38 | 7.9 | 26.0 | 90.8 |
| | 5,000 | 50 | 1,028 | 435 | 0.42 | 8.7 | 26.0 | 100.0 |
| Cyclophosphamide | 1.5 | 50 | 1,042 | 1,430 | 1.37 | 28.6 | 26.0 | 328.7 |

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY DIMETHYL METHYLPHOSPHONATE (Continued)

| | Dose ($\mu\text{g/ml}$) | Total Cells | No. of Chromo- somes | No. of SCEs | SCEs/ Chromo- some | SCEs/ Cell | Hours in BrdU | Relative SCEs/cell (percent) (b) |
|--|------------------------------|----------------|----------------------------|----------------|--------------------------|---------------|------------------|--|
| -S9 (c) | | | | | | | | |
| Study performed at Litton Bionetics, Inc. | | | | | | | | |
| Trial No. 1--Summary: Positive | | | | | | | | |
| Water | | 50 | 1,021 | 383 | 0.38 | 7.7 | 25.5 | |
| Dimethyl methyl- phosphonate | 1,100 | 50 | 1,034 | 402 | 0.39 | 8.0 | 25.5 | 103.9 |
| | 3,667 | 50 | 1,031 | 679 | 0.66 | 13.6 | 25.5 | 176.6 |
| | 11,000 | 50 | 1,024 | 1,365 | 1.33 | 27.3 | 25.5 | 354.5 |
| Mitomycin C | 0.001 | 50 | 1,040 | 545 | 0.52 | 10.9 | 25.5 | 141.6 |
| | 0.010 | 5 | 104 | 202 | 1.94 | 40.4 | 25.5 | 524.7 |
| +S9 (d) | | | | | | | | |
| Study performed at Litton Bionetics, Inc. | | | | | | | | |
| Trial No. 1--Summary: Weakly positive | | | | | | | | |
| Water | | 50 | 1,035 | 420 | 0.41 | 8.4 | 25.5 | |
| Dimethyl methyl- phosphonate | 1,100 | 50 | 1,031 | 403 | 0.39 | 8.1 | 25.5 | 96.4 |
| | 3,670 | 50 | 1,036 | 409 | 0.39 | 8.2 | 25.5 | 97.6 |
| | 11,000 | 50 | 1,036 | 504 | 0.49 | 10.1 | 25.5 | 120.2 |
| Cyclophosphamide | 0.3 | 50 | 1,040 | 628 | 0.60 | 12.6 | 25.5 | 150.0 |
| | 2 | 5 | 103 | 129 | 1.25 | 25.8 | 25.5 | 307.1 |
| Trial No. 2--Summary: Positive | | | | | | | | |
| Medium | | 50 | 1,033 | 409 | 0.40 | 8.2 | 25.5 | |
| Dimethyl methyl- phosphonate | 14,300 | 50 | 1,019 | 515 | 0.51 | 10.3 | 25.5 | 125.6 |
| | 17,600 | 50 | 1,037 | 617 | 0.59 | 12.3 | 25.5 | 150.0 |
| | 22,000 | 50 | 1,032 | 624 | 0.60 | 12.5 | 25.5 | 152.4 |
| Cyclophosphamide | 0.3 | 50 | 1,031 | 564 | 0.55 | 11.3 | 25.5 | 137.8 |
| | 2 | 5 | 103 | 104 | 1.01 | 20.8 | 25.5 | 253.7 |

(a) SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent as described in (c) and (d) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake off, fixed, air dried, and stained.

(b) SCEs/cell in treated culture expressed as a percent of the SCEs/cell in the control culture

(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.

(d) In the presence of S9, cells were incubated with study compound or solvent for 2 hours at 37° C. Cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE E4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY DIMETHYL METHYLPHOSPHONATE (a)

