NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 284

# TOXICOLOGY AND CARCINOGENESIS STUDIES OF DIALLYLPHTHALATE

## (CAS NO. 131-17-9)

## IN F344/N RATS

## (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 DIALLYLPHTHALATE

(CAS NO. 131-17-9)

# IN F344/N RATS

(GAVAGE STUDIES)


NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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## NOTE TO THE READER

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 testing in the NTP Carcinogenesis Program 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 test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical has the potential for hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

Five categories of interpretative conclusions were adopted for use in June 1983 in the Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice), one of the following quintet will be 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 Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of malignant neoplasms, studies that exhibit a substantially increased incidence of benign neoplasms, or studies that exhibit an increased incidence of a combination of malignant and benign neoplasms where each increases with dose.
- **Some Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of benign neoplasms, studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.
- **Equivocal Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related marginal increase of neoplasms.
- **No Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenicity** demonstrates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

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 earlier induction by chemicals of neoplasms that are commonly observed, or the induction by chemicals of more neoplasms than are generally found. 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.

This study was initiated by the National Cancer Institute's Carcinogenesis Bioassay Program, now part of the National Institute of Environmental Health Sciences, 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. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for 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).

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

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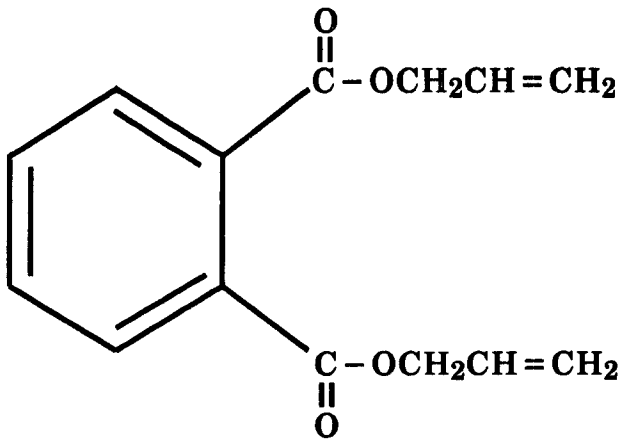

# DIALLYLPHTHALATE

CAS NO. 131-17-9

$C_{14}H_{14}O_4$ Mol Wt 246.24

Synonym: DAP

## ABSTRACT


Toxicology and carcinogenesis studies of diallylphthalate (approximately 99% pure) were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats at doses of 0 (vehicle control), 50, or 100 mg/kg 5 days per week for 103 weeks. The diallylphthalate doses used in the 2-year studies were chosen on the basis of 13-week studies, wherein doses of 200 or 400 mg/kg caused death, reductions in body weight gains, or periportal hepatocellular necrosis and fibrosis in both sexes.

Mean body weights and survival of male and female rats administered diallylphthalate were essentially the same as those of the vehicle controls throughout the 2-year studies, although hepatotoxicity was produced in both sexes by the 100 mg/kg dose. Based on the results of the prechronic studies and the effects on the liver in the 2-year studies, the doses used in the 2-year studies were considered to be adequate for carcinogenicity testing.

Male and female rats receiving the 100 mg/kg dose of diallylphthalate in the 2-year studies developed chronic liver disease characterized by periportal fibrosis, periportal accumulation of pigment, and severe bile duct hyperplasia. Pigment accumulation also occurred at the 50 mg/kg dose in both sexes.

Diallylphthalate administration increased the occurrence of mononuclear cell leukemia in female rats ($P<0.05$ by trend tests), and the incidence in the 100 mg/kg dose female rats was greater ($P\leq0.05$) than in the vehicle controls by pairwise comparisons (vehicle control, 15/50, 30%; low dose, 15/43, 35%; high dose, 25/49, 51%). An increased occurrence of mononuclear cell leukemia was not observed in male rats receiving diallylphthalate.

A previous NTP carcinogenesis study (NTP TR 242) reported an increased incidence of lymphomas in male B6C3F$_1$ mice receiving diallylphthalate by gavage for 2 years at doses of 0, 150, or 300 mg/kg. This increase was considered to be equivocally related to diallylphthalate administration. The incidences of hyperplasia and inflammatory lesions of the forestomach were increased in a dose-related fashion in both sexes of mice in that study, and uncommon forestomach papillomas were observed in 0%, 2%, and 4% of both sexes of mice. Because of the numerical increase in forestomach papillomas, the concomitant presence of forestomach hyperplasia, and the rarity of forestomach papillomas in vehicle control (corn oil gavage) B6C3F$_1$ mice, the development of these proliferative lesions of the forestomach in mice may have been related to diallylphthalate administration. In the current study in rats, a squamous cell carcinoma was found in one high dose male rat.

Diallylphthalate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without activation by a 9,000 $\times$ *g* supernatant fraction from the livers of Aroclor 1254-treated male Sprague-Dawley rats or Syrian hamsters. Diallylphthalate did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster.*

An audit of the experimental data was conducted for these carcinogenesis studies on diallylphthalate. No data discrepancies were found that influenced the final interpretations.

Under the conditions of this study, the administration of diallylphthalate by gavage in corn oil to male and female F344/N rats for 2 years caused chronic liver disease characterized by periportal fibrosis and pigment accumulation and an increased severity of bile duct hyperplasia. The incidence of mononuclear cell leukemia was significantly increased in female rats receiving 100 mg/kg. Because of the variability in the incidence of this neoplasm in aged Fischer 344 rats and the difficulty in definitively diagnosing this lesion in Fischer 344 rats, this increase was considered to be *equivocal evidence of carcinogenicity** of diallylphthalate in female rats. There was *no evidence of carcinogenicity* in male rats.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

# CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Diallylphthalate is based on the 13-week studies that began in July 1977 and ended in October 1977 and on the 2-year studies that began in February 1980 and ended in February 1982 at Litton Bionetics, Inc.

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The members of the Peer Review Panel who evaluated this draft Technical Report on March 23, 1984, 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 DIALLYLPHTHALATE

On March 23, 1984, the draft Technical Report on the toxicology and carcinogenesis studies of diallylphthalate received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9:00 a.m. in the First Floor Auditorium, Hubert Humphrey Building, Washington, DC.

Mr. L. Beliczky, a principal reviewer, stated that the data supported a finding of clear evidence of carcinogenicity for female rats. He noted that these findings were supported not only by the current data but also by the increased incidences of hematopoietic system tumors in rodents from other NTP studies of "allyl" compounds. Mr. Beliczky also felt the study of diallylphthalate in mice (NTP TR 242) should have been reviewed concurrently with the study in rats, under the currently used criteria for strength of evidence. Noting that the Panel had received summary information from a recently completed comparative disposition and metabolism study in rats and mice, Mr. Beliczky asked that the study results be fully incorporated into any final report. Dr. T. Slaga and Dr. R. Kociba, also principal reviewers, said that the rat study should stand on its own. In response, Dr. W. Kluwe, NTP, explained that the mouse and rat studies were started concurrently, but the study in rats had to be restarted because of a dosing error. Dr. Kluwe said that the data from the comparative disposition studies suggest a species difference in hepatotoxic effects but do not explain the hematopoietic system tumor incidences. Dr. Kluwe said a program decision had been made not to apply the current categories for strength of evidence retroactively. Dr. D. Rall, NTP, proposed that an appendix describing the mouse study design, results, and conclusions be added to the report of the rat studies [Appendix L].

As a second principal reviewer, Dr. Slaga stated that the increased incidence of mononuclear cell leukemia in high dose female rats should be considered equivocal evidence of carcinogenicity rather than some evidence of carcinogenicity as originally stated in the draft Technical Report because an increased incidence was not observed in male rats and because this neoplasm occurs at a moderate rate in historical control rats.

As a third principal reviewer, Dr. Kociba believed that the data supported a designation of equivocal evidence of carcinogenicity in female rats since this leukemia is common in historical control rats (more so in males) and the historical control rate is variable; however, the high dose female rat group did have an incidence greater than that ever seen in female vehicle historical control rats. Mr. Beliczky noted that the definition of equivocal evidence of carcinogenicity was different now than when the mouse study was reviewed. Dr. J. Swenberg thought the results of the mouse study would still be considered equivocal evidence of carcinogenicity. Dr. Kociba commented that the 13-week studies could have included histopathologic examination of the liver in the lowest dose group and a more complete toxicologic evaluation to aid in setting doses for the 2-year studies and that the 2-year studies could have included more hematology to aid in the interpretation of the leukemias.

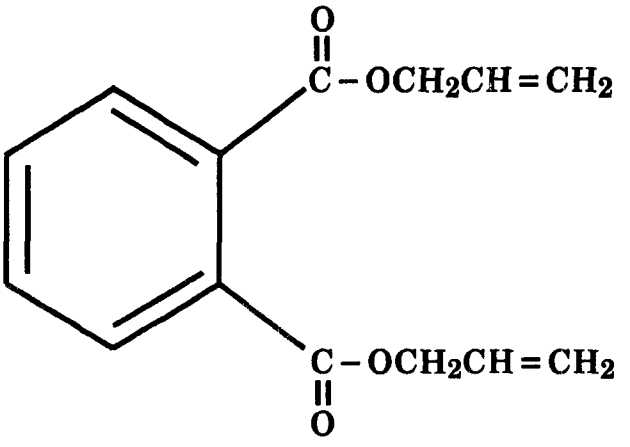
The interpretative issue was if the increased incidence of mononuclear cell leukemia in female rats best supported an evaluation of some evidence of carcinogenicity or equivocal evidence of carcinogenicity. Dr. G. Boorman, NTP, pointed out that there were some borderline diagnoses, more in the high dose group than in the vehicle controls. Dr. T. Jones commented that the greater numbers of borderline diagnoses in dosed animals supported a more conservative interpretation. Dr. Kluwe listed reasons supporting each interpretation and said that the NTP staff could support either choice. Dr. Swenberg suggested that Dr. Kluwe adjust the report discussion to reflect the difficulty in coming

to a decision. [ See pages 52-53.] In response to a request by Mr. Beliczky, Dr. Boorman said a table with a grading of the toxic liver lesions would be added to the report [Table 11].

Dr. Jones moved that the Technical Report be accepted with the conclusions as written (some evidence of carcinogenicity in female rats). Dr. Swenberg seconded the motion. The motion was not accepted (five affirmative to six negative votes). Dr. Jones then made the motion that the Technical Report on the toxicology and carcinogenesis studies of diallylphthalate in rats be accepted with the conclusion of equivocal evidence of carcinogenicity in female rats and with other modifications as discussed. Dr. Slaga seconded the motion, and the report was approved by eight affirmative votes. There were two negative votes (Mr. Beliczky and Dr. Davis) and one abstention (Dr. Kotelchuck).

# I. INTRODUCTION

# I. INTRODUCTION



DIALLYLPHTHALATE

CAS NO. 131-17-9

$C_{14}H_{14}O_4$ Mol Wt 246.24

Synonym: DAP

Diallylphthalate is a widely used crosslinking agent for unsaturated polyesters. Diallylphthalate or diallylphthalate polyester blends are used primarily as plasticizers and carriers for adding catalysts and pigments to polyesters and in molding, electrical parts, laminating compounds, and impregnation of metal castings (Modern Plastics Encyclopedia, 1979; Kirk-Othmer, 1979). Rubber compounds, epoxy formulations, and polyurethane foams may also contain diallylphthalate. Precise figures are not currently available, although annual production of diallylphthalate in the United States is known to exceed 5,000 pounds (USITC, 1980), and an estimated 57,000 pounds were imported into the United States in 1982 (USITC, 1983).

Little information is available in the literature concerning the pharmacokinetics of diallylphthalate. In general, however, dialkyl phthalate esters appear to be easily hydrolyzed to their corresponding alcohols and monoalkyl phthalates (Carter et al., 1974; Rowland, 1974; Rowland et al., 1977), possibly in the gut before intestinal absorption (Albro and Thomas, 1973; Rowland, 1974; Rowland et al., 1977). The NTP has recently shown that diallylphthalate is readily hydrolyzed in vivo (Appendix K). The parent compound was not isolated from the tissues of rats 4 hours after oral administration of near-lethal amounts of diallylphthalate (Carter et al., 1978).

The metabolite 3-hydroxypropylmercapturic acid has been isolated from the urine of rats administered diallylphthalate (Kaye and Young, 1972). Since allyl alcohol and acrolein (allyl aldehyde, 2-propenal) are also excreted as 3-hydroxypropylmercapturic acid in the urine of rats, it was hypothesized that one or both ester linkages of diallylphthalate are initially hydrolyzed and that the released allyl alcohol is then oxidized to acrolein (Kaye and Young, 1972; Kaye, 1973). Acrolein reacts with glutathione (forming 2-aldehydoethylglutathione) and is then reduced to an alcohol and excreted as the N-acetylcysteine conjugate (mercapturic acid). The metabolism of diallylphthalate is illustrated in Figure 1. Conjugation of acrolein with glutathione occurs in the liver in vivo (Giles, 1979) but has not been demonstrated in other tissues.

Liver tissue from phenobarbital-pretreated rats metabolizes allyl alcohol to acrolein and allylic acid (2-propenoic acid) (Patel et al., 1980). The characteristics of the oxidation of allyl alcohol to

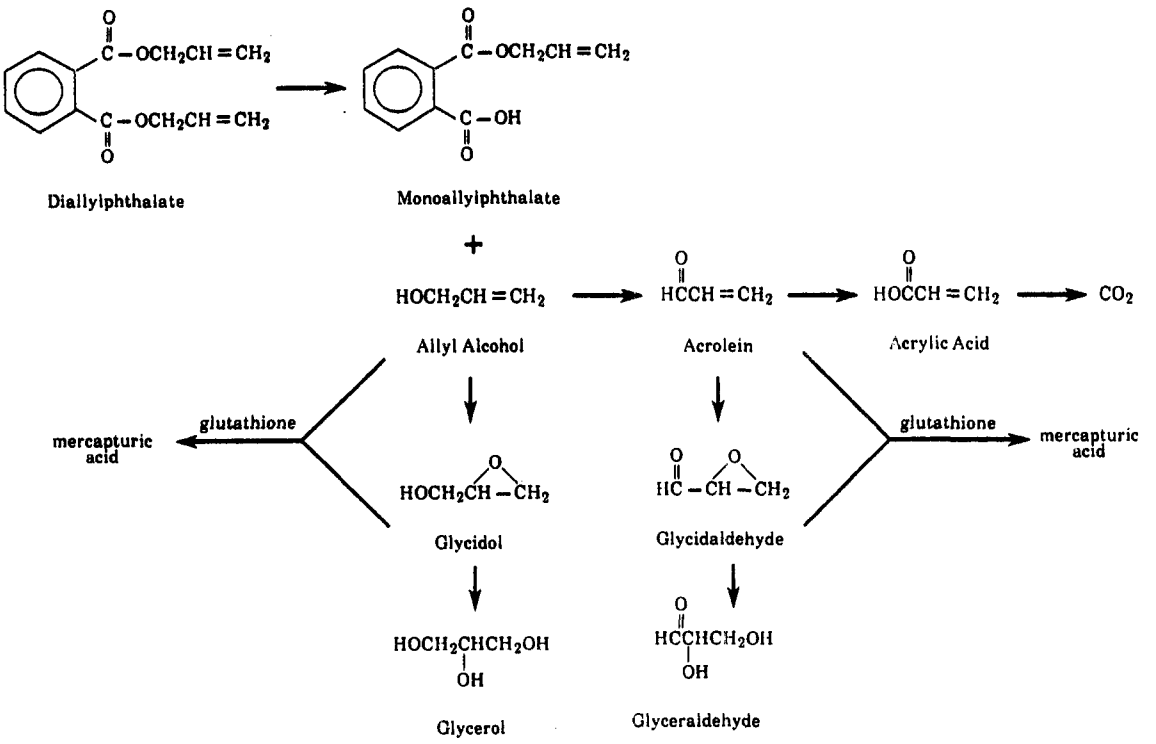



FIGURE 1. METABOLISM OF DIALLYLPHTHALATE

acrolein were consistent with catalysis by alcohol dehydrogenase, whereas those of oxidation of acrolein to acrylic acid were consistent with catalysis by aldehyde dehydrogenase. Allyl alcohol and acrolein were also shown to undergo hepatic microsomal oxidation to the epoxides glycidol and glycidaldehyde. These epoxides were subsequently hydrolyzed to glycerol and glyceraldehyde or conjugated with glutathione. The products of the latter reaction were not isolated or identified.

The conjugation of the reactive aldehyde, acrolein, with glutathione occurs in vitro in the absence of enzyme mediation (Giles, 1979) but may be catalyzed by glutathione transferases in vivo. Conjugation of an allyl alcohol metabolite with glutathione appears to be a detoxification reaction, since Hanson and Anders (1978) reported that diethyl maleate-induced depletion of glutathione enhances the lethal potency of allyl alcohol in rats.