| Environmental Health Research and Testing Laboratory Study | | | | | Litton Bionetics, Inc., Study | | | | |
|---|----------------|---------------|--------------|------------------------------|--------------------------------------|----------------|---------------|--------------|------------------------------|
| Dose (µg/ml) | Total Cells | No. of Abs | Abs/ Cell | Percent Cells with Abs | Dose (µg/ml) | Total Cells | No. of Abs | Abs/ Cell | Percent Cells with Abs |
| -S9 (b)--Harvest time: 12.0 hours | | | | | -S9--Harvest time: 10.5 hours | | | | |
| Medium | 100 | 0 | 0.00 | 0 | Medium | 100 | 2 | 0.02 | 2 |
| Dimethyl methylphosphonate | | | | | Dimethyl methylphosphonate | | | | |
| 2,000 | 100 | 0 | 0.00 | 0 | 16,500 | 100 | 1 | 0.01 | 1 |
| 3,000 | 100 | 2 | 0.02 | 2 | 19,300 | 100 | 5 | 0.05 | 4 |
| 4,000 | 100 | 2 | 0.02 | 2 | 22,000 | 100 | 5 | 0.05 | 5 |
| 5,000 | 100 | 3 | 0.03 | 3 | | | | | |
| Summary: Negative | | | | | Summary: Negative | | | | |
| Mitomycin C | | | | | Mitomycin C | | | | |
| 0.500 | 100 | 96 | 0.96 | 57 | 0.500 | 50 | 9 | 0.18 | 16 |
| +S9 (c)--Harvest time: 12.0 hours | | | | | +S9--Harvest time: 10.5 hours | | | | |
| Medium | 100 | 0 | 0.00 | 0 | Medium | 100 | 0 | 0.00 | 0 |
| Dimethyl methylphosphonate | | | | | Dimethyl methylphosphonate | | | | |
| 2,000 | 100 | 0 | 0.00 | 0 | 14,300 | 100 | 1 | 0.01 | 1 |
| 3,000 | 100 | 4 | 0.04 | 3 | 17,600 | 100 | 0 | 0.00 | 0 |
| 4,000 | 100 | 3 | 0.03 | 3 | 22,000 | 100 | 3 | 0.03 | 3 |
| 5,000 | 100 | 2 | 0.02 | 2 | | | | | |
| Summary: Negative | | | | | Summary: Negative | | | | |
| Cyclophosphamide | | | | | Cyclophosphamide | | | | |
| 50 | 100 | 57 | 0.57 | 39 | 25 | 50 | 23 | 0.46 | 26 |

(a) Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or medium as indicated in (b) and (c). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake off, fixed, and stained in 6% Giemsa.

(b) In the absence of S9, cells were incubated with study compound or medium for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.

(c) In the presence of S9, cells were incubated with study compound or medium for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation prior to harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

TABLE E5. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY DIMETHYL METHYLPHOSPHONATE (a)

| Route of Exposure | Dose (ppm) | Incidence of Deaths (percent) | Incidence of Sterility (percent) | No. of Lethals/No. of X Chromosomes Tested | | | Overall Total (b) |
|-------------------|------------|-------------------------------|----------------------------------|--|----------|----------|-------------------|
| | | | | Mating 1 | Mating 2 | Mating 3 | |
| Feeding | 23,735 | 57 | 0 | 19/2,012 | 21/3,250 | 2/716 | 42/5,978 (0.70%) |
| | 0 | | | 1/2,003 | 3/2,929 | 1/942 | 5/5,874 (0.09%) |

(a) Study performed at Brown University. A detailed protocol of the sex-linked recessive lethal assay is presented in Zimmering et al. (1985). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the study chemical dissolved in 5% sucrose. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F₁ heterozygous females were crossed to their siblings and placed in individual vials. F₁ daughters from the same parental male were kept together to identify clusters; no clusters were found. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were significant at the 5% level (Margolin et al., 1983).

(b) Combined total of number of lethal mutations/number of X chromosomes tested for three mating trials

TABLE E6. INDUCTION OF RECIPROCAL TRANSLOCATIONS IN DROSOPHILA BY DIMETHYL METHYLPHOSPHONATE (a)

| Route of Exposure | Dose (ppm) | Transfers (translocations/total F ₁ tested) | | | | | Total No. of Tests | Total No. of Translocations | Total Translocations (percent) |
|--------------------|------------|--|---------|---------|-------|-------|--------------------|-----------------------------|--------------------------------|
| | | 1 | 2 | 3 | 4 | 5 | | | |
| Feeding | 23,500 | 0/2,466 | 0/2,323 | 0/2,953 | 0/900 | 0/131 | 8,773 | 0 | 0.0000 |
| Historical control | 0 | | | | | | 104,844 | 2 | 0.0019 |

(a) Study performed at Brown University. A detailed protocol of the reciprocal translocation assay is presented in Zimmering et al. (1985). Exposed males were mated to three *bw;st* females for 3 days and discarded. The females were transferred to fresh medium every 3-4 days to produce a total of five cultures, and then they were discarded. In this manner, sample sperm from successive cultures were stored for increasing lengths of time. Individual F₁ males were backcrossed to *bw;st* females, and the F₂ generation was screened for pseudolinkage. This procedure allows the recovery of translocations involving the Y, second, or third chromosomes in any combination. Presumptive translocations were retested. Results were not significant at the 5% level (Kastenbaum and Bowman, 1970).

APPENDIX F

SENTINEL ANIMAL PROGRAM

APPENDIX F. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen B6C3F₁ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected vehicle control animals of each sex and species. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the antibody titers. The following tests are performed:

| | <u>Hemagglutination Inhibition</u> | <u>Complement Fixation</u> | <u>ELISA</u> |
|------|---|---|---|
| Mice | PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai | M.Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) MHV (mouse hepatitis virus) | MHV <i>M. Pul. (Mycoplasma pulmonis)</i> |
| Rats | PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai (6, 18, 24 mo) | RCV (rat coronavirus) Sendai (12 mo) | |

II. Results

Five of 10 mice tested at 18 months had a positive serologic reaction for *Mycoplasma pulmonis*. No positive results were seen at 6, 12, 18, or 24 months in rats or at 6, 12, or 24 months in mice.

Mycoplasma pulmonis infection-related lesions were not observed in the rats and mice in these studies. Further evaluation of the reagents used for detection of *M. pulmonis* by ELISA indicated that the reagents may not be specific for detection of antibodies to *M. pulmonis*.

APPENDIX G

INGREDIENTS, NUTRIENT COMPOSITION, AND

CONTAMINANT LEVELS

IN NIH 07 RAT AND MOUSE RATION

Pellet Diet: April 1981 to September 1983
(Manufactured by Zeigler Bros., Inc., Gardners, PA)

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TABLE G1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

| Ingredients (b) | Percent by Weight |
|--|-------------------|
| Ground #2 yellow shelled corn | 24.50 |
| Ground hard winter wheat | 23.00 |
| Soybean meal (49% protein) | 12.00 |
| Fish meal (60% protein) | 10.00 |
| Wheat middlings | 10.00 |
| Dried skim milk | 5.00 |
| Alfalfa meal (dehydrated, 17% protein) | 4.00 |
| Corn gluten meal (60% protein) | 3.00 |
| Soy oil | 2.50 |
| Brewer's dried yeast | 2.00 |
| Dry molasses | 1.50 |
| Dicalcium phosphate | 1.25 |
| Ground limestone | 0.50 |
| Salt | 0.50 |
| Premixes (vitamin and mineral) | 0.25 |

(a) NIH, 1978; NCI, 1976

(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE G2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

| | Amount | Source |
|---|---------------|---|
| Vitamins | | |
| A | 5,500,000 IU | Stabilized vitamin A palmitate or acetate |
| D ₃ | 4,600,000 IU | D-activated animal sterol |
| K ₃ | 2.8 g | Menadione activity |
| <i>d</i> - α -Tocopheryl acetate | 20,000 IU | |
| Choline | 560.0 g | Choline chloride |
| Folic acid | 2.2 g | |
| Niacin | 30.0 g | |
| <i>d</i> -Pantothenic acid | 18.0 g | <i>d</i> -Calcium pantothenate |
| Riboflavin | 3.4 g | |
| Thiamine | 10.0 g | Thiamine mononitrate |
| B ₁₂ | 4,000 μ g | |
| Pyridoxine | 1.7 g | Pyridoxine hydrochloride |
| Biotin | 140.0 mg | <i>d</i> -Biotin |
| Minerals | | |
| Iron | 120.0 g | Iron sulfate |
| Manganese | 60.0 g | Manganous oxide |
| Zinc | 16.0 g | Zinc oxide |
| Copper | 4.0 g | Copper sulfate |
| Iodine | 1.4 g | Calcium iodate |
| Cobalt | 0.4 g | Cobalt carbonate |