The NTP had contracted for disposition and metabolism studies of diallylphthalate in mice and rats concurrently with the evaluation of the toxicity data; the results of that pharmacokinetic study are summarized in Appendix K. In brief, within 24 hours after receiving a single oral dose of $^{14}C$ diallylphthalate, male rats excreted approximately 60% of the $^{14}C$ equivalents in urine and approximately 30% in expired air. Excretion was independent of dose over the range studied, 1-100 mg/kg body weight. The parent compound was labeled in the allyl moiety rather than in the ring because of the NTP's interest in the toxic potential of the allyl portion of the molecule.

Radioactivity in expired air was in the form of $^{14}C$-carbon dioxide, whereas that in urine was found in (in descending order) monoallylphthalate, 3-hydroxypropylmercapturic acid, an unidentified polar metabolite, and allyl alcohol. The presence of monoallylphthalate and allyl alcohol in urine confirms the initial hydrolysis reaction.

The urinary 3-hydroxypropylmercapturic acid probably represents detoxification of allyl alcohol or acrolein via glutathione conjugation, apparently a major pathway of metabolism in the rat, although a small amount of allyl alcohol is also excreted unchanged. Excretion as carbon dioxide in expired air probably represents successive oxidation of allyl alcohol to acrolein, acrylic acid, and finally carbon dioxide.

Oral $LD_{50}$ values for diallylphthalate in rats (strain and sex unspecified) ranged from 0.77 to 1.7 g/kg (Hagan et al., 1949; Peakall, 1975; Carter et al., 1978). The major toxic effect in rats of the diallylphthalate metabolite allyl alcohol is periportal hepatocellular necrosis, a lesion believed to be caused by acrolein, the product of allyl alcohol oxidation (Rees and Tarlow, 1967; Reid, 1972). The hepatotoxic effects of allyl alcohol in rats regress despite continued chemical administration over several days, a process suggesting adaptation of the liver to the presence of allyl alcohol or acrolein (Butterworth et al., 1978; Lake et al., 1978). The mechanism of this apparent acquired resistance to allyl alcohol is not known.

Diallylphthalate was not mutagenic to *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in either the presence or absence of a 9,000 × *g* supernatant fraction from the livers of Aroclor 1254-treated male Sprague-Dawley rats or Syrian hamsters (NTP, 1982a; Appendix E) and did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Appendix E). Although little information is available on the mutagenicity of diallylphthalate, a large and comprehensive literature base does exist on the genetic toxicology of its metabolites allyl alcohol, acrolein, glycidol, and glycidaldehyde. Table 1 summarizes the genetic toxicology of diallylphthalate and these four metabolites. The results of these studies, reviewed in detail below, indicate that the epoxides glycidol and glycidaldehyde are clearly mutagenic in a wide variety of short-term tests, whereas allyl alcohol and acrolein exhibit limited mutagenic activity.

Allyl alcohol was reported to be negative in *S. typhimurium* in the presence or absence of Aroclor-induced rat liver S9 (Rosen et al., 1980; Yamaguchi, 1980; Principe et al., 1981). It also was negative in the bacterium *Streptomyces coelicolor* and the fungus *Aspergillus nidulans* (Principe et al., 1981). Other investigators, however, have reported that allyl alcohol was weakly mutagenic in *S. typhimurium* strain TA100 only in the absence of S9 (Lutz et al., 1982) and in strain TA1535 only in the presence of Aroclor-induced hamster liver S9 (Lijinsky and Andrews, 1980). Thus, allyl alcohol appears to have limited mutagenic activity.

Acrolein was reported to be negative in tests with *S. typhimurium* (Andersen et al., 1972; Bartsch et al., 1980; Florin et al., 1980; Rosen et al., 1980; Loquet et al., 1981) and *Escherichia coli* (Ellenberger and Mohn, 1977). Other workers, however, reported that acrolein was weakly mutagenic in *E. coli* in the absence of S9 (Hemminki et al., 1980a) and in various strains of *S. typhimurium*: TA98 without S9 (Lijinsky and Andrews, 1980); TA1535 with S9 (Hales, 1982); TA100 without S9 (Lutz et al., 1982); and TA100 with S9 (Appendix E). Although acrolein was not mutagenic in Drosophila (Appendix E) and did not induce dominant lethal mutations in mice (Epstein et al., 1972), it did induce chromosomal aberrations and sister-chromatid exchanges (SCE's) in Chinese hamster ovary cells (Au et al., 1980; Appendix E). Acrolein also has been reported to alkylate *E. coli* DNA (Hemminki et al., 1980a). Thus, acrolein appears to be weakly mutagenic in bacteria and causes cytogenetic damage in mammalian cells in vitro.

Glycidol was mutagenic in *S. typhimurium* in strains TA100 and TA1535, indicating that it is a base-pair substitution mutagen, and the addition of S9 had no effect on its mutagenic activity (Dorange et al., 1977; Wade et al., 1978, 1979; Hemminki and Falck, 1979; Simmon, 1979a; Stolzenberg and Hine, 1979; Ivie et al., 1980; Thompson et al., 1981; Lutz et al., 1982; Yamaguchi, 1982; Appendix E). It was also mutagenic in *E. coli* (Hemminki and Falck, 1979) and *Klebsiella pneumoniae* (Voogd et al., 1981) and caused DNA damage in *E. coli* (Leifer et al., 1981; McCarroll et al., 1981b; Mamber et al., 1983) and *Bacillus subtilis* (McCarroll et al., 1981a). In addition to inducing mitotic recombination (Simmon, 1979b) and mutation in yeast

TABLE 1. SUMMARY OF THE GENETIC TOXICOLOGY OF DIALLYLPHTHALATE AND FOUR OF ITS METABOLITES

| Compound | Test System | Endpoint | Result | Reference |
|---|---|---|---|---|
| **Diallylphthalate** | **Bacteria** | | | |
| | *Salmonella typhimurium* | Gene mutation | – | Appendix E |
| | **Nonmammalian Eukaryotes** | | | |
| | *Drosophila melanogaster* | Sex-linked recessive lethal mutations | – | Appendix E |
| **Allyl Alcohol** | **Bacteria** | | | |
| | *S. typhimurium* | Gene mutation | – | Rosen et al., 1980 |
| | | | – | Yamaguchi, 1980 |
| | | | – | Principe et al., 1981 |
| | | | + | Lijinsky and Andrews, 1980 |
| | | | + | Lutz et al., 1982 |
| | *Streptomyces coelicolor* | Gene mutation | – | Principe et al., 1981 |
| | **Nonmammalian Eukaryotes** | | | |
| | *Aspergillus nidulans* | Gene mutation | – | Principe et al., 1981 |
| **Acrolein** | **Bacteria** | | | |
| | *S. typhimurium* | Gene mutation | – | Andersen et al., 1972 |
| | | | – | Bartsch et al., 1980 |
| | | | – | Florin et al., 1980 |
| | | | – | Rosen et al., 1980 |
| | | | – | Loquet et al., 1981 |
| | | | + | Lijinsky and Andrews, 1980 |
| | | | + | Hales, 1982 |
| | | | + | Lutz et al., 1982 |
| | | | + | Appendix E |
| | *Escherichia coli* | Gene mutation | – | Ellenberger and Mohn, 1977 |
| | | | + | Hemminki et al., 1980a |
| | **Nonmammalian Eukaryotes** | | | |
| | *D. melanogaster* | Sex-linked recessive lethal mutations | – | Appendix E |
| | **Mammalian Cells (in vitro)** | | | |
| | Chinese hamster ovary cells | Chromosomal aberrations | + | Au et al., 1980 |
| | | | + | Appendix E |
| | | Sister chromatid exchanges (SCE's) | + | Au et al., 1980 |
| | | | + | Appendix E |
| | **Mammals (in vivo)** | | | |
| | Mice | Dominant lethal mutations | – | Epstein et al., 1972 |
| | **Chemical Reactivity** | | | |
| | *E. coli* DNA | Alkylation | + | Hemminki et al., 1980a |
| **Glycidol** | **Bacteria** | | | |
| | *S. typhimurium* | Gene mutation | + | Dorange et al., 1977 |
| | | | + | Wade et al., 1978, 1979 |
| | | | + | Hemminki and Falck, 1979 |
| | | | + | Simmon, 1979a |
| | | | + | Stolzenberg and Hine, 1979 |
| | | | + | Ivie et al., 1980 |
| | | | + | Thompson et al., 1981 |
| | | | + | Lutz et al., 1982 |
| | | | + | Yamaguchi, 1982 |
| | | | + | Appendix E |

TABLE 1. SUMMARY OF THE GENETIC TOXICOLOGY OF DIALLYLPHTHALATE AND FOUR OF ITS METABOLITES (Continued)

| Compound | Test System | Endpoint | Result | Reference |
|---|---|---|---|---|
| **Glycidol** (Continued) | **Bacteria** | | | |
| | *E. coli* | Gene mutation | + | Hemminki and Falck, 1979 |
| | *Klebsiella pneumoniae* | | + | Voogd et al., 1981 |
| | *E. coli* | DNA damage | + | Leifer et al., 1981 |
| | | | + | McCarroll et al., 1981b |
| | | | + | Mamber et al., 1983 |
| | *Bacillus subtilis* | | + | McCarroll et al., 1981a |
| | **Nonmammalian Eukaryotes** | | | |
| | *Saccharomyces cerevisiae* | Mitotic recombination | + | Simmon, 1979b |
| | | Gene mutation | + | Pittman and Brusick, 1971 |
| | | | + | Migliore et al., 1982 |
| | **Mammalian Cells (in vitro)** | | | |
| | Mouse lymphoma | | + | Thompson et al., 1981 |
| | Human lymphocytes | Chromosomal aberrations | + | Norppa et al., 1981 |
| | | SCE's | + | Norppa et al., 1981 |
| | Syrian hamster embryo (SHE) cells | Morphological transformation | + | Pienta, 1980 |
| | Human fibroblasts | Unscheduled DNA synthesis | + | Thompson et al., 1981 |
| | **Mammals (in vivo)** | | | |
| | Rats | Chromosomal aberrations | – | Thompson and Hiles, 1981 |
| | **Chemical Reactivity** | | | |
| | DNA | Alkylation | + | Hemminki, 1979 |
| | | | + | Hemminki et al., 1980b |
| | | | + | Hemminki and Vainio, 1980 |
| | | | + | Archer and Eng, 1981 |
| | | | + | Chen and Carlson, 1981 |
| **Glycidaldehyde** | **Bacteria** | | | |
| | *S. typhimurium* | Gene mutation | + | Wade et al., 1978, 1979 |
| | | | + | Simmon, 1979a |
| | | | + | Simmon et al., 1979 |
| | | | + | Bartsch et al., 1980, 1983 |
| | | | + | Appendix E |
| | *K. pneumoniae* | Gene mutation | + | Voogd et al., 1981 |
| | | | + | Knaap et al., 1982 |
| | *E. coli* | DNA damage | + | Leifer et al., 1981 |
| | **Nonmammalian Eukaryotes** | | | |
| | *S. cerevisiae* | Mitotic recombination | + | Simmon, 1979b |
| | | Gene mutation | + | Izard, 1973 |
| | *D. melanogaster* | Sex-linked recessive lethal mutations | + | Knaap et al., 1982 |
| | **Mammalian Cells (in vitro)** | | | |
| | Mouse lymphoma | Gene mutation | + | Amacher and Turner, 1982 |
| | SHE cells | Morphological transformation | + | Pienta, 1980 |

(Pittman and Brusick, 1971; Migliore et al., 1982), glycidol was mutagenic in the mouse lymphoma assay (Thompson et al., 1981).

Although glycidol reportedly did not induce chromosomal aberrations in rats (Thompson and Hiles, 1981), it did induce chromosomal aberrations and SCE's in human lymphocytes in vitro in the absence of S9 (Norppa et al., 1981). Glycidol also transformed Syrian hamster embryo cells in vitro (Pienta, 1980) and caused unscheduled DNA synthesis in human cells in vitro (Thompson et al., 1981). Consistent with its demonstrated genotoxicity, glycidol has been reported to alkylate DNA (Hemminki, 1979; Hemminki et al., 1980b; Hemminki and Vainio, 1980; Archer and Eng, 1981; Chen and Carlson, 1981). Thus, glycidol is clearly genotoxic, as demonstrated by mutagenic activity in bacteria, yeast, and mammalian cells; cytogenetic damage and transformation in mammalian cells; and alkylation of DNA.

Glycidaldehyde was mutagenic in *S. typhimurium* strains TA100 and TA1535, indicating that it is a base-pair substitution mutagen; the addition of S9 had no effect on its mutagenic activity (Wade et al., 1978, 1979; Simmon, 1979a; Simmon et al., 1979; Bartsch et al., 1980, 1983; Appendix E). It was also mutagenic in *K. pneumoniae* (Voogd et al., 1981; Knaap et al., 1982) and caused DNA damage in *E. coli* (Leifer et al., 1981). In addition to causing mutation (Izard, 1973) and mitotic recombination (Simmon, 1979b) in yeast, glycidaldehyde was mutagenic in Drosophila (Knaap et al., 1982) and in the mouse lymphoma assay in the presence (Amacher and Turner, 1982) but not in the absence (Knaap et al., 1982) of uninduced rat liver S9. Glycidaldehyde transformed Syrian hamster embryo cells in vitro (Pienta, 1980). Thus, glycidaldehyde is mutagenic in a wide variety of test systems (bacteria, yeast, Drosophila, and mammalian cells) and causes transformation in mammalian cells.

In summary, the genetic toxicity studies show that several of the metabolites of diallylphthalate are mutagenic, with the epoxides (glycidol, glycidaldehyde) exhibiting greater activity than acrolein or allyl alcohol. The absence of an effect of added S9 on the mutagenic activity of the epoxide metabolites suggests that they are direct-acting mutagens and are possibly the ultimate mutagenic metabolites of allyl alcohol and acrolein.

Two-year carcinogenicity studies of diallylphthalate in male and female B6C3F$_1$ mice were conducted with oral (gavage, in corn oil) doses of 0 (vehicle control), 150, or 300 mg/kg, 5 days per week for 103 weeks; each dose was administered to 50 mice of each sex (NTP, 1983b; Appendix L). The results of those studies demonstrated that diallylphthalate caused chronic inflammation and hyperplasia of the forestomach in both male and female mice. Squamous cell papillomas of the forestomach occurred in 0/50 of both the male and female vehicle controls, 1/50 of both the males and females at 150 mg/kg, and 2/50 of both the males and females at 300 mg/kg. Although this pattern of occurrence of rare forestomach papillomas in mice (historical incidences of 3/638, 0.5%, in male, and 3/656, 0.5%, in female mice) was suggestive of a chemically induced effect, the data were judged insufficient to clearly indicate a cause-and-effect relationship. An increased occurrence of lymphomas in male mice (300 mg/kg) was observed in that study (overall incidences of 6/50 for the vehicle controls; 5/50 for the 150 mg/kg group; and 12/50 for the 300 mg/kg group), but the increase was considered equivocally related to diallylphthalate administration because of the absence of statistical significance ($P>0.05$) of the pairwise comparisons and the absence of a similar effect in female mice. The incidence of lymphomas in vehicle control male mice from that study (6/50, 12%) was similar to that of male historical control mice (administered corn oil by gavage) from the testing laboratory (19/120, 16%) and programwide (80/661, 12%).

A carcinogenicity study of acrolein in Fisher 344 rats is currently in progress (IARC, 1982). Inhalation by hamsters of the respiratory tract irritant acrolein at 4 ppm for 1 year or at 10 ppm throughout life (5 days per week) failed to cause an increase in tumors of the respiratory tract (Personal communication, Dr. P. Nettesheim, National Institute of Environmental Health

Sciences, 1983; Feron and Kruysse, 1977). No information is currently available on the potential carcinogenic effect of acrolein on nonrespiratory tract tissues or of the chronic effects of oral administration of this compound. Glycidaldehyde was reported to cause both benign and malignant tumors of the skin when applied dermally to female mice throughout their lifetime (Van Duuren et al., 1965). There is limited evidence, therefore, of carcinogenic potential for one diallylphthalate metabolite (glycidaldehyde); the carcinogenic potential of another metabolite (acrolein) is currently under study.

Other *ortho* phthalate esters and related compounds tested for carcinogenic potential in rodents include di(2-ethylhexyl)phthalate, di(2-ethylhexyl)adipate, and butyl benzyl phthalate. Di(2-ethylhexyl)phthalate administration was associated with increased incidences of hepatocellular carcinomas in rats and mice (NTP, 1982e), di(2-ethylhexyl)adipate administration with increased incidences of hepatocellular carcinomas in mice (NTP, 1982d), and butyl benzyl phthalate administration with increased incidences of mononuclear cell leukemia in female rats (NTP, 1982c).

Diallylphthalate was tested in the Carcinogenesis Program because of its widespread use, potential for human exposure, and the lack of prior chronic toxicity testing.

# II. MATERIALS AND METHODS

## PROCUREMENT AND CHARACTERIZATION OF DIALLYLPHTHALATE

Diallylphthalate, a pale yellow liquid, was obtained in a single batch (lot no. 25-121) from Hardwicke Chemical Company (Elgin, South Carolina). The identity of diallylphthalate was confirmed by infrared, ultraviolet/visible, and nuclear magnetic resonance analyses (Appendix F). All spectroscopic data were in agreement with literature values and were consistent with those expected for the substance.

The purity of diallylphthalate was determined by elemental analyses and thin-layer and gas chromatographic analyses (Appendix F). Results of elemental analyses were consistent with the theoretical values. Only a single component was detected by thin-layer chromatography in two different solvent systems. Eight impurities (comprising a total area of approximately 1% that of the major peak) were detected by gas chromatography. These trace impurities were not characterized further. According to the overall data obtained in these studies, the test material was approximately 99% pure diallylphthalate.

Diallylphthalate was found to be stable for 2 weeks at temperatures up to 60° C in the presence of light (Appendix F). After receiving the chemical from the analytical laboratory, the testing laboratory stored several portions at −20° C as reference samples and the bulk chemical at 4° C.

No change in the purity of the bulk chemical supply was observed throughout the studies when the chemical was analyzed periodically by gas chromatography and infrared spectroscopy (Appendix F).

## PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

Diallylphthalate in corn oil was found to be stable for 2 weeks at 25° C in the presence of light (Appendix G). Dosing solutions were prepared on a weight/volume or volume/volume basis in a glass container and mixed by inversion of the container. The solutions were stored at ambient temperatures before use (Table 2).

Analyses for diallylphthalate in the dosing solutions were performed periodically to confirm that the correct doses were administered (Appendix H). The analyses involved extraction of the test chemical from corn oil followed by gas chromatography. Two different extraction methods were used during the studies. A procedure with carbon disulfide as the extracting solvent was used for the 13-week studies, and one with methanol was used for the 2-year studies.

One set of dose mixtures prepared for the 13-week studies was analyzed and found to be within 10% of their target concentrations (Appendix I). Sets of samples were analyzed at approximately 8-week intervals during the 2-year studies. Because 2/33 samples were not within ±10% of the target concentrations, it is estimated that the mixes were formulated within specifications (±10%) approximately 94% of the time during the 2-year studies (Appendix I). A statistical summary of the analyses of dose mixtures for the 2-year studies is presented below:

| | Target Concentration (mg/ml) | |
|---|---|---|
| | 15.0 | 30.0 |
| Mean (mg/ml) | 14.8 | 28.8 |
| Standard deviation | 0.59 | 3.4 |
| Coefficient of variation (percent) | 4.0 | 11.9 |
| Range (mg/ml) | 13.4-16.0 | 15.8-31.5 |
| Number of samples | 16 | 17 |

## SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats were obtained from Frederick Cancer Research Center 6 weeks before the test began. Animals were approximately 10 weeks old when placed on study. Details of animal maintenance are presented in Table 2.

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF DIALLYLPHTHALATE

| Single-Administration Studies | Fourteen-Day Repeated-Administration Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|---|
| **EXPERIMENTAL DESIGN** | | | |
| **Size of Test Groups** | | | |
| 5 males and 5 females | 5 males and 5 females | 10 males and 10 females | 50 males and 50 females |
| **Doses** | | | |
| 316 (females only), 464, 681, 1,000, or 1,470 mg/kg diallylphthalate in corn oil by gavage; dose vol: variable from a 50 mg/ml stock solution | 0, 50, 100, 200, 400, or 600 mg/kg diallylphthalate in corn oil by gavage; dose vol: variable from a 50 mg/ml stock solution | 0, 25, 50, 100, 200, or 400 mg/kg diallylphthalate in corn oil by gavage; dose vol: 3.33 ml/kg | 0, 50, or 100 mg/kg diallylphthalate in corn oil by gavage; dose vol: 3.33 ml/kg |
| **Date of First Dose** | | | |
| 1,470 mg/kg and 464 mg/kg on 1/5/76; 1,000 mg/kg and 681 mg/kg on 1/8/76; 316 mg/kg on 1/12/76 | 9/8/76 | 7/21/77 | 2/21/80 |
| **Date of Last Dose** | | | |
| Same as date of first dose | 9/21/76 | 10/17/77 | 2/12/82 |
| **Duration of Dosing** | | | |
| Single administration | 1 × d for 14 d | 5 × wk for 13 wk | 5 × wk for 103 wk |
| **Type and Frequency of Observation** | | | |
| Observed every 1/2 h on day of dosing and then 1 × d for 13 d; weighed on d 0, 7, 14 | Observed 1 × d for mortality; weighed on d 0, 7, 14 | Observed 2 × d M-F and 1 × d on weekends for mortality and signs of moribundity; body weight measured 1 × wk | Observed 2 × d for signs of moribundity and mortality; clinical signs and body weights recorded 1 × wk for 12 wk, then 1 × mo thereafter |
| **Necropsy and Histologic Examination** | | | |
| Necropsy performed on all animals. No tissues examined microscopically. | Necropsy performed on all animals. No tissues examined microscopically. | Necropsy performed on all animals; histologic exam performed on kidneys, colon, and liver of 0, 50, 100, 200, and 400 mg/kg groups; in addition, the following tissues were examined in the vehicle control (0) and 400 mg/kg groups: mandibular lymph node, salivary gland, sternebrae (including marrow), thyroid gland, parathyroids, colon, small intestine, prostate/testes or ovaries/uterus, lungs and bronchi, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, pituitary gland, eyes (if grossly abnormal), mammary gland, gross lesions, urinary bladder, and adrenal glands | Necropsy performed on all animals; histologic exam performed on the following tissues of all animals: gross lesions and tissue masses, mandibular and mesenteric lymph nodes, salivary gland, sternebrae (including marrow), parathyroids, thyroid gland, liver, urinary bladder, colon, prostate/testes/seminal vesicles or ovaries/uterus, lungs, bronchi, skin, cecum, thigh muscle, costochondral junction (rib), larynx, nasal cavity, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, adrenal glands, pituitary gland, spinal cord, eyes, mammary gland, rectum jejunum, duodenum, ileum, and sciatic nerve |

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF DIALLYLPHTHALATE (Continued)

| Single-Administration Studies | Fourteen-Day Repeated-Administration Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|---|
| **ANIMALS AND ANIMAL MAINTENANCE** | | | |
| **Strain and Species** | | | |
| F344/N rats | F344/N rats | F344/N rats | F344/N rats |
| **Animal Source** | | | |
| Frederick Cancer Research Center (Frederick, MD) | Same as single-administration studies | Same as single-administration studies | Charles River Breeding Laboratories (Portage, MI) |
| **Time Held Before Test** | | | |
| 6 wk | 18 d | 4 wk | 2 wk |
| **Age When Placed on Study** | | | |
| 10 wk | 10 wk | 8 wk | 6 wk |
| **Method of Identification** | | | |
| Ear tag | Ear tag | Ear tag | Ear tag |
| **Age When Killed** | | | |
| 12 wk | 12 wk | 21 wk | 110-111 wk |
| **Necropsy Dates** | | | |
| 1/18/76-1/26/76 | 9/22/76 | 10/18/77 | 2/22-2/26/82 |
| **Method of Animal Distribution** | | | |
| -- | -- | Randomized so that the average cage weights for each sex and species were approximately equal | Assigned to cages, then to groups according to two tables of random numbers |
| **Feed** | | | |
| Purina Lab Chow® (ground); provided ad libitum except for night before dosing when feed was removed from cages (Ralston Purina Co., St. Louis, MO) | Purina Lab Chow® (ground); provided ad libitum | Same as 14-d repeated-administration studies | Purina Lab Chow 5001® pellets (Ralston Purina Co., St. Louis, MO); provided ad libitum |
| **Bedding** | | | |
| Ab-sorb-dri® (Lab Products; Garfield, NJ) | Same as single-administration studies | Same as single-administration studies | Ab-sorb-dri® (Williams Feed and Bedding Co., Gaithersburg, MD) until 9/23/81; SANI-CHIPS® (P.J. Murphy Forest Products, Rochelle Park, NJ) until termination |
| **Water** | | | |
| Tap water in bottles, acidified to pH 2.5 with HCl; provided ad libitum | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies |

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF DIALLYLPHTHALATE (Continued)

| Single-Administration Studies | Fourteen-Day Repeated-Administration Studies | Thirteen-Week Studies | Two-Year Studies |
|---|---|---|---|
| **Cages** | | | |
| Polycarbonate (Lab Products, Garfield, NJ, and Hazleton Systems, Aberdeen, MD) | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies |
| **Cage Filters** | | | |
| Nonwoven polyester (Snow Filtration, Cincinnati, OH) | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies |
| **Animals per Cage** | | | |
| 2-3 | 2-3 | 5 | 5 |
| **Animal Room Environment** | | | |
| 12-15 room air changes/h; temp: 23° ± 1° C; humidity: 30%-70%; fluorescent light 12 h/d | Same as single-administration studies | Same as single-administration studies | Temp: 24° ± 2° C 98% of time (a); humidity: 23%-79% 99% of time (b) |
| **Other Chemicals on Test in Same Room** | | | |
| Toluene diisocyanate, 2,6-dichloro-*p*-phenylene-diamine | None | None | None |
| **CHEMISTRY** | | | |
| **Lot Numbers Used** | | | |
| 25-121 | 25-121 | 25-121 | 25-121 |
| **Supplier** | | | |
| Hardwicke Chemical Co. (Elgin, SC) | Same as single-administration studies | Same as single-administration studies | Same as single-administration studies |

(a) Fourteen readings at 26°-27.2° C; one reading at 28.9° C
(b) Three readings of 83%-100%; five readings of 18%-20%

Diallylphthalate in corn oil was administered to groups of five male rats in single doses of 464, 681, 1,000, or 1,470 mg/kg and to groups of five female rats in single doses of 316, 464, 681, 1,000, or 1,470 mg/kg. The concentration of diallylphthalate in the dosing solution was 50 mg/ml. Different volumes were administered to achieve the various doses. All animals were observed every half hour on the day of dosing and then daily for the next 13 days. Weights were measured on the day of dosing and on days 7 and 14 after dosing. Necropsies were performed on all animals found dead or killed (by carbon dioxide asphyxiation) at study termination. Tissues were not examined histopathologically.

The survival data from these studies were used to determine $LD_{50}$ values by the Spearman-Karber method (Finney, 1964) and to select the doses for the 14-day repeated-administration studies.

### FOURTEEN-DAY REPEATED-ADMINISTRATION STUDIES

Male and female F344/N rats were obtained from the Frederick Cancer Research Center 18 days before the study began. Animals were approximately 10 weeks old when placed on study.

Groups of five males and five females received 0, 50, 100, 200, 400, or 600 mg/kg diallylphthalate in corn oil by gavage for 14 consecutive days. The concentration of diallylphthalate in the dosing solution was 50 mg/ml. Different volumes were administered to achieve the various doses; vehicle controls received the same dose volume as did the 600 mg/kg animals. Animals were housed two or three per cage and received water and feed ad libitum. Details of animal maintenance are presented in Table 2. The rats were observed daily for clinical signs and mortality and were weighed on days 0 (prestudy), 7, and 14 of the experiment. Dose volumes were based on the day 0 and day 7 weights. Necropsies were performed on all animals found dead or killed (by carbon dioxide asphyxiation) after 14 days of chemical administration. Tissues were not examined histopathologically.

### THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxicity of diallylphthalate, to characterize lesions, and to determine the doses to be used in the 2-year studies.

Four-week-old male and female F344/N rats were obtained from the Frederick Cancer Research Center 4 weeks before being assigned to cages and dose groups (randomized by weight). The animals were approximately 8 weeks old when placed on study. Rats were housed five per cage in polycarbonate cages. Feed and water (acidified with hydrochloric acid to pH 2.5 for bacterial control) were available ad libitum. Further experimental details are summarized in Table 2.

Diallylphthalate was administered by gavage to groups of 10 rats of each sex at doses of 0, 25, 50, 100, 200, or 400 mg/kg in corn oil, 5 days per week for 13 weeks. Dose solutions were formulated such that each animal received a total gavage volume of 3.33 ml/kg, based on weekly group mean body weights.

Animals were checked twice daily Monday through Friday and once per day on the weekends for signs of moribundity and mortality and were given a clinical examination daily. Individual body weights and feed consumption by cage were recorded once per week.

Moribund animals and survivors at the end of the 13-week studies were killed by carbon dioxide asphyxiation. The 13-week survivors were fasted overnight before being killed. All survivors from the 13-week studies were killed 1 day after the final chemical administration. Necropsies were performed on all animals, including those found dead except when excessively autolyzed or cannibalized. Tissues examined histopathologically from various dose groups are listed in Table 2.

## TWO-YEAR STUDIES

### Study Design

Groups of 50 rats of each sex received 0 (vehicle control), 50, or 100 mg/kg diallylphthalate by gavage in corn oil 5 days per week for 103 weeks. Dose solutions were formulated such that each animal received a total gavage volume of 3.33 ml/kg.

### Source and Specifications of Test Animals

The male and female F344/N rats used in this study were produced under strict barrier conditions at Charles River Breeding Laboratories (Portage, Michigan) under a contract to the Carcinogenesis Program. Breeding starts for the foundation colony at the production facility originated at the National Institutes of Health Repository. Animals shipped for testing were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the testing laboratory at 4 weeks of age. The animals were quarantined at the testing facility for 2 weeks. Thereafter, a complete necropsy was performed on five animals of each sex to assess their health. The rodents were 53 days old when placed on study. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program (Appendix J). Although the significance of elevated antibody titers to the interpretation of toxicity studies is not known at the present time, the data are included in Appendix J for future reference.

The animals were assigned to cages according to a table of random numbers, and the cages randomized to dose groups according to another table of random numbers.

### Animal Maintenance

Rats were housed five per cage. Feed and water were available ad libitum. Details of animal maintenance are summarized in Table 2. Room temperature and relative humidity generally ranged from 22° to 24° C and 30% to 70%, respectively.

### Clinical Examinations and Pathology

All animals were observed twice daily for signs of moribundity or mortality. Clinical signs and individual body weights were recorded weekly for the initial 12 weeks of the studies and once every 4 weeks thereafter. Feed consumption was not measured. Moribund animals and animals that survived to the end of the study were killed with carbon dioxide. Necropsies were performed on all animals, including those found dead, unless they were excessively autolyzed or cannibalized. 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 in each group.

Examinations for grossly visible lesions were performed on major tissues or organs. 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 2.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assurance pathologist. Slides of all target tissues and those about which the original and quality assurance pathologists disagreed were submitted to the Chairperson of the Pathology Working Group (PWG) for evaluation. Representative coded slides selected by the Chairperson were reviewed by PWG pathologists, who reached a consensus and compared their findings with the original and quality assurance diagnoses. When diagnostic differences were found, the PWG sent the appropriate slides and comments to the original pathologist for review. 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.

Nonneoplastic lesions are not examined routinely by the quality assurance pathologist or PWG. Certain nonneoplastic findings are reviewed by the quality assurance pathologist and PWG if they are considered part of the toxic response to a chemical or if they are deemed of special interest.

### 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) extensions of Cox's method for testing for a dose-related trend. 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 necropsies were 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. All reported P values for tumor analyses 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 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 mor-tality is the life table method of Cox (1972) and of Tarone (1975).

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 control groups were compared in each of five time intervals: 0-52 weeks, 53-78 weeks, 79-92 weeks, 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 on which necropsies were actually performed 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's exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in Appendix C. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

### Liver Weights

Because of the gross observation of an apparent increase in liver size at necropsy, individual liver weights were measured during the latter half of the terminal kill. Group mean liver weights were compared statistically by Dunnett's test (Dunnett, 1955).

# III. RESULTS

SINGLE-ADMINISTRATION STUDIES
FOURTEEN-DAY REPEATED-ADMINISTRATION STUDIES
THIRTEEN-WEEK STUDIES
TWO-YEAR STUDIES
- Body Weights and Clinical Signs
- Sentinel Animal Serology
- Survival
- Pathology and Statistical Analyses of Results

## SINGLE-ADMINISTRATION STUDIES

All rats that received 1,470 mg/kg, 4/5 males and 3/5 females that received 1,000 mg/kg, and 5/5 females that received 681 mg/kg diallylphthalate died during the study (Table 3). Diarrhea, inactivity, hunched posture, hyperpnea, and watery secretions around the nose and mouth were observed in nearly all animals of both sexes at 1,470 mg/kg before they died. These clinical signs occurred less frequently at 1,000 mg/kg. Female rats receiving 681 mg/kg exhibited reduced activity on the day of dosing only.

At necropsy, apparent hemorrhagic lesions were noted in the urinary bladder, and the lungs appeared dark in animals receiving the 1,470 mg/kg dose (chemical-induced deaths). The darkened appearance of the lungs was also noted frequently at 1,000, 681, and 464 mg/kg. Fluid was found in the thoracic cavity, and the intestines appeared to be reddened in two females in the 1,000 mg/kg group that died early.