(a) Per ton (2,000 lb) of finished product

TABLE G3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION

| Nutrient | Mean \pm Standard Deviation | Range | Number of Samples |
|--|-------------------------------|-----------------|-------------------|
| Crude protein (percent by weight) | 23.63 \pm 0.85 | 22.2-25.3 | 29 |
| Crude fat (percent by weight) | 4.9 \pm 0.51 | 3.3-5.7 | 29 |
| Crude fiber (percent by weight) | 3.33 \pm 0.25 | 2.9-3.8 | 29 |
| Ash (percent by weight) | 6.51 \pm 0.43 | 5.7-7.31 | 29 |
| Essential Amino Acids (percent of total diet) | | | |
| Arginine | 1.323 \pm 0.830 | 1.21-1.39 | 4 |
| Cystine | 0.310 \pm 0.099 | 0.218-0.400 | 4 |
| Glycine | 1.155 \pm 0.069 | 1.06-1.21 | 4 |
| Histidine | 0.572 \pm 0.030 | 0.530-0.603 | 4 |
| Isoleucine | 0.910 \pm 0.033 | 0.881-0.944 | 4 |
| Leucine | 1.949 \pm 0.065 | 1.85-1.99 | 4 |
| Lysine | 1.275 \pm 0.076 | 1.20-1.37 | 4 |
| Methionine | 0.422 \pm 0.187 | 0.306-0.699 | 4 |
| Phenylalanine | 0.909 \pm 0.167 | 0.665-1.04 | 4 |
| Threonine | 0.844 \pm 0.029 | 0.824-0.886 | 4 |
| Tryptophan | 0.187 | 0.171-0.211 | 3 |
| Tyrosine | 0.631 \pm 0.094 | 0.566-0.769 | 4 |
| Valine | 1.11 \pm 0.050 | 1.05-1.17 | 4 |
| Essential Fatty Acids (percent of total diet) | | | |
| Linoleic | 2.44 | 2.37-2.52 | 3 |
| Linolenic | 0.274 | 0.256-0.308 | 3 |
| Arachidonic | 0.008 | | 1 |
| Vitamins | | | |
| Vitamin A (IU/kg) | 11,655 \pm 4,113 | 7,500-24,000 | 29 |
| Vitamin D (IU/kg) | 4,650 | 3,000-6,300 | 2 |
| α -Tocopherol (ppm) | 41.53 \pm 7.52 | 31.1-48.9 | 4 |
| Thiamine (ppm) | 16.2 \pm 2.17 | 12.0-21.0 | 28 |
| Riboflavin (ppm) | 7.5 \pm 0.96 | 6.1-8.2 | 4 |
| Niacin (ppm) | 85.0 \pm 14.2 | 65.0-97.0 | 4 |
| Pantothenic acid (ppm) | 29.3 \pm 4.6 | 23.0-34.0 | 4 |
| Pyridoxine (ppm) | 7.6 \pm 1.5 | 5.6-8.8 | 4 |
| Folic acid (ppm) | 2.8 \pm 0.88 | 1.8-3.7 | 4 |
| Biotin (ppm) | 0.27 \pm 0.05 | 0.21-0.32 | 4 |
| Vitamin B ₁₂ (ppb) | 21.0 \pm 11.9 | 11.0-38.0 | 4 |
| Choline (ppm) | 3,302.0 \pm 120.0 | 3,200.0-3,430.0 | 4 |
| Minerals | | | |
| Calcium (percent) | 1.25 \pm 0.12 | 1.08-1.53 | 29 |
| Phosphorus (percent) | 0.99 \pm 0.05 | 0.88-1.11 | 29 |
| Potassium (percent) | 0.862 \pm 0.100 | 0.772-0.974 | 3 |
| Chloride (percent) | 0.546 \pm 0.100 | 0.442-0.635 | 4 |
| Sodium (percent) | 0.311 \pm 0.038 | 0.258-0.350 | 4 |
| Magnesium (percent) | 0.169 \pm 0.133 | 0.151-0.181 | 4 |
| Sulfur (percent) | 0.316 \pm 0.070 | 0.270-0.420 | 4 |
| Iron (ppm) | 447.0 \pm 57.3 | 409.0-523.0 | 4 |
| Manganese (ppm) | 90.6 \pm 8.20 | 81.7-95.5 | 4 |
| Zinc (ppm) | 53.6 \pm 5.27 | 46.1-58.6 | 4 |
| Copper (ppm) | 10.77 \pm 3.19 | 8.09-15.39 | 4 |
| Iodine (ppm) | 2.95 \pm 1.05 | 1.52-3.82 | 4 |
| Chromium (ppm) | 1.81 \pm 0.28 | 1.44-2.09 | 4 |
| Cobalt (ppm) | 0.68 \pm 0.14 | 0.49-0.80 | 4 |