The calculated $LD_{50}$ values and 95% confidence intervals were 891 (766-1,036) mg/kg and 656 (545-789) mg/kg for male and female rats, respectively. Based on these data, a maximum dose of 600 mg/kg was chosen for both male and female rats in the 14-day repeated-administration studies.

**TABLE 3. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF DIALLYLPHTHALATE**

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) | | |
|---|---|---|---|---|
| | | Initial | Final | Change |
| **MALE** | | | | |
| 464 | 5/5 | 246 | 252 | + 6 |
| 681 | 5/5 | 250 | 275 | +25 |
| 1,000 | (b) 1/5 | 247 | 245 | − 2 |
| 1,470 | (c) 0/5 | 247 | (d) | (d) |
| **FEMALE** | | | | |
| 316 | 5/5 | 151 | 154 | + 3 |
| 464 | 5/5 | 150 | 161 | +11 |
| 681 | (e) 0/5 | 154 | (d) | (d) |
| 1,000 | (f) 2/5 | 151 | 146 | − 5 |
| 1,470 | (g) 0/5 | 149 | (d) | (d) |

(a) Number surviving/number initially in group
(b) Days of death: 1, 2, 2, 4
(c) Days of death: 2, 2, 2, 2, 2
(d) No data are presented due to the 100% mortality in this group.
(e) Days of death: 2, 2, 2, 2, 3
(f) Days of death: 2, 2, 3
(g) Days of death: 2, 2, 2, 2, 3

## FOURTEEN-DAY REPEATED-ADMINISTRATION STUDIES

All the rats that received 600 mg/kg and 3/5 males and 1/5 females that received 400 mg/kg diallylphthalate died during the studies (Table 4). Males that received 400 mg/kg initially lost weight; during the second week, mean body weight gains for males that received 200 or 400 mg/kg and for females that received 400 mg/kg appeared to be lower than those of the vehicle controls. Some animals at 600 mg/kg were inactive between day 4 of dosing and death. Scattered occurrences of inactivity and hunched posture also were observed at 400 mg/kg, but the effects appeared to reverse with time, and less than half of the animals in each dose group appeared to be affected. Clinical signs were not observed in other dose groups.

At necropsy, dark, mottled lungs and distended stomachs were observed in both males and females at 600 and 400 mg/kg (observed both in animals dying early and in those killed after 14 days). Enlarged cecums (approximately twice normal size) were observed in both sexes at 200 mg/kg and in male rats at 50 and 100 mg/kg. Spleens also appeared to be enlarged in males and females at 200 mg/kg but not at lower doses. Abnormalities in the appearance of the liver were observed in all but one of the males and in all of the females at doses of 200 mg/kg and higher, in a few of the animals of each sex at 100 mg/kg, and in two males at 50 mg/kg. At 200-600 mg/kg, the livers appeared to be enlarged, dark, and mottled. Small, yellowish spots were observed on the surface of the liver in many rats. The mottling was observed as well in males at 50 and 100 mg/kg, but it was less severe, and enlargement of the liver was not grossly apparent. Yellowish spots were observed infrequently at these doses. In contrast to the single-administration studies, hemorrhagic lesions of the urinary bladder were not observed. No histologic evaluations of the apparent target organs (liver, lung, stomach, cecum, spleen) were performed.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY REPEATED-ADMINISTRATION GAVAGE STUDIES OF DIALLYLPHTHALATE

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) Initial | Interim (7 day) | Final | Change | Final Weight Relative to Vehicle Controls (percent) |
|---|---|---|---|---|---|---|
| **MALE** | | | | | | |
| 0 | 5/5 | 128 | 161 | 191 | + 63 | -- |
| 50 | 5/5 | 128 | 159 | 186 | + 58 | 97 |
| 100 | 5/5 | 128 | 161 | 182 | + 54 | 95 |
| 200 | 5/5 | 128 | 159 | 169 | + 41 | 88 |
| 400 | (b) 2/5 | 128 | 112 | 124 | - 4 | 65 |
| 600 | (c) 0/5 | 128 | (d) | (d) | (d) | (d) |
| **FEMALE** | | | | | | |
| 0 | 5/5 | 103 | 119 | 134 | + 31 | -- |
| 50 | 5/5 | 103 | 121 | 131 | + 28 | 98 |
| 100 | 5/5 | 103 | 121 | 136 | + 33 | 101 |
| 200 | 5/5 | 103 | 121 | 133 | + 30 | 99 |
| 400 | (e) 4/5 | 103 | 97 | 111 | + 8 | 83 |
| 600 | (f) 0/5 | 103 | (d) | (d) | (d) | (d) |

(a) Number surviving/number initially in group
(b) Days of death: 7,13,14
(c) Days of death: 3,4,4,5,5
(d) No data are presented due to the 100% mortality in this group.
(e) Day of death: 8
(f) Days of death: 4,4,5,5,5

Based on these data, doses of 0 (vehicle control), 25, 50, 100, 200, and 400 mg/kg diallylphthalate were chosen for both male and female rats in the 13-week studies.

## THIRTEEN-WEEK STUDIES

Six of 10 male rats that received 400 mg/kg diallylphthalate died during the study, and another 2 were killed when found in a moribund condition (Table 5). Mean body weight gain for male rats at 400 mg/kg appeared to be depressed relative to that of the vehicle controls. No other differences in body weights or weight gains were observed.

Overall feed consumption averaged 24 g per animal per day for males (all dose groups) and 14 g per animal per day for females (all dose groups) (data not shown). Feed consumption was lower in the 400 mg/kg groups of both sexes than in other groups for weeks 1-3 of study but matched or exceeded the feed consumption for vehicle control groups thereafter.

Clinical signs of chemical toxicity were commonly observed in both males and females at 400 mg/kg throughout the studies, and less frequently in both sexes at 200 mg/kg. The clinical signs consisted of diarrhea, rough hair coat or alopecia around the head, hunched posture, and general emaciation; the emaciation occurred most frequently in males at 400 mg/kg. No clinical signs were observed in lower dose groups.

At necropsy, gross abnormalities of the liver were observed in all eight 400 mg/kg male rats that died early. The livers appeared to be enlarged, mottled (many with yellow blotches on the surface), and pale; the surface texture was rough, granular, or pitted. The lungs in many of these male rats appeared darkened or bright red.

Similar liver lesions were observed grossly in the two surviving males at 400 mg/kg, in most females at 400 mg/kg, and in 5/10 of the males at

TABLE 5. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF DIALLYLPHTHALATE

| Dose (mg/kg) | Survival (a) | Mean Body Weights (grams) Initial | Final | Change | Final Weight Relative to Vehicle Controls (percent) |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| 0 | 10/10 | 186 | 311 | +125 | -- |
| 25 | 10/10 | 185 | 321 | +136 | 103 |
| 50 | 10/10 | 189 | 322 | +133 | 104 |
| 100 | 10/10 | 188 | 312 | +124 | 100 |
| 200 | 10/10 | 186 | 303 | +117 | 97 |
| 400 | (b) 2/10 | 184 | 273 | + 89 | 88 |
| **FEMALE** | | | | | |
| 0 | 10/10 | 134 | 192 | + 58 | -- |
| 25 | 10/10 | 133 | 202 | + 69 | 105 |
| 50 | 10/10 | 134 | 197 | + 63 | 103 |
| 100 | 10/10 | 135 | 199 | + 64 | 104 |
| 200 | 10/10 | 133 | 197 | + 64 | 103 |
| 400 | 10/10 | 134 | 191 | + 57 | 101 |

(a) Number surviving/number initially in group
(b) Weeks of death: 2,2,8,10,10,10 (moribund kill),12,12 (moribund kill)

200 mg/kg. The severity appeared to be dose related in males and greater in males than in females. The kidneys of female rats at 400 mg/kg were considered to have an abnormal (greenish-brown) coloration.

Histologic examination indicated that the liver was the primary target organ. The lesions occurred principally in the periportal regions of the hepatic lobules (Table 6). Periportal hepatocellular necrosis and fibrosis, bile duct hyperplasia, and hepatocellular nodular hyperplasia occurred in both males and females at 200 and 400 mg/kg. The relatively greater severity and universal frequency of the liver lesions in rats at 400 mg/kg resulted in the use of the term "cirrhosis" to describe the hepatic alterations at this dose. Necrosis, fibrosis, and biliary hyperplasia were not observed at doses lower than 200 mg/kg, but hepatocellular alterations in the periportal region were observed with decreasing frequency and severity at doses as low as 50 mg/kg (males) or 100 mg/kg (females) (Table 6). The cellular alterations were characterized by hepatocellular basophilia, cellular and nuclear hypertrophy, and nuclear hyperchromatism. The severity of the hepatocellular alterations was subjectively graded as moderate to severe in both sexes at 200 mg/kg and mild at lower doses. (The extent of cirrhosis precluded any diagnosis of cellular alterations at 400 mg/kg.)

Acute, necrotizing colitis, characterized by the loss of surface and glandular epithelium, varying degrees of mucosal and submucosal edema, and acute inflammatory cell infiltration, was diagnosed in seven of the eight early-death males at 400 mg/kg. In addition, three of these male rats exhibited multifocal renal cortical tubular necrosis.

Doses of 0 (vehicle control), 50, and 100 mg/kg diallylphthalate were chosen for the 2-year studies based on the following observations from the 13-week studies:

1. Decreased body weight gains and early deaths in male rats at 400 mg/kg.
2. Clinical signs of chemical toxicity in both male and female rats at 200 and 400 mg/kg.
3. Severe liver lesions in both male and female rats at 200 and 400 mg/kg; only mild hepatocellular alterations at 100 mg/kg.

**TABLE 6. INCIDENCES OF LIVER LESIONS IN RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF DIALLYLPHTHALATE**

| Dose (mg/kg) | Lesions and Incidences (a) | | | | |
|---|---|---|---|---|---|
| | Periportal Hepatocellular Alterations | Periportal Necrosis | Periportal Fibrosis | Periportal Cirrhosis | Bile Duct Hyperplasia |
| **MALE** | | | | | |
| (b) 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| (c) 50 | 3/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 100 | 2/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 200 | 9/10 | 5/10 | 5/10 | 0/10 | 8/10 |
| (d) 400 | -- | -- | -- | 10/10 | -- |
| **FEMALE** | | | | | |
| (b) 0 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| (c) 50 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 100 | 8/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 200 | 10/10 | 7/10 | 4/10 | 0/10 | 9/10 |
| (d) 400 | -- | -- | -- | 10/10 | -- |

(a) Incidences are presented as the number of animals with the specified lesion over the number of animals in the group.
(b) Vehicle controls
(c) Livers from the lowest dose group (25 mg/kg) were not examined because of the absence of (or the presence of only minimal) hepatic changes at 50 mg/kg.
(d) The presence of cirrhosis precluded the diagnosis of the other liver lesions listed in this table.

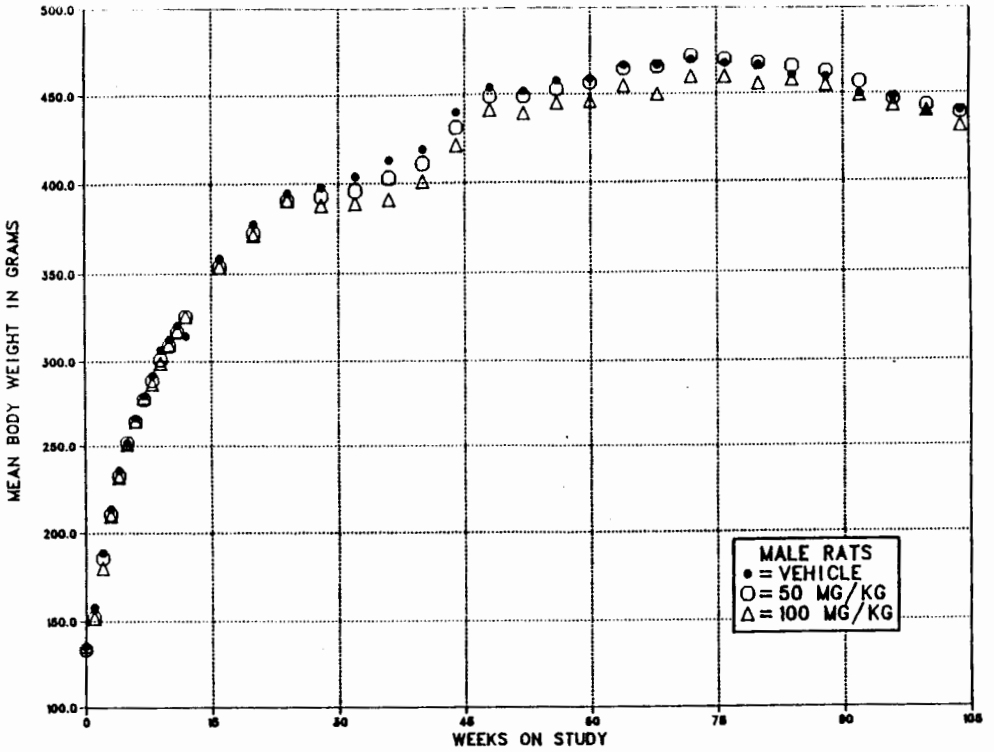
## TWO-YEAR STUDIES

### Body Weights and Clinical Signs

Mean body weights of dosed male or female rats were not notably different from those of the vehicle controls throughout the studies (Table 7 and Figure 2). No clinical signs clearly attributable to diallylphthalate administration were observed in the studies.

TABLE 7. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

| Weeks on Study | Vehicle Control | | 50 mg/kg | | | 100 mg/kg | | |
|---|---|---|---|---|---|---|---|---|
| | Av. Wt. (grams) | No. of Survivors | Av. Wt. (grams) | Wt. (percent of controls) | No. of Survivors | Av. Wt. (grams) | Wt. (percent of controls) | No. of Survivors |
| MALE | | | | | | | | |
| 0 | 136 | 50 | 134 | 98.5 | 50 | 136 | 100.0 | 50 |
| 1 | 158 | 50 | 152 | 96.2 | 49 | 152 | 96.2 | 50 |
| 2 | 189 | 50 | 186 | 98.4 | 49 | 180 | 95.2 | 49 |
| 3 | 214 | 50 | 211 | 98.6 | 49 | 210 | 98.1 | 49 |
| 4 | 236 | 50 | 233 | 98.7 | 49 | 232 | 98.3 | 49 |
| 5 | 252 | 50 | 252 | 100.0 | 49 | 251 | 99.6 | 49 |
| 6 | 267 | 50 | 265 | 99.3 | 49 | 265 | 99.3 | 49 |
| 7 | 280 | 50 | 278 | 99.3 | 49 | 279 | 99.6 | 49 |
| 8 | 292 | 50 | 289 | 99.0 | 49 | 287 | 98.3 | 49 |
| 9 | 307 | 50 | 301 | 98.0 | 49 | 299 | 97.4 | 49 |
| 10 | 313 | 50 | 309 | 98.7 | 49 | 310 | 99.0 | 49 |
| 11 | 321 | 50 | 317 | 98.8 | 49 | 318 | 99.1 | 49 |
| 12 | 315 | 50 | 326 | 103.5 | 49 | 326 | 103.5 | 49 |
| 16 | 359 | 50 | 354 | 98.6 | 49 | 354 | 98.6 | 49 |
| 20 | 378 | 50 | 373 | 98.7 | 49 | 372 | 98.4 | 49 |
| 24 | 395 | 50 | 391 | 99.0 | 49 | 391 | 99.0 | 49 |
| 28 | 398 | 50 | 393 | 98.7 | 49 | 388 | 97.5 | 49 |
| 32 | 404 | 50 | 396 | 98.0 | 49 | 389 | 96.3 | 49 |
| 36 | 413 | 50 | 403 | 97.6 | 49 | 391 | 94.7 | 49 |
| 40 | 419 | 50 | 411 | 98.1 | 49 | 401 | 95.7 | 49 |
| 44 | 440 | 50 | 431 | 98.0 | 48 | 421 | 95.7 | 49 |
| 48 | 454 | 50 | 449 | 98.9 | 48 | 441 | 97.1 | 49 |
| 52 | 452 | 50 | 449 | 99.3 | 48 | 439 | 97.1 | 49 |
| 56 | 458 | 50 | 453 | 98.9 | 48 | 445 | 97.2 | 48 |
| 60 | 459 | 50 | 457 | 99.6 | 47 | 446 | 97.2 | 48 |
| 64 | 467 | 50 | 465 | 99.6 | 47 | 455 | 97.4 | 48 |
| 68 | 467 | 50 | 466 | 99.8 | 47 | 450 | 96.4 | 48 |
| 72 | 470 | 50 | 472 | 100.4 | 47 | 460 | 97.9 | 48 |
| 76 | 468 | 50 | 470 | 100.4 | 47 | 460 | 98.3 | 48 |
| 80 | 467 | 50 | 468 | 100.2 | 47 | 456 | 97.6 | 47 |
| 84 | 461 | 50 | 466 | 101.1 | 47 | 458 | 99.3 | 45 |
| 88 | 460 | 48 | 463 | 100.7 | 46 | 455 | 98.9 | 41 |
| 92 | 450 | 46 | 457 | 101.6 | 46 | 449 | 99.8 | 39 |
| 96 | 448 | 44 | 447 | 99.8 | 43 | 443 | 98.9 | 37 |
| 100 | 439 | 42 | 443 | 100.9 | 41 | 440 | 100.2 | 35 |
| 104 | 440 | 40 | 439 | 99.8 | 34 | 431 | 98.0 | 31 |
| FEMALE | | | | | | | | |
| 0 | 112 | 50 | 110 | 98.2 | 50 | 113 | 100.9 | 50 |
| 1 | 123 | 50 | 117 | 95.1 | 46 | 121 | 98.4 | 49 |
| 2 | 139 | 50 | 136 | 97.8 | 43 | 136 | 97.8 | 49 |
| 3 | 151 | 50 | 147 | 97.4 | 43 | 148 | 98.0 | 49 |
| 4 | 160 | 50 | 158 | 98.8 | 43 | 161 | 100.6 | 49 |
| 5 | 167 | 50 | 168 | 100.6 | 43 | 169 | 101.2 | 49 |
| 6 | 176 | 50 | 174 | 98.9 | 43 | 175 | 99.4 | 49 |
| 7 | 181 | 50 | 181 | 100.0 | 43 | 180 | 99.4 | 49 |
| 8 | 183 | 50 | 184 | 100.5 | 43 | 186 | 101.6 | 49 |
| 9 | 191 | 50 | 189 | 99.0 | 43 | 190 | 99.5 | 49 |
| 10 | 193 | 50 | 192 | 99.5 | 43 | 196 | 101.6 | 49 |
| 11 | 195 | 50 | 195 | 100.0 | 43 | 197 | 101.0 | 49 |
| 12 | 200 | 50 | 198 | 99.0 | 43 | 200 | 100.0 | 49 |
| 16 | 214 | 50 | 212 | 99.1 | 43 | 213 | 99.5 | 49 |
| 20 | 219 | 50 | 217 | 99.1 | 43 | 219 | 100.0 | 49 |
| 24 | 222 | 50 | 222 | 100.0 | 43 | 226 | 101.8 | 49 |
| 28 | 221 | 50 | 221 | 100.0 | 43 | 226 | 102.3 | 49 |
| 32 | 226 | 50 | 223 | 98.7 | 43 | 231 | 102.2 | 49 |
| 36 | 231 | 50 | 226 | 97.8 | 42 | 231 | 100.0 | 49 |
| 40 | 234 | 50 | 231 | 98.7 | 42 | 236 | 100.9 | 49 |
| 44 | 247 | 50 | 244 | 98.8 | 42 | 246 | 99.6 | 48 |
| 48 | 248 | 50 | 249 | 100.4 | 42 | 254 | 102.4 | 48 |
| 52 | 249 | 50 | 249 | 100.0 | 42 | 257 | 103.2 | 48 |
| 56 | 253 | 50 | 255 | 100.8 | 41 | 259 | 102.4 | 48 |
| 60 | 253 | 50 | 258 | 102.0 | 41 | 265 | 104.7 | 48 |
| 64 | 262 | 50 | 266 | 101.5 | 41 | 273 | 104.2 | 48 |
| 68 | 270 | 50 | 273 | 101.1 | 41 | 277 | 102.6 | 48 |
| 72 | 279 | 50 | 282 | 101.1 | 41 | 281 | 100.7 | 48 |
| 76 | 286 | 50 | 290 | 101.4 | 40 | 288 | 100.7 | 46 |
| 80 | 290 | 49 | 292 | 100.7 | 39 | 290 | 100.0 | 44 |
| 84 | 294 | 49 | 295 | 100.3 | 39 | 295 | 100.3 | 43 |
| 88 | 297 | 49 | 300 | 101.0 | 38 | 297 | 100.0 | 43 |
| 92 | 294 | 47 | 297 | 101.0 | 37 | 296 | 100.7 | 40 |
| 96 | 296 | 47 | 296 | 100.0 | 33 | 299 | 101.0 | 38 |
| 100 | 295 | 42 | 299 | 101.4 | 33 | 298 | 101.0 | 37 |
| 104 | 295 | 40 | 297 | 100.7 | 32 | 294 | 99.7 | 33 |



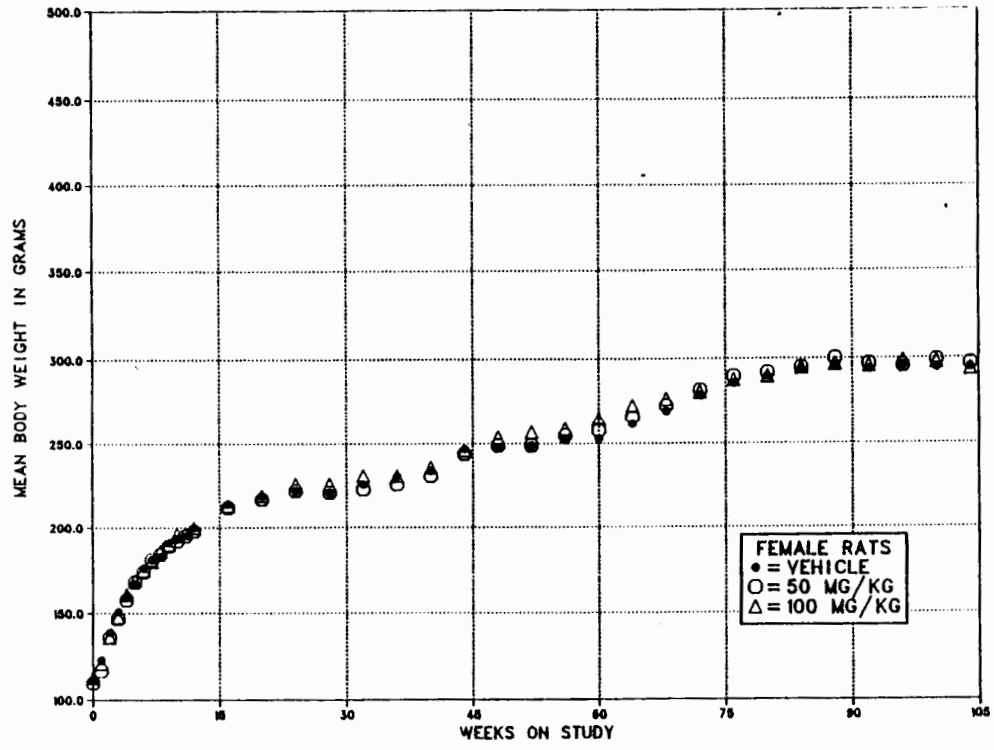




FIGURE 2. GROWTH CURVES FOR RATS ADMINISTERED DIALLYLPHTHALATE IN CORN OIL BY GAVAGE FOR TWO YEARS

### Sentinel Animal Serology

Groups of 10 rats (5 males and 5 females) were killed at 6, 12, 18, and 24 months, and sera were collected and tested for the presence of the following viral antibodies: pneumonia virus of mice (PVM), Sendai, Kilham rat virus (KRV), Toolan's H-1 Virus (H-1), and rat coronavirus (RCV) (Appendix J). Positive titers were observed for RCV at the following times and incidences: 6 months, 10/10 animals; 12 months, 1/10; 18 months, 10/10; and 24 months, 1/10. (Although the significance of elevated antibody titers to the interpretation of toxicity studies is not known at the present time, the data are included in this report for future reference.)

### Survival

Estimates of the probabilities of survival for male and female rats administered diallylphthalate by gavage at the doses used in these studies and those of the vehicle controls are shown in the Kaplan and Meier curves in Figure 3. No significant differences in survival were observed between any groups of either sex. Additional survival data are summarized in Table 8.

A total of 13 rats died accidentally during the studies, including 10 within the initial week of dosing. Twelve of the 13 deaths were considered to be gavage related, and one was due to an apparent crushing of the rat. The distribution of the accidental deaths was as follows: one low dose male, one high dose male, one vehicle control female, eight low dose females, and two high dose females.

The 10 animals that died during the first week of the studies (one low dose male, one high dose male, seven low dose females, and one high dose female) have been removed from the statistical analyses of tumor incidences; however, incidences of neoplastic and nonneoplastic lesions for these animals are included in Appendixes A and B.

TABLE 8. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **MALE (a)** | | | |
| Animals initially in study | 50 | 50 | 50 |
| Nonaccidental deaths before termination (b) | 9 | 13 | 17 |
| Accidentally killed | 0 | 1 | 1 |
| Killed at termination | 40 | 34 | 31 |
| Died during termination period | 1 | 2 | 1 |
| Survival P values (c) | 0.064 | 0.371 | 0.082 |
| **FEMALE (a)** | | | |
| Animals initially in study | 50 | 50 | 50 |
| Nonaccidental deaths before termination (b) | 8 | 10 | 15 |
| Accidentally killed | 1 | 8 | 2 |
| Killed at termination | 40 | 32 | 33 |
| Died during termination period | 1 | 0 | 2 |
| Survival P values (c) | 0.150 | 0.569 | 0.176 |

(a) Terminal kill period: weeks 104-105
(b) Includes animals killed in a moribund condition
(c) The vehicle control column contains the results of the life table trend test; the columns for dosed groups contain the life table exact pairwise comparisons with the vehicle controls.

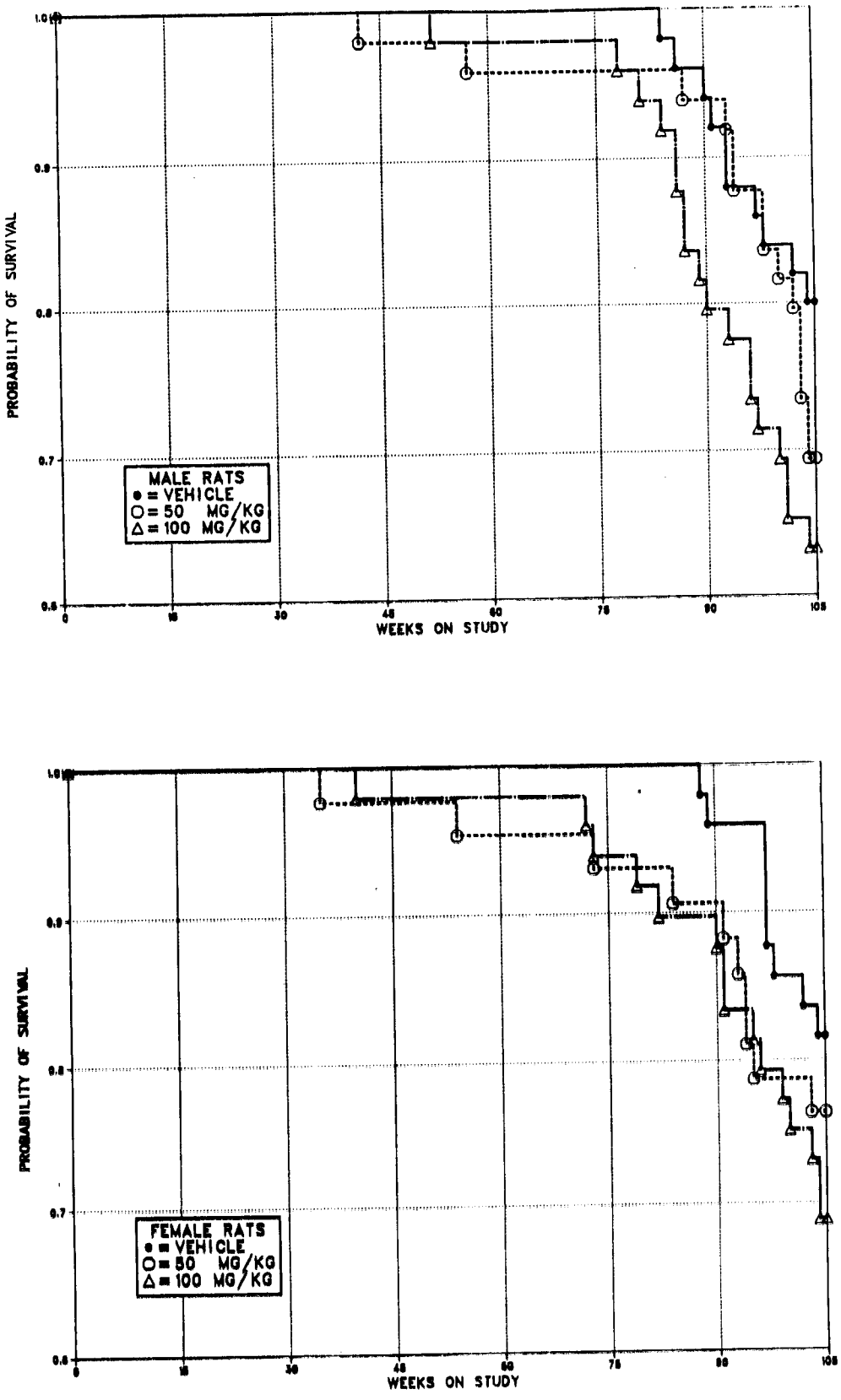



FIGURE 3. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED DIALLYLPHTHALATE IN CORN OIL BY GAVAGE FOR TWO YEARS

### Pathology and Statistical Analyses of Results

This section describes significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions. Histopathologic diagnoses of neoplasms in rats are summarized in Appendix A, Tables A1 and A2; Tables A3 and A4 give the survival and tumor status for individual male and female rats. Diagnoses of nonneoplastic lesions are summarized in Appendix B, Tables B1 and B2. Appendix C, Tables C1 and C2, contain 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 in this study are discussed in Chapter II (Statistical Methods) and Appendix C (footnotes). Historical incidences of tumors in corn oil vehicle control animals are listed in Appendix D.

*Hematopoietic System:* Mononuclear cell leukemia in female rats occurred with a significant positive trend, and the incidence in the high dose group was significantly greater than that in the vehicle controls (Table 9). This leukemia was recognized as a diffuse infiltration of atypical mononuclear white blood cells into the liver sinusoids and the interfollicular pulp of the spleen. Infiltrations into virtually all organs and tissues were observed in more advanced cases. No other types of leukemia were diagnosed in this study.

Mononuclear cell leukemia was detected in several female rats that died before the terminal kill (Table 9). Three female rats in the high dose group (deaths occurring at 40, 72, and 73 weeks on study) and two in the low dose group (deaths occurring at 35 and 54 weeks on study) died with mononuclear cell leukemia before the first death of a vehicle control female rat with this lesion (76 weeks on study). The life table test indicated a stronger statistical association between diallylphthalate administration and leukemia incidence than did the incidental tumor test. Since mononuclear cell leukemia in the F344/N rat is generally considered a lethal tumor, the life table test (which considers the tumor as the cause of death) is more appropriate than the incidental tumor test (which considers the tumor to be coincidental to death) for analyzing the association between chemical administration and the occurrence of mononuclear cell leukemia.

TABLE 9. ANALYSIS OF HEMATOPOIETIC SYSTEM TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (a)

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **Mononuclear Cell Leukemia (b)** | | | |
| Overall Rates | 15/50 (30%) | 15/43 (35%) | 25/49 (51%) |
| Adjusted Rates | 32.2% | 39.6% | 56.0% |
| Terminal Rates | 10/41 (24%) | 10/32 (31%) | 16/35 (46%) |
| Life Table Tests | P=0.013 | P=0.293 | P=0.017 |
| Incidental Tumor Tests | P=0.038 | P=0.513 | P=0.052 |
| **Week of Observation:** | 76 | 35 | 40 |
| | 89 | 54 | 72 |
| | 97 | 91 | 73 |
| | 97 | 93 | 79 |
| | 102 | 94 | 82 |
| | | | 90 |
| | (c) Terminal (10) | (c) Terminal (10) | 95 |
| | | | 96 |
| | | | 100 |
| | | | (c) Terminal (16) |

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).
(b) Mean historical incidence of all leukemias at Litton Bionetics, Inc.: 29.3% ± 13.01% (no data on mononuclear cell leukemia only)
(c) Number of animals found to have mononuclear cell leukemia at the terminal kill

An increased incidence of mononuclear cell leukemia was not observed in male rats (Appendix C, Table C1).

*Liver:* A subjective conclusion was made partway through the terminal kill that diallylphthalate may have increased the size of the liver. Therefore, liver weights were recorded for the remaining animals. The data on absolute and relative liver weights are presented in Table 10, along with incidences of nonneoplastic liver lesions. Although a statistically significant ($P<0.05$) increase in absolute liver and liver-to-body weight ratio was observed in low dose female rats, the magnitude of the increase was not striking. Furthermore, the significance of increased liver weights in aged F344/N rats is clouded by the common occurrence of leukemic infiltrates of the liver sinusoids.