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (a)

| Contaminant | Mean \pm Standard Deviation | Range | Number of Samples |
|--|-------------------------------|--------------------------------|-------------------|
| Arsenic (ppm) | 0.50 \pm 0.13 | 0.29-0.77 | 29 |
| Cadmium (ppm) (a) | <0.10 | <0.1-0.1 | 29 |
| Lead (ppm) (b) | 0.71 \pm 0.39 | 0.33-1.97 | 27 |
| Lead (ppm) (c) | 0.87 \pm 0.71 | 0.33-3.37 | 29 |
| Mercury (ppm) (a) | <0.05 | | 29 |
| Selenium (ppm) | 0.29 \pm 0.06 | 0.13-0.40 | 29 |
| Aflatoxins (ppb) (d) | <10 | <5.0- <10.0 | 29 |
| Nitrate nitrogen (ppm) (e) | 9.55 \pm 4.46 | <0.1-22.0 | 29 |
| Nitrite nitrogen (ppm) (f) | 2.25 \pm 1.77 | <0.1-7.2 | 29 |
| BHA (ppm) (g) | 5.43 \pm 4.72 | 0.4-17.0 | 29 |
| BHT (ppm) (h) | 2.7 \pm 1.82 | <1.0-12.0 | 28 |
| | 3.0 \pm 2.5 | <1.0-5.9 | 29 |
| Aerobic plate count (CFU/g) | 46,810 \pm 34,504 | 6,600-130,000 | 29 |
| Coliform (MPN/g) (i) | 13.25 \pm 21.07 | <3-93 | 28 |
| Coliform (MPN/g) (j) | 28.66 \pm 85.50 | <3-460 | 29 |
| <i>E. coli</i> (MPN/g) | <3 | <2-3 | 29 |
| Total nitrosamines (ppb) (k,l) | 3.44 \pm 2.68 | 0.8-9.3 | 28 |
| Total nitrosamines (ppb) (k,m) | 12.96 \pm 51.33 | 0.8-279.5 | 29 |
| <i>N</i> -Nitrosodimethylamine (ppb) (k,n) | 2.78 \pm 2.39 | 0.8-8.3 | 28 |
| <i>N</i> -Nitrosodimethylamine (ppb) (k,o) | 12.27 \pm 51.16 | 0.8-278.0 | 29 |
| <i>N</i> -Nitrosopyrrolidine (ppb) (p) | 1.16 \pm 0.49 | <0.9-2.9 | 25 |
| Pesticides (ppm) | | | |
| α -BHC (a,q) | <0.01 | | 29 |
| β -BHC (a) | <0.02 | | 29 |
| γ -BHC-Lindane (a) | <0.01 | | 29 |
| δ -BHC (a) | <0.01 | | 29 |
| Heptachlor (a) | <0.01 | | 29 |
| Aldrin (a) | <0.01 | | 29 |
| Heptachlor epoxide (a) | <0.01 | | 29 |
| DDE (a) | <0.01 | | 29 |
| DDD (a) | <0.01 | | 29 |
| DDT (a) | <0.01 | | 29 |
| HCB (a) | <0.01 | | 29 |
| Mirex (a) | <0.01 | | 29 |
| Methoxychlor (r) | <0.05 | 0.09 (8/26/81); 0.06 (7/26/83) | 29 |
| Dieldrin (a) | <0.01 | | 29 |
| Endrin (a) | <0.01 | | 29 |
| Telodrin (a) | <0.01 | | 29 |
| Chlordane (a) | <0.05 | | 29 |
| Toxaphene (a) | <0.1 | | 29 |
| Estimated PCBs (a) | <0.2 | | 29 |
| Ronnel (a) | <0.01 | | 29 |
| Ethion (a) | <0.02 | | 29 |
| Trithion (a) | <0.05 | | 29 |
| Diazinon (a) | <0.1 | | 29 |
| Methyl parathion (a) | <0.02 | | 29 |
| Ethyl parathion (a) | <0.02 | | 29 |
| Malathion (s) | 0.10 \pm 0.09 | <0.05-0.42 | 29 |
| Endosulfan I (a) | <0.01 | | 29 |
| Endosulfan II (a) | <0.01 | | 29 |
| Endosulfan sulfate (a) | <0.03 | | 29 |