Diallylphthalate administration produced a dose-dependent chronic liver injury characterized by periportal necrosis and fibrosis, pigment accumulation in periportal histiocytes, and excessive bile duct hyperplasia (Tables 10 and 11). Bile duct hyperplasia is frequently observed in aged rats, but the severity of the lesion in the high dose (100 mg/kg) rats in this study was much greater than that in the vehicle controls (Table 11). This complex of liver lesions was often more apparent in some areas of the liver than in others from the same animal, suggesting a possible lobe specificity.

Despite the occurrence of chemically induced nonneoplastic pathologic lesions in the livers of diallylphthalate-dosed rats, no increased occurrences of neoplastic lesions of the liver were observed in either male or female rats (Appendix A, Tables A1 and A2).

**TABLE 10. LIVER WEIGHTS AND INCIDENCES OF LIVER LESIONS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE**

| Dose (mg/kg) | Liver Wt (a) (grams) | Liver/Body Wt (a) (percent) | Lesions and Incidences (percent) | | | |
|---|---|---|---|---|---|---|
| | | | Pigmentation | Necrosis | Fibrosis | Bile Duct Hyperplasia |
| **MALE** | | | | | | |
| 0 | 12.8 ± 0.4 (16) | 3.06 ± 0.10 (16) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 43/50 (86%) |
| 50 | (b) 14.6 ± 0.5 (15) | (b) 3.61 ± 0.14 (15) | 7/49 (14%) | 0/49 (0%) | 3/49 (6%) | 19/49 (39%) |
| 100 | 13.5 ± 0.7 (10) | 3.25 ± 0.14 (10) | 45/49 (92%) | 1/49 (2%) | 43/49 (88%) | 44/49 (90%) |
| **FEMALE** | | | | | | |
| 0 | 9.2 ± 0.3 (16) | 3.33 ± 0.11 (16) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) | 17/50 (34%) |
| 50 | 8.7 ± 0.2 (14) | 3.10 ± 0.08 (14) | 25/43 (58%) | 0/43 (0%) | 0/43 (0%) | 24/43 (56%) |
| 100 | 10.3 ± 1.4 (12) | 3.67 ± 0.51 (12) | 46/49 (94%) | 1/49 (2%) | 34/49 (69%) | 47/49 (96%) |

(a) Mean ± standard error (no. of animals)
(b) Significantly greater than vehicle control ($P< 0.05$) by Dunnett's test

TABLE 11. RELATIVE SEVERITY OF LIVER LESIONS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE (a)

| Severity of Lesion | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Vehicle Control | 50 mg/kg | 100 mg/kg | Vehicle Control | 50 mg/kg | 100 mg/kg |
| **Bile Duct Hyperplasia** | | | | | | |
| None | 7/50 (14%) | 31/50 (62%) | 6/49 (12%) | 32/50 (64%) | 19/43 (44%) | 2/49 (4%) |
| Minimal | 2/50 (4%) | 3/50 (6%) | 5/49 (10%) | 7/50 (14%) | 11/43 (26%) | 10/49 (20%) |
| Mild | 41/50 (82%) | 15/50 (30%) | 14/49 (29%) | 10/50 (20%) | 13/43 (30%) | 19/49 (39%) |
| Moderate | -- | 1/50 (2%) | 24/49 (49%) | 1/50 (2%) | -- | 17/49 (35%) |
| Severe | -- | -- | -- | -- | -- | 1/49 (2%) |
| **Periportal Fibrosis** | | | | | | |
| None | 50/50 (100%) | 47/50 (94%) | 6/49 (12%) | 50/50 (100%) | 43/43 (100%) | 15/49 (31%) |
| Minimal | -- | 2/50 (4%) | 11/49 (23%) | -- | -- | 8/49 (16%) |
| Mild | -- | 1/50 (2%) | 28/49 (57%) | -- | -- | 25/49 (51%) |
| Moderate | -- | -- | 4/49 (8%) | -- | -- | 1/49 (2%) |
| Severe | -- | -- | -- | -- | -- | -- |
| **Pigmentation** | | | | | | |
| None | 50/50 (100%) | 42/50 (84%) | 4/49 (8%) | 45/50 (90%) | 18/43 (42%) | 3/49 (6%) |
| Minimal | -- | 7/50 (14%) | 18/49 (37%) | 5/50 (10%) | 24/43 (56%) | 18/49 (37%) |
| Mild | -- | 1/50 (2%) | 22/49 (45%) | -- | 1/43 (2%) | 28/49 (57%) |
| Moderate | -- | -- | 5/49 (10%) | -- | -- | -- |
| Severe | -- | -- | -- | -- | -- | -- |

(a) These diagnoses for severity were conducted independently of the principal histopathology diagnoses for this study and by different pathologists.

*Other Organs:* The following tumors occurred with marginal but statistically significant ($P<0.05$) negative trends in dosed rats, or at marginal but significantly ($P<0.05$) lower incidences in high dose animals than in vehicle controls: pituitary adenomas and pituitary adenomas or carcinomas (combined) in male and female rats and keratoacanthomas (skin) in male rats (Table 12).

TABLE 12. ANALYSIS OF PITUITARY GLAND AND SKIN TUMORS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **MALE (a)** | | | |
| **Skin: Keratoacanthoma** | | | |
| Overall Rates | 5/50 (10%) | 2/49 (4%) | 0/49 (0%) |
| Adjusted Rates | 12.2% | 5.6% | 0.0% |
| Terminal Rates | 5/41 (12%) | 2/36 (6%) | 0/32 (0%) |
| Life Table Tests | P=0.031N | P=0.271N | P=0.058N |
| Incidental Tumor Tests | P=0.031N | P=0.271N | P=0.058N |
| **Pituitary: Adenoma** | | | |
| Overall Rates | 8/50 (16%) | 6/48 (13%) | 1/49 (2%) |
| Adjusted Rates | 18.9% | 16.7% | 3.1% |
| Terminal Rates | 7/41 (17%) | 6/36 (17%) | 1/32 (3%) |
| Life Table Tests | P=0.037N | P=0.482N | P=0.042N |
| Incidental Tumor Tests | P=0.033N | P=0.443N | P=0.034N |
| **Pituitary: Carcinoma** | | | |
| Overall Rates | 0/50 (0%) | 2/48 (4%) | 0/49 (0%) |
| **Pituitary: Adenoma or Carcinoma** | | | |
| Overall Rates | 8/50 (16%) | 8/48 (17%) | 1/49 (2%) |
| Adjusted Rates | 18.9% | 21.4% | 3.1% |
| Terminal Rates | 7/41 (17%) | 7/36 (19%) | 1/32 (3%) |
| Life Table Tests | P=0.051N | P=0.509 | P=0.042N |
| Incidental Tumor Tests | P=0.040N | P=0.583 | P=0.034N |
| **FEMALE (b)** | | | |
| **Pituitary: Adenoma** | | | |
| Overall Rates | 23/49 (47%) | 12/41 (29%) | 14/48 (29%) |
| Adjusted Rates | 50.8% | 32.9% | 37.0% |
| Terminal Rates | 18/40 (45%) | 8/32 (25%) | 11/34 (32%) |
| Life Table Tests | P=0.125N | P=0.122N | P=0.160N |
| Incidental Tumor Tests | P=0.034N | P=0.074N | P=0.061N |
| **Pituitary: Carcinoma** | | | |
| Overall Rates | 0/49 (0%) | 1/41 (2%) | 0/48 (0%) |
| **Pituitary: Adenoma or Carcinoma** | | | |
| Overall Rates | 23/49 (47%) | 13/41 (32%) | 14/48 (29%) |
| Adjusted Rates | 50.8% | 35.7% | 37.0% |
| Terminal Rates | 18/40 (45%) | 9/32 (28%) | 11/34 (32%) |
| Life Table Tests | P=0.129N | P=0.173N | P=0.160N |
| Incidental Tumor Tests | P=0.036N | P=0.116N | P=0.061N |

(a) Mean historical incidence (± standard deviation) at Litton Bionetics, Inc.: skin keratocanthoma--4.7% ± 4.62%; pituitary adenoma--13.4% ± 3.09%; pituitary carcinoma--2.0% ± 2.04%; pituitary adenoma or carcinoma--15.4% ± 3.22%
(b) Mean historical incidence (± standard deviation) at Litton Bionetics, Inc.: pituitary adenoma--44.3% ±7.36%; pituitary carcinoma--2.0% ± 2.0%; pituitary adenoma or carcinoma--46.3% ± 8.02%

# IV. DISCUSSION AND CONCLUSIONS

*Prechronic Studies:* The 14-day $LD_{50}$ values for diallylphthalate administered once, by gavage in corn oil, were 891 mg/kg for male rats and 656 mg/kg for female rats. These values are within or close to the range of 0.77-1.7 g/kg for acute oral $LD_{50}$ values reported in the literature (see Introduction).

The liver was the major target organ in the prechronic studies. Gross changes consisting of a mottled appearance and yellow blotches on the surface of the liver were detected at necropsy of male and female rats administered doses as low as 100 mg/kg in the 14-day repeated-administration studies. Similar gross changes were observed at necropsy during the 13-week studies. Microscopic examination of the livers from the 13-week studies revealed a chemically induced complex of lesions in the periportal area of the hepatic lobules. The liver lesions consisted of hepatocellular necrosis and fibrosis and bile duct hyperplasia in both sexes at 200 and 400 mg/kg. The lesions were more severe at 400 mg/kg than at 200 mg/kg, and nodular hyperplasia was observed as well at 400 mg/kg, leading to the diagnosis of liver cirrhosis at this dose level. More subtle changes in hepatocytes (basophilia, hypertrophy, and nuclear hyperchromatism) were observed with doses as low as 50 mg/kg.

As discussed in the Introduction of this report, hydrolysis of diallylphthalate would release allyl alcohol, a chemical known to produce acute periportal necrosis similar to that described for diallylphthalate. Allyl alcohol produces liver injury following oxidation to acrolein. It can be speculated, therefore, that hydrolysis of diallylphthalate to allyl alcohol, and further oxidation to acrolein, are responsible for the periportal injury observed in this study. Although supporting histopathologic data are not currently available, it is likely that diallylphthalate administration initially produced periportal hepatocellular necrosis. Continued necrotic insult with successive chemical administration, however, resulted in the mixed lesion of periportal necrosis, periportal fibrosis, and nodular hyperplasia in the 13-week studies.

The same general type of hepatotoxic response was observed in rats receiving allylisovalerate (NTP, 1983a). Whether allylisovalerate is metabolized to allyl alcohol or acrolein is unknown.

A previous report (NTP, 1983b) indicated that diallylphthalate did not cause liver injury in 13week or 2-year studies in $B6C3F_1$ mice. Data on acute allyl alcohol or acrolein toxicity in mice are not available. However, the pharmacokinetic data in Appendix K support metabolic differences as a probable reason for the species difference in hepatotoxic response to diallylphthalate. Both rats and mice produce a similar spectrum of metabolites, but the relative percentages of metabolites differ greatly. Rats metabolize a greater percentage of diallylphthalate to carbon dioxide than do mice. Metabolism to carbon dioxide is through the intermediates allyl alcohol and acrolein, potent hepatotoxicants known to cause the periportal hepatocellular necrosis observed in diallylphthalate-treated rats. In contrast, mice excrete a relatively greater percentage of diallylphthalate as urinary 3-hydroxypropylmercapturic acid, a product of the conjugation (with further metabolism) of allyl alcohol or acrolein with hepatic glutathione. Thus, mice may be more efficient than rats at detoxifying the reactive intermediate acrolein via conjugation with glutathione and are therefore more resistant to diallylphthalate hepatotoxicity.

Two other lesions, acute necrotizing colitis and multifocal renal cortical necrosis, occurred in several male rats at 400 mg/kg in the 13-week study. All of these lesions occurred in male rats that died before the end of the study, and the renal necrosis did not appear to be sufficiently severe to cause death. Thus, the kidney injury may have been an indirect result of circulatory collapse in the moribund animals (ischemia), rather than a direct nephrotoxic effect. Toxic nephropathy, however, cannot be excluded on the basis of these data. The pathogenesis of the acute necrotizing colitis is also unclear. The loss of surface epithelium and the presence of mucosal and submucosal edema and acute inflammatory cell infiltration suggest an irritant effect or other localized response to the chemical. It should be noted that diallylphthalate caused inflammation and hyperplasia of the forestomach when administered for 2 years to male and female $B6C3F_1$ mice by gavage (NTP, 1983b).

No gastric lesions were observed in the rat studies.

*Two-Year Studies:* Maximum doses of 100 mg/kg for both male and female rats were chosen for the 2-year studies because of the presence of severe diallylphthalate-induced hepatotoxic lesions in both sexes at 200 and 400 mg/kg in the 13-week studies and because of the relative absence of hepatotoxic effects, other tissue injuries, clinical signs of toxicity, or reductions in survival or mean body weight gains at doses of 100 mg/kg or lower in the 13-week studies. Doses of 50 or 100 mg/kg of diallylphthalate did not significantly reduce survival or alter body weight gains of either male or female rats during the 2-year studies. For these reasons, the doses were considered to be appropriate to test for chronic toxic potential, including carcinogenic activity.

A total of 13 animals in the 2-year studies died of accidental causes, 12 by gavage-related trauma. Although this number by itself does not seem excessive, 10/13 animals were killed during the initial week of the studies, effectively reducing the number of animals at risk. (These 10 animals were censored from the statistical comparisons of tumor incidences.) Seven of the 10 deaths occurring during the initial week of the studies were from the low dose group of female rats. The effective number of animals in this group, therefore, was reduced from 50 to 43. Since the survival of the high dose females and the remaining low dose females was good and the diallylphthalate-induced liver lesions occurring in the low dose females were consistent with those occurring in the high dose females, the loss of these seven animals does not appear to have had a major adverse impact on the study results or interpretations.

The only nonneoplastic toxic injury observed in the 2-year studies was the development of chronic liver disease characterized by periportal fibrosis, periportal accumulation of pigmented material, and increased incidence (females only) and severity (males and females) of bile duct hyperplasia. The nature of these lesions, primarily fibrosis, is consistent with the complex of liver lesions observed at higher doses in the 13-week studies, and its pathogenesis is probably similar, although the lesion is in a more advanced form than that seen in the 13-week studies. The presence of fibrosis at 100 mg/kg in the 2-year studies, but not at 100 mg/kg in the 13-week studies, may indicate progression of the lesion with prolonged exposure to the chemical or increased susceptibility of aging animals.

The marked hepatic fibrosis in dosed rats did not reduce survival, alter body weight gains, or produce other clinical signs of toxicity. Its impact on animal health, therefore, is unknown. Examination of different areas of the same liver led to a subjective impression that the presence and severity of the chronic liver injury were not uniformly distributed throughout the liver. Sufficient liver tissue may have been spared, therefore, to allow continued good health and survival of the affected animals.

Despite the occurrence of chronic liver injury (fibrosis) indicative of prior necrosis and cellular regeneration (see the Results section of this report and the Discussion on the 13-week studies), increased incidences of liver tumors were not observed in dosed rats. Hepatocellular carcinomas occurred in one rat in each of the male vehicle control and high dose groups, and neoplastic nodules of the liver occurred in one vehicle control male, five vehicle control females, and three high dose females (Appendix A, Tables A1 and A2).

The only tumor occurring at significantly increased incidence in dosed rats in these studies was mononuclear cell leukemia (Table 9). The increase in female rats was statistically significant ($P<0.05$) by trend tests and in high dose rats by pairwise comparisons. In addition, there appeared to be a decreased latency for the occurrence of mononuclear cell leukemia in female rats receiving diallylphthalate (Table 9). The significance of this increased tumor incidence was more apparent by the life table test than by the incidental tumor test. The former regards the tumor as the cause of death, whereas the latter regards it as coincidental. Since mononuclear cell leukemia is considered to be a lethal tumor in F344/N rats, the life table test would seem to be more appropriate than the incidental tumor test for evaluating survival-adjusted

incidences of mononuclear cell leukemia in this study. (A more detailed comparison of the various statistical tests is contained in the Materials and Methods section.) All of the statistical tests, however, indicated a significant ($P<0.05$) relationship between diallylphthalate administration and the incidence of mononuclear cell leukemia in female rats.

The incidence of mononuclear cell leukemia in vehicle (corn oil) control female rats from this study, 15/50 (30%), was similar to the historical rate for leukemia for corn oil-gavaged vehicle control female rats at the performing laboratory (incidence: 44/150; mean ± SD: 29% ± 13%). It was somewhat greater than that observed programwide in corn oil-gavaged, control female rats (185/1,147; 16% ± 9%) (Appendix D, Table D1). The statistically significant increase in mononuclear cell leukemia in high dose female rats, therefore, cannot be attributed to a low incidence in the concurrent controls . Moreover, the incidence of mononuclear cell leukemia in high dose females in this study (25/49, 51%) is substantially greater than that for corn oil-gavaged vehicle control female rats, both in the testing laboratory (29%) and programwide (16%), and is greater than the highest incidence ever recorded in this program's historical data base for corn oil-gavaged vehicle control female rats (21/50, 42%).

No lymphomas and no other types of leukemia were detected in female rats in this study. Differential diagnoses of hematopoietic system tumors, therefore, did not contribute to the increased occurrence of mononuclear cell leukemia in dosed female rats. Tumors of the hematopoietic system did not occur at increased incidences in dosed male rats.

Adenomas and adenomas or carcinomas (combined) of the pituitary gland occurred in dosed male and female rats at marginally but statistically significant ($P<0.05$) decreased incidences by trend tests or pairwise comparisons. Incidences in vehicle controls from these studies were consistent with programwide historical controls (Appendix D, Tables D2 and D4). Keratoacanthomas (skin) also occurred in dosed male rats at marginally but statistically significant ($P<0.05$) decreased incidences by trend tests or pairwise comparisons. However, the incidence in concurrent vehicle control males (5/50, 10%) was greater than that in programwide historical controls (24/1,146; 2.1% ± 2.4%) and in previous studies at the performing laboratory (2/100, 2%) (Appendix D, Table D3. The cause(s) and significance of these decreased tumor incidences in dosed rats are unknown.

*Comparisons Between These Studies and Other Studies:* Diallylphthalate was administered by gavage in corn oil to male and female $B6C3F_1$ mice for 2 years in a recently reported companion study to this experiment (NTP, 1983b; Appendix M) and was found to cause forestomach hyperplasia and chronic inflammation. Rare forestomach papillomas also occurred in both sexes of dosed mice, leading to the conclusion that the development of forestomach papillomas may have been related to chemical administration but that the available data were insufficient to indicate a clear cause-and-effect relationship. In addition, a statistically significant positive trend occurred in the incidence of lymphomas in male mice, although a pairwise comparison between high dose and vehicle control groups did not reveal a statistically significant ($P<0.05$) difference (Table 13). Thus, the increased occurrence of lymphomas in male mice was regarded as equivocally related to diallylphthalate administration. When considered collectively, however, the data from the diallylphthalate mouse and rat studies provide evidence that suggests an effect of this chemical on the rodent hematopoietic system.

Three other chemicals with an allyl moiety ($CH_2=CH-CH_2-$) have been tested in the NTP/NCI carcinogenesis program (all by gavage in corn oil): allyl chloride (NCI, 1978), allylisovalerate (NTP, 1983a), and allylisothiocyanate (NTP, 1982b). Allyl chloride caused squamous cell papillomas or carcinomas of the forestomach in both male and female mice, as did allylisovalerate in male mice. Allylisothiocyanate caused transitional cell papillomas of the urinary bladder in male rats. In addition, allylisovalerate and allylisothiocyanate increased

TABLE 13. OCCURRENCES OF HEMATOPOIETIC SYSTEM TUMORS IN THE ALLYLISOTHIOCYANATE, ALLYLISOVALERATE, BUTYL BENZYL PHTHALATE, AND DIALLYLPHTHALATE (MOUSE) STUDIES

| Chemical and Tumor Type | Vehicle Control | Low Dose | High Dose |
|---|---|---|---|
| **Allylisothiocyanate (a) (F344/N rat, male)** | (b) 0 | 12 mg/kg | 25 mg/kg |
| **Mononuclear Cell Leukemia** | | | |
| Overall Rates | 2/50 (4%) | 6/50 (12%) | 8/50 (16%) |
| Adjusted Rates | 5% | 17% | 22% |
| Terminal Rates | 0/38 (0%) | 4/33 (12%) | 5/33 (15%) |
| Life Table Tests | P=0.024 | P=0.093 | P=0.030 |
| Incidental Tumor Tests | P=0.006 | P=0.070 | P=0.009 |
| **Allylisovalerate (c) (F344/N rat, male)** | (b) 0 | 31 mg/kg | 62 mg/kg |
| **Mononuclear Cell Leukemia** | | | |
| Overall Rates | 1/50 (2%) | 4/50 (8%) | 7/50 (14%) |
| Adjusted Rates | 3% | 11% | 22% |
| Terminal Rates | 0/34 (0%) | 0/30 (0%) | 4/28 (14%) |
| Life Table Tests | P=0.015 | P=0.183 | P=0.022 |
| Incidental Tumor Tests | P=0.023 | P=0.482 | P=0.044 |
| **Allylisovalerate (c) ($B6C3F_1$ mouse, female)** | (b) 0 | 31 mg/kg | 62 mg/kg |
| **Malignant Lymphoma** | | | |
| Overall Rates | 11/50 (22%) | 11/50 (22%) | 18/50 (36%) |
| Adjusted Rates | 30% | 46% | 55% |
| Terminal Rates | 8/32 (25%) | 6/17 (35%) | 10/24 (42%) |
| Life Table Tests | P=0.026 | P=0.172 | P=0.034 |
| Incidental Tumor Tests | P=0.037 | P=0.360 | P=0.052 |
| **Diallylphthalate (d) ($B6C3F_1$ mouse, male)** | (b) 0 | 150 mg/kg | 300 mg/kg |
| **Lymphoma** | | | |
| Overall Rates | 6/50 (12%) | 5/50 (10%) | 12/50 (24%) |
| Adjusted Rates | 15.8% | 13.2% | 32.7% |
| Terminal Rates | 6/38 (16%) | 5/38 (13%) | 8/32 (25%) |
| Life Table Tests | P=0.031 | P=0.500N | P=0.051 |
| Incidental Tumor Tests | P=0.037 | P=0.500N | P=0.058 |
| **Butyl Benzyl Phthalate (e) (F344/N rat, female)** | (b) 0 | (f) 6,000 ppm | (f) 12,000 ppm |
| **Mononuclear Cell Leukemia** | | | |
| Overall Rates | 7/49 (14%) | 7/49 (14%) | 18/50 (36%) |
| Adjusted Rates | 19% | 18% | 42% |
| Terminal Rates | 4/32 (12%) | 2/31 (6%) | 8/32 (25%) |
| Life Table Tests | P=0.013 | P=0.600N | P=0.024 |
| Incidental Tumor Tests | P=0.004 | P=0.611N | P=0.009 |

(a) Data derived from NTP, 1982a
(b) Vehicle control
(c) Data derived from NTP, 1983a
(d) Data derived from NTP, 1983b
(e) Data derived from NTP, 1982c
(f) Concentration in feed

the occurrences of mononuclear cell leukemia in male rats, and the former increased the occurrence of malignant lymphomas in female mice (Table 13). Thus, some other compounds containing an allyl group appear to be associated with malignancies of the hematopoietic system in rodents.

Allylisovalerate also caused a chronic liver lesion characterized by periportal cirrhosis (fibrosis), focal necrosis, nodular regeneration, and periportal accumulation of pigment in male and female rats (NTP, 1983a). Thus, the morphological characteristics, and perhaps the pathogenesis of the liver disease caused by allylisovalerate, appear to be similar to or the same as that caused by diallylphthalate; allyl alcohol (or acrolein) may be the causative agent.

Several other *ortho* phthalate esters and structurally related compounds have been tested in the NTP/NCI carcinogenesis program: di(2-ethylhexyl)phthalate (NTP, 1982e), di(2-ethylhexyl)adipate (NTP, 1982d), butyl benzyl phthalate (NTP, 1982c), phthalamide (NCI, 1979a), and phthalic anhydride (NCI, 1979b). Both di(2-ethylhexyl)phthalate and di(2-ethylhexyl)adipate increased the occurrence of hepatocellular carcinomas in rodents, the former in both rats and mice and the latter in mice only. Butyl benzyl phthalate was not considered adequately tested in male rats because of excessive numbers of early deaths, but increased incidences of mononuclear cell leukemia occurred in female rats (Table 13). Butyl benzyl phthalate increased the occurrence of mononuclear cell leukemia in female rats; similar effects were seen in the current diallylphthalate study. No tumors occurred at increased incidences in the phthalamide or phthalic anhydride 2-year studies. Therefore, carcinogenic activities have been associated with some *ortho* phthalate esters and related compounds.

The genetic toxicity of diallylphthalate and purported metabolites is discussed in detail in the Introduction section of this report. In brief, mutagenic activity has been reported for several diallylphthalate metabolites, especially the epoxides glycidol and glycidaldehyde. Glycidaldehyde also caused skin tumors upon repeated dermal application to mice (Van Duuren et al., 1965). Mutagenic activity has not been reported for the parent compound diallylphthalate.

*Interpretation Difficulties:* In June 1983, the NTP adopted the use of specific categories of evidence of carcinogenicity in order to define more precisely the relative strength of experimental evidence of carcinogenic response in each adequately tested male and female animal species, as outlined on page 2 of this Technical Report. Although these categories are of substantial generic value to the interpretation of NTP studies, some experimental data, such as those for diallylphthalate, do not easily fit into any single category. If great confidence is placed in the statistical association of diallylphthalate administration with the increased occurrence of mononuclear cell leukemia in the female rats, then the most appropriate category would appear to be *clear evidence of carcinogenicity*, defined as a "chemically related increased incidence of malignant neoplasms." If less confidence is placed in this statistical association, then the category of *equivocal evidence of carcinogenicity*, defined as a "chemically related marginal increase of neoplasms," would be more appropriate. The definitions for the category of *some evidence of carcinogenicity*--a "chemically related increased incidence of benign neoplasms," a "marginal increase in neoplasms of several organs or tissues," a "slight increase in uncommon tumors"--do not appear superficially to describe the diallylphthalate-mononuclear cell leukemia relationship. However, this category implies, at least by its descriptive title of *some evidence*, a strength of evidence intermediate between *clear evidence* and *equivocal evidence*.

In its deliberations, the NTP has considered arguments both supporting and detracting from the significance of the observed increase in mononuclear cell leukemia in female rats as evidence of a carcinogenic response. These arguments are as follows:

Supporting Evidence

1. The apparent increase in mononuclear cell leukemia was statistically significant by both trend tests and pairwise comparisons. Moreover, the strongest statistical evidence of an effect was provided by the survival-adjusted life table test,

the analysis most appropriate for a lethal neoplasm such as mononuclear cell leukemia. The results of this test suggest that mononuclear cell leukemia occurred earlier in the higher dose female rats than in the vehicle controls.

2. The occurrence of mononuclear cell leukemia in vehicle control female rats in this study was within the range observed for historical controls at the performing laboratory and somewhat greater than the historical control rate program-wide. Thus, the apparent increase in mononuclear cell leukemia in the higher dose animals was not a result of an abnormally low rate in the controls.

3. Although the historical incidence of mononuclear cell leukemia in control female rats appears to be highly variable, the overall rate observed for the higher dose female rats in this study, 25/49 or 51%, exceeded the highest overall rate ever observed in control female rats in this program, 21/50 or 42%.

4. As indicated in Table 13, other compounds containing an allylic moiety, allylisovalerate and allylisothiocyanate, have been associated with increased occurrences of mononuclear cell leukemia or lymphomas in at least one sex or species in recent NTP studies. Also, diallylphthalate was considered to be equivocally related to a marginally increased occurrence of lymphomas in male mice in a previous NTP study.

5. Some diallylphthalate metabolites are recognized as strong (glycidol, glycidaldehyde) or weak (acrolein, allyl alcohol) mutagens.

Mitigating Evidence

1. There was no evidence for a diallylphthalate-related increased occurrence of mononuclear cell leukemia in male rats or in the lower dose female rats.

2. Mononuclear cell leukemia is a rapidly progressing disease in Fischer 344 rats and can be difficult to diagnose in a definitive fashion. Thus, the rates cited may not be absolute and, upon reanalysis, could change somewhat depending on the subjective criteria used by the examiner. Any interpretive disagreements would not be expected to totally negate the apparent increased occurrence of mononuclear cell leukemia in the higher dose female rats, but the statistical evidence of an association between mononuclear cell leukemia and chemical administration could be reduced to a more marginal level. All the mononuclear cell leukemias in female rats in this study were graded according to severity in an independent, "blind" (coded slide) review. In that review, with grade I being the least severe and grade III the most severe, the numbers of leukemias in each grade were as follows: grade I--vehicle control, 2; low dose, 7; high dose, 6; grade II--3, 2, 1; grade III--10, 7, 18. Statistical analyses of these data were considered unwarranted because the distinctions between grades were arbitrary and the grading was not peer reviewed.

3. The relatively high and variable spontaneous occurrence of mononuclear cell leukemia in aged Fischer 344 rats confounds the interpretation of an increased occurrence of this tumor type in dosed animals as evidence of a carcinogenic response. That is, statistical evidence of an increased occurrence of mononuclear cell leukemia in dosed animals as an indication of carcinogenicity may appropriately be regarded with less confidence than would similar incidence data for other tumor types in the F344 rat.

4. No other chemically related increased incidences of neoplasia were observed in rats.

The NTP debated the respective merits of all of these arguments and came to the consensus that, although none of the designated categories of evidence clearly described the diallylphthalate results, the data could arguably be supported in either the categories of *some evidence* or *equivocal evidence*. This equivocation in choosing the most appropriate category of evidence was reflected as well in the opinions of the Peer Review Panel, which ranged from support for *clear evidence* to support for *equivocal evidence*. Following a lengthy discussion, however, the consensus of the Panel was that *equivocal evidence* was the most appropriate category of evidence due to the nondefinitive nature of the diagnosis of mononuclear cell leukemia in aged F344 rats.

# IV. DISCUSSION AND CONCLUSIONS

*Conclusions:* Under the conditions of this study, the administration of diallylphthalate by gavage in corn oil to male and female F344/N rats for 2 years caused chronic liver disease characterized by periportal fibrosis and pigment accumulation and an increased severity of bile duct hyperplasia. The incidence of mononuclear cell leukemia was significantly increased in female rats receiving 100 mg/kg. Because of the variability in incidence of this neoplasm in aged Fischer 344 rats and the difficulty in definitively diagnosing this lesion in Fischer 344 rats, this increase was considered to be *equivocal evidence of carcinogenicity** of diallylphthalate in female rats. There was *no evidence of carcinogenicity* in male rats.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

# V. REFERENCES

# V. REFERENCES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# APPENDIX A

# SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

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

| | CONTROL (VEH) | 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 | | | 1 (2%) |
| SQUAMOUS CELL CARCINOMA | | 1 (2%) | |
| BASAL-CELL TUMOR | 1 (2%) | | |
| KERATOACANTHOMA | 5 (10%) | 2 (4%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| SARCOMA, NOS | | | 1 (2%) |
| FIBROMA | 3 (6%) | 2 (4%) | 1 (2%) |
| FIBROSARCOMA | 2 (4%) | | |
| MYXOSARCOMA | | 1 (2%) | |
| GRANULAR-CELL TUMOR, NOS | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (50) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 1 (2%) | 1 (2%) | |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 1 (2%) | | 1 (2%) |
| FIBROSARCOMA, METASTATIC | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIG. LYMPHOMA, HISTIOCYTIC TYPE | | | 1 (2%) |
| LEUKEMIA, MONONUCLEAR CELL | 13 (26%) | 12 (24%) | 14 (28%) |
| #MEDIASTINAL L. NODE | (50) | (50) | (49) |
| MESOTHELIOMA, METASTATIC | | 1 (2%) | |
| #ABDOMINAL LYMPH NODE | (50) | (50) | (49) |
| CARCINOSARCOMA, UNC PRIM OR META | | 1 (2%) | |
| #MESENTERIC L. NODE | (50) | (50) | (49) |
| CARCINOSARCOMA, UNC PRIM OR META | | 1 (2%) | |
| #THYMUS | (42) | (44) | (44) |
| CYSTADENOMA, NOS | | | 1 (2%) |
| THYMOMA | | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| HEMANGIOMA | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #SALIVARY GLAND | (50) | (50) | (49) |
| UNDIFFERENTIATED CARCINOMA | | 1 (2%) | |
| FIBROSARCOMA, INVASIVE | 1 (2%) | | |
| #LIVER | (50) | (50) | (50) |
| NEOPLASTIC NODULE | 1 (2%) | | |
| HEPATOCELLULAR CARCINOMA | 1 (2%) | | 1 (2%) |
| #PANCREAS | (50) | (50) | (49) |
| ACINAR-CELL ADENOMA | | 2 (4%) | |
| #FORESTOMACH | (50) | (50) | (50) |
| SQUAMOUS CELL CARCINOMA | | | 1 (2%) |
| URINARY SYSTEM | | | |
| NONE | | | |