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

- (a) All values were less than the detection limit. The detection limit is given as the mean.
- (b) Excludes two high values of 2.65 ppm and 3.37 ppm obtained in batches produced on 8/26/81 and on 7/21/82.
- (c) Includes the high values given in b.
- (d) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
- (e) Sources of contamination: alfalfa, grains, and fish meal
- (f) Sources of contamination: soy oil and fish meal
- (g) Mean, standard deviation, and range exclude one very high value of 12.0 obtained for the batch produced on 11/23/82.
- (h) Mean, standard deviation, and range include the high value listed in footnote g.
- (i) MPN = most probable number; mean, standard deviation, and range exclude one very high value of 460 MPN/g obtained in the batch produced on 9/23/82.
- (j) Mean, standard deviation, and range include the high value listed in footnote i.
- (k) All values were corrected for percent recovery.
- (l) Mean, standard deviation, and range exclude one very high value of 279.5 ppb obtained for the batch produced on 4/27/81.
- (m) Mean, standard deviation, and range include the high value listed in footnote l.
- (n) Mean, standard deviation, and range exclude one very high value of 278 obtained for the batch produced on 4/27/81.
- (o) Mean, standard deviation, and range include the high value given in footnote n.
- (p) Samples analyzed from batches produced on 6/22/83, 7/26/83, 8/17/83, and 9/20/83 were below the detection limit (1.0 ppb).
- (q) BHC = hexachlorocyclohexane or benzene hexachloride
- (r) Two observations were above the detection limit. The values and the date are given under the range.
- (s) Fourteen batches contained more than 0.05 ppm.

APPENDIX H

DATA AUDIT SUMMARY

APPENDIX H. DATA AUDIT SUMMARY

The experimental data, documents, pathology materials, and draft NTP Technical Report for the 2-year toxicology and carcinogenesis studies of dimethyl methylphosphonate in rats and mice were audited for accuracy, consistency, completeness, and compliance with Good Laboratory Practice regulations of the Food and Drug Administration (implemented by the NTP beginning October 1, 1981). The laboratory experiments were conducted for the NTP by Litton Bionetics, Inc., Rockville, Maryland, under a subcontract with Tracor Jitco, Inc. Animal dosing with dimethyl methylphosphonate began in July 1981 and ended November 1983. The retrospective audit was conducted at the NTP Archives in February 1986 by Program Resources, Inc. The following individuals were involved with the audit: W.L. Oller, Ph.D. (Principal Investigator); K.A. Connor; J.E. Kovach, B.A.; S.A. Corson, H.T. (ASCP); K.M. Pace, B.S.; and C.D. Rafferty, A.S.; and the following personnel from Veritas Laboratories: J.W. Sagartz, D.V.M., ACVP; and N.J. MacLachlan, D.V.M., ACVP.

The full report of the audit is on file at the NIEHS. The audit included a review of:

- (1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
- (2) All chemistry records.
- (3) Body weights and clinical observation data for a random 10% sample of the study animals.
- (4) All inlife records concerning environmental conditions, palpable masses, mortality, and animal identification.
- (5) All postmortem records for individual animals concerning identification, disposition codes, condition codes, correct data entry, and correlation between gross observations and microscopic diagnoses.
- (6) Wet tissues from a random 10% sample of the study animals and from animals that had a gross observation without a corresponding microscopic diagnosis to verify animal identification and to examine for untrimmed lesions.
- (7) Slides and blocks of tissues from all vehicle control and high dose animals to examine for proper match and inventory.
- (8) Tabulated pathology diagnoses for a random 10% of study animals to verify computer data entry.

The audit showed that the data in the Technical Report (including inlife observations and chemistry data) reflect the data at the NTP Archives.

Animal identification was confirmed. The audit revealed untrimmed lesions in the wet tissues. All wet tissues were then examined by an NTP pathology support contractor, and any lesions found were sectioned. NTP pathology staff provided the diagnoses for these additional lesions, and these are incorporated in the Technical Report. This additional pathology evaluation and review did not change the interpretation of the studies.

The NIEHS/NTP concludes that the documents and materials at the NTP Archives support the data and results presented in this Technical Report.

**NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PUBLISHED AS OF OCTOBER 1987**

| TR No. | CHEMICAL | TR No. | CHEMICAL |
|--------|---|--------|---|
| 201 | 2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin (Dermal) | 261 | Chlorobenzene |
| 206 | Dibromochloropropane | 263 | 1,2-Dichloropropane |
| 207 | Cytembena | 267 | Propylene Oxide |
| 208 | FD & C Yellow No. 6 | 269 | Telone II® |
| 209 | 2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin (Gavage) | 271 | HC Blue No. 1 |
| 210 | 1,2-Dibromoethane (Inhalation) | 272 | Propylene |
| 211 | C.I. Acid Orange 10 | 274 | Tris(2-ethylhexyl)phosphate |
| 212 | Di(2-ethylhexyl)adipate | 275 | 2-Chloroethanol |
| 213 | Butylbenzyl Phthalate | 276 | 8-Hydroxyquinoline |
| 214 | Caprolactam | 281 | H.C. Red No. 3 |
| 215 | Bisphenol A | 282 | Chlorodibromomethane |
| 216 | 11-Aminoundecanoic Acid | 284 | Diallylphthalate (Rats) |
| 217 | Di(2-ethylhexyl)phthalate | 285 | C.I. Basic Red 9 Monohydrochloride |
| 219 | 2,6-Dichloro- <i>p</i> -phenylenediamine | 287 | Dimethyl Hydrogen Phosphite |
| 220 | C.I. Acid Red 14 | 288 | 1,3-Butadiene |
| 221 | Locust Bean Gum | 289 | Benzene |
| 222 | C.I. Disperse Yellow 3 | 291 | Isophorone |
| 223 | Eugenol | 293 | HC Blue No. 2 |
| 224 | Tara Gum | 294 | Chlorinated Trisodium Phosphate |
| 225 | D & C Red No. 9 | 295 | Chrysotile Asbestos (Rats) |
| 226 | C.I. Solvent Yellow 14 | 296 | Tetrakis(hydroxymethyl)phosphonium Sulfate and Tetrakis(hydroxymethyl)phosphonium Chloride |
| 227 | Gum Arabic | 298 | Dimethyl Morpholinophosphoramidate |
| 228 | Vinylidene Chloride | 299 | C.I. Disperse Blue 1 |
| 229 | Guar Gum | 300 | 3-Chloro-2-methylpropene |
| 230 | Agar | 301 | <i>o</i> -Phenylphenol |
| 231 | Stannous Chloride | 303 | 4-Vinylcyclohexene |
| 232 | Pentachloroethane | 304 | Chlorendic Acid |
| 233 | 2-Biphenylamine Hydrochloride | 305 | Chlorinated Paraffins (C ₂₃ , 43% chlorine) |
| 234 | Allyl Isothiocyanate | 306 | Dichloromethane |
| 235 | Zearalenone | 307 | Ephedrine Sulfate |
| 236 | D-Mannitol | 308 | Chlorinated Paraffins (C ₁₂ , 60% chlorine) |
| 237 | 1,1,1,2-Tetrachloroethane | 309 | Decabromodiphenyl Oxide |
| 238 | Ziram | 310 | Marine Diesel Fuel and JP-5 Navy Fuel |
| 239 | Bis(2-chloro-1-methylethyl)ether | 311 | Tetrachloroethylene (Inhalation) |
| 240 | Propyl Gallate | 312 | <i>n</i> -Butyl Chloride |
| 242 | Diallyl Phthalate (Mice) | 314 | Methyl Methacrylate |
| 244 | Polybrominated Biphenyl Mixture | 315 | Oxytetracycline Hydrochloride |
| 245 | Melamine | 316 | 1-Chloro-2-methylpropene |
| 247 | L-Ascorbic Acid | 317 | Chlorpheniramine Maleate |
| 248 | 4,4'-Methylenedianiline Dihydrochloride | 318 | Ampicillin Trihydrate |
| 249 | Amosite Asbestos | 319 | 1,4-Dichlorobenzene |
| 250 | Benzyl Acetate | 321 | Bromodichloromethane |
| 251 | Toluene Diisocyanate | 322 | Phenylephrine Hydrochloride |
| 252 | Geranyl Acetate | 324 | Boric Acid |
| 253 | Allyl Isovalerate | 325 | Pentachloronitrobenzene |
| 255 | 1,2-Dichlorobenzene | 327 | Xylenes (Mixed) |
| 257 | Diglycidyl Resorcinol Ether | | |
| 259 | Ethyl Acrylate | | |

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