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

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (50) | (49) | (50) |
| ADENOMA, NOS | 8 (16%) | 6 (12%) | 1 (2%) |
| #ANTERIOR PITUITARY | (50) | (49) | (50) |
| CARCINOMA, NOS | | 2 (4%) | |
| #ADRENAL | (50) | (50) | (49) |
| PHEOCHROMOCYTOMA | 13 (26%) | 10 (20%) | 6 (12%) |
| GANGLIONEUROMA | | 1 (2%) | |
| #THYROID | (49) | (49) | (49) |
| FOLLICULAR-CELL ADENOMA | | | 1 (2%) |
| C-CELL ADENOMA | 2 (4%) | 3 (6%) | 1 (2%) |
| C-CELL CARCINOMA | | 1 (2%) | |
| #PARATHYROID | (44) | (46) | (45) |
| ADENOMA, NOS | | | 1 (2%) |
| #PANCREATIC ISLETS | (50) | (50) | (49) |
| ISLET-CELL ADENOMA | 1 (2%) | 1 (2%) | 1 (2%) |
| ISLET-CELL CARCINOMA | 2 (4%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| FIBROADENOMA | 1 (2%) | 2 (4%) | 1 (2%) |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| CARCINOMA, NOS | 1 (2%) | 2 (4%) | 1 (2%) |
| #TESTIS | (50) | (50) | (50) |
| INTERSTITIAL-CELL TUMOR | 48 (96%) | 45 (90%) | 46 (92%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (50) | (50) | (50) |
| ASTROCYTOMA | 1 (2%) | 1 (2%) | 1 (2%) |
| MENINGIOMA | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *ZYMBAL GLAND | (50) | (50) | (50) |
| CARCINOMA, NOS | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (50) | (50) | (50) |
| MYXOMA | 1 (2%) | | |
| MYXOSARCOMA | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MESOTHELIOMA, NOS | 1 (2%) | 1 (2%) | |
| MESOTHELIOMA, MALIGNANT | | 1 (2%) | |
| TAIL | | | |
| FIBROMA | | | 1 |

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

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

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH | 2 | 4 | 14 |
| MORIBUND SACRIFICE | 8 | 11 | 4 |
| SCHEDULED SACRIFICE | | | |
| TERMINAL SACRIFICE | 40 | 34 | 31 |
| DOSING ACCIDENT | | 1 | 1 |
| ACCIDENTALLY KILLED, NDA | | | |
| ACCIDENTALLY KILLED, NOS | | | |
| ANIMAL MISSING | | | |
| ANIMAL MISSEXED | | | |
| OTHER CASES | | | |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS** | 50 | 48 | 48 |
| TOTAL PRIMARY TUMORS | 109 | 102 | 85 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 50 | 47 | 46 |
| TOTAL BENIGN TUMORS | 85 | 75 | 63 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 21 | 20 | 20 |
| TOTAL MALIGNANT TUMORS | 22 | 23 | 22 |
| TOTAL ANIMALS WITH SECONDARY TUMORS## | 1 | 1 | |
| TOTAL SECONDARY TUMORS | 2 | 1 | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 2 | 2 | |
| TOTAL UNCERTAIN TUMORS | 2 | 2 | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | 1 | |
| TOTAL UNCERTAIN TUMORS | | 2 | |

** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
## SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

**TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE**

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| FIBROMA | 1 (2%) | 1 (2%) | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (50) |
| ADENOCARCINOMA, NOS, METASTATIC | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| LEUKEMIA, MONONUCLEAR CELL | 15 (30%) | 15 (30%) | 25 (50%) |
| #THYMUS | (43) | (44) | (31) |
| ADENOMA, NOS | | | 1 (3%) |
| THYMOMA | 1 (2%) | | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| *TONGUE | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILLOMA | | 1 (2%) | |
| #LIVER | (50) | (50) | (50) |
| NEOPLASTIC NODULE | 5 (10%) | | 3 (6%) |
| #JEJUNUM | (49) | (48) | (49) |
| LEIOMYOSARCOMA | 1 (2%) | | |
| URINARY SYSTEM | | | |
| NONE | | | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (49) | (48) | (49) |
| ADENOMA, NOS | 23 (47%) | 12 (25%) | 14 (29%) |
| #PITUITARY INTERMEDIA | (49) | (48) | (49) |
| ADENOMA, NOS | | | 1 (2%) |
| #ANTERIOR PITUITARY | (49) | (48) | (49) |
| CARCINOMA, NOS | | 1 (2%) | |
| #ADRENAL | (50) | (50) | (50) |
| PHEOCHROMOCYTOMA | 2 (4%) | 3 (6%) | 5 (10%) |
| #ADRENAL MEDULLA | (50) | (50) | (50) |
| PHEOCHROMOCYTOMA | 2 (4%) | | |
| #THYROID | (48) | (49) | (50) |
| FOLLICULAR-CELL ADENOMA | | 1 (2%) | |
| C-CELL ADENOMA | 1 (2%) | | 3 (6%) |
| C-CELL CARCINOMA | 1 (2%) | | |
| #PANCREATIC ISLETS | (49) | (49) | (49) |
| ISLET-CELL ADENOMA | | 1 (2%) | |

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | | 1 (2%) | |
| ADENOCARCINOMA, NOS | 1 (2%) | 1 (2%) | |
| CYSTADENOMA, NOS | | | 1 (2%) |
| FIBROADENOMA | 12 (24%) | 11 (22%) | 5 (10%) |
| *CLITORAL GLAND | (50) | (50) | (50) |
| CARCINOMA, NOS | 1 (2%) | | 1 (2%) |
| ADENOMA, NOS | 1 (2%) | | |
| *VAGINA | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILLOMA | 1 (2%) | | |
| #UTERUS | (50) | (50) | (50) |
| LEIOMYOMA | | 1 (2%) | |
| ENDOMETRIAL STROMAL POLYP | 9 (18%) | 8 (16%) | 10 (20%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (49) | (50) | (50) |
| ASTROCYTOMA | 1 (2%) | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH | 5 | 3 | 6 |
| MORIBUND SACRIFICE | 4 | 7 | 9 |
| SCHEDULED SACRIFICE | | | |
| TERMINAL SACRIFICE | 40 | 32 | 33 |
| DOSING ACCIDENT | 1 | 7 | 2 |
| ACCIDENTALLY KILLED, NDA | | 1 | |
| ACCIDENTALLY KILLED, NOS | | | |
| ANIMAL MISSING | | | |
| ANIMAL MISSEXED | | | |
| OTHER CASES | | | |

**TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)**

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS** | 41 | 37 | 42 |
| TOTAL PRIMARY TUMORS | 78 | 58 | 70 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 35 | 30 | 30 |
| TOTAL BENIGN TUMORS | 53 | 40 | 41 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 19 | 16 | 25 |
| TOTAL MALIGNANT TUMORS | 20 | 18 | 26 |
| TOTAL ANIMALS WITH SECONDARY TUMORS## | | 1 | |
| TOTAL SECONDARY TUMORS | | 1 | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 5 | | 3 |
| TOTAL UNCERTAIN TUMORS | 5 | | 3 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |

** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
## SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

## TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE: VEHICLE CONTROL

| ANIMAL NUMBER | 50011 | 50012 | 50013 | 50014 | 50015 | 50016 | 50017 | 50018 | 50019 | 50020 | 50021 | 50022 | 50023 | 50024 | 50025 | 50026 | 50027 | 50028 | 50029 | 50030 | 50031 | 50032 | 50033 | 50034 | 50035 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10304 | 10404 | 10404 | 10404 | 10404 | 08404 | 10404 | 10404 | 10405 | 10505 | 10202 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 10205 | 08806 | 10205 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Basal-cell tumor | | | | | | | | | | X | | | | | | | | | | | | | | | |
| Keratoacanthoma | | | | | | | | | | | | X | | X | X | X | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibroma | | | | | | | | | X | | | | | | | | | | X | | | | | | |
| Fibrosarcoma | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Hemangioma | X | | | | | | | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibrosarcoma, metastatic | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Trachea | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibrosarcoma, invasive | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Neoplastic nodule | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular carcinoma | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | 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 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | X | | | | | | | X | X | | | | | X | | | | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | | X | | | | X | X | | X | | X | | | | | | | X | X | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Islet-cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | N |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 | X | X | X |
| Prostate | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + |
| 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 | N | N | N |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **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 | N | N |
| Carcinoma, NOS | | | | | | | | | | | | | | | X | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | 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 |
| Myxoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **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 |
| Mesothelioma, NOS | | | | | X | | | | | | | | | | | | | | | | | | | | |
| Leukemia, mononuclear cell | | | | | X | | | | | | X | | | | X | 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 A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: VEHICLE CONTROL (Continued)

| ANIMAL NUMBER | 52126 | 52127 | 52128 | 52129 | 53030 | 53031 | 53032 | 53033 | 53034 | 53035 | 53036 | 53037 | 53038 | 53039 | 54010 | 54011 | 54012 | 54013 | 54014 | 54015 | 54016 | 54017 | 54018 | 54019 | 55050 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 09707 | 10505 | 09303 | 10505 | 10505 | 10505 | 09101 | 10505 | 10505 | 10505 | 10505 | 10505 | 10505 | 10505 | 10505 | 10505 | 10505 | 09303 | 10404 | 10505 | 09005 | 10505 | 09808 | 10105 | 10005 | TISSUES TUMORS |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Basal-cell tumor | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Keratoacanthoma | | | | | | | | | | | | | | | | | | | X | | | | | | | 5 |
| Subcutaneous tissue | N | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroma | | | | | | | | X | | | | | | | | | | | | | | | | | | 3 |
| Fibrosarcoma | | | | | | | | | | | | | | | | | | X | | | | | | | | 2 |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | X | | | | | | | | 1 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Fibrosarcoma, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | – | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | – | – | – | – | + | + | | 42 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Fibrosarcoma, invasive | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Neoplastic nodule | | | | | | | | | | | | | | | | | | | X | | | | | | | 1 |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | 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 | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | X | | | | | | | | | | | | X | | X | | | | | | | | X | 3 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | | X | | | | | | X | X | | X | | | | | | X | | | | | | | | X | 13 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | 49 |
| C-cell adenoma | | | | | X | | | | X | | | | | | | | | | | | | | | | | 2 |
| Parathyroid | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | + | 44 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | X | | 1 |
| Islet-cell carcinoma | | | | | | | | | | | X | | | | | | | | | | | | X | | | 2 |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | 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 | X | X | | 48 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| 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 | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | X | | | | | | | 1 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | X | | | | | | | | | | | | | | | | 1 |
| **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 | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | 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 | *50 |
| Myxoma | | | | | | | | | | | | | | | | | | | | | | | | | 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 | N | N | N | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia, mononuclear cell | X | X | | | | | X | | | X | | | | | | | | | X | X | | | X | X | | 13 |

* Animals Necropsied

## TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE: LOW DOSE

| ANIMAL NUMBER | 50101 | 50201 | 50301 | 50401 | 50501 | 50601 | 50701 | 50801 | 50901 | 51001 | 51101 | 51201 | 51301 | 51401 | 51501 | 51601 | 51701 | 51801 | 51901 | 52001 | 52101 | 52201 | 52301 | 52401 | 52501 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10401 | 10401 | 10401 | 10401 | 10201 | 10301 | 10301 | 10401 | 10401 | 10401 | 10501 | 10501 | 10401 | 09401 | 10501 | 10401 | 10501 | 10401 | 09401 | 10501 | 10501 | 10501 | 10501 | 10501 | 10501 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Keratoacanthoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibroma | | | | | | | | | | | | | | | | | | | | | X | | | | |
| Myxosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Granular-cell tumor, NOS | | | | | | | | | | | X | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | X | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinosarcoma, unc prim or meta | | | | | | | | | X@ | | | | | | | | | | | | | | | | |
| Mesothelioma, metastatic | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Thymus | + | + | + | + | – | + | + | – | + | + | + | + | + | + | – | + | – | + | + | + | + | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Undifferentiated carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | 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 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Acinar-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Esophagus | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | X | | | | X | | | | | | | | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | X | | | | | | X | | | | | | | | | X | | | | | | X | | |
| Ganglioneuroma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | | | | | X | | | | | | | | | | | | X | | | | |
| C-cell carcinoma | | | | | X | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | – | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | X | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | N | + | + | N | + | + | + | + | + | + | N | + | + | + | + | X | + | + | + | N | + | + |
| Fibroadenoma | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Testis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 | X | X | |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 | N | N | N |
| Carcinoma, NOS | | | | | | | | X | | | | | | | | | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **BODY CAVITIES** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | 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 |
| Myxosarcoma | | | | | X | | | | | | | | | | | | | | | | | | | | |
| **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 |
| Mesothelioma, NOS | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Mesothelioma, malignant | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Leukemia, mononuclear cell | | | | | | | | | | | | | | X | X | | X | X | | X | | | | | |

+ : Tissue Examined Microscopically
– : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Missexed
@ : Multiple Occurrence of Morphology
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

## TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

| ANIMAL NUMBER | 52 66 | 52 77 | 52 88 | 52 99 | 53 01 | 53 11 | 53 22 | 53 33 | 53 44 | 53 55 | 53 66 | 53 77 | 53 83 | 53 93 | 54 04 | 54 14 | 54 21 | 54 32 | 54 43 | 54 51 | 54 65 | 54 76 | 54 88 | 54 91 | 55 00 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10 00 | 08 77 | 09 55 | 10 55 | 09 55 | 10 55 | 10 55 | 10 55 | 04 21 | 00 51 | 10 05 | 10 55 | 09 33 | 05 77 | 09 98 | 09 88 | 10 51 | 10 55 | 10 01 | 10 55 | 10 05 | 10 55 | 10 05 | 10 55 | 10 33 | 10 55 | TISSUES TUMORS |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Squamous cell carcinoma | | | | | | | | X | | | | | | | | | | | | | | | | | | 1 |
| Keratoacanthoma | | | | | X | | | | | | | | | | | | | | | | X | | | | | 2 |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroma | | | | | | | | | | | | | X | | | | | | | | | | | | | 2 |
| Myxosarcoma | | X | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Granular-cell tumor, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | 49 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Carcinosarcoma, unc prim or meta | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| *Mesothelioma, metastatic* | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Thymus | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | 44 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Undifferentiated carcinoma | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | 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 | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Acinar-cell adenoma | | | | | | | | | | | | | X | | | | | | | | | | | | | 2 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Carcinoma, NOS | X | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Adenoma, NOS | | | | | | X | | | | | | | | | | | | | | | X | | X | | X | 6 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | X | | | | | | | | | | X | X | | | | | | X | | X | | | X | | | 10 |
| Ganglioneuroma | | | | | | | | | | | | | | X | | | | | | | | | | | | 1 |
| Thyroid | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | X | | | 3 |
| C-cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | + | + | – | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | N | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | *50 |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| 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 | 46 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| 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 | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | X | | | | | | 2 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | X | | | | | | | | | | | | | | | | | 1 |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | 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 | *50 |
| Myxosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 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 | N | N | N | *50 |
| Mesothelioma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Mesothelioma, malignant | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia, mononuclear cell | | | | | | X | X | X | | | | | | X | X | X | | | | X | | | | | | 12 |

* Animals Necropsied

## TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE: HIGH DOSE

| ANIMAL NUMBER | 50 01 | 50 02 | 50 03 | 50 04 | 50 05 | 50 06 | 50 07 | 50 08 | 50 09 | 50 10 | 51 11 | 51 12 | 51 13 | 51 14 | 51 15 | 51 16 | 51 17 | 51 18 | 51 19 | 52 20 | 52 21 | 52 22 | 52 23 | 52 24 | 52 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10 04 | 10 04 | 10 04 | 10 04 | 10 04 | 10 04 | 10 04 | 10 05 | 09 00 | 10 05 | 10 05 | 05 21 | 07 08 | 08 01 | 08 06 | 08 11 | 10 00 | 08 06 | 10 05 | 10 05 | 10 05 | 08 71 | 10 05 | 10 05 | 10 05 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Sarcoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | X | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trachea | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | - | + | + | + |
| Cystadenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thymoma | | | | | | | | X | | | | | | | | | | | | | | | | | |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | 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 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + |
| Pheochromocytoma | | X | | | | | | | | | | | | | | | | | | | | | | X | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Follicular-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | N | N | + | + | + | + | N | + | + | + | + | + |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Interstitial-cell tumor | X | X | X | X | X | X | X | X | X | X | X | | | | X | X | X | | X | X | X | X | X | X | X |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + |
| 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 | N | N | N |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Meningioma | | | | | | | | | | | | | | | | | | X | | | | | | | |
| **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 |
| Malig. lymphoma, histiocytic type | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Leukemia, mononuclear cell | | | | | | | | | | X | | | X | | | | | X | | | | X | | |
| Tail | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | |

+ : 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 A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

| ANIMAL NUMBER | 52 66 | 52 77 | 52 88 | 52 99 | 53 00 | 53 11 | 53 22 | 53 33 | 53 44 | 53 55 | 53 66 | 53 77 | 53 88 | 53 99 | 54 00 | 54 11 | 54 22 | 54 33 | 54 44 | 54 55 | 54 66 | 54 77 | 54 88 | 54 91 | 55 00 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10 05 | 10 11 | 09 15 | 09 33 | 09 05 | 10 11 | 08 44 | 10 05 | 10 05 | 10 05 | 09 66 | 10 05 | 10 05 | 10 05 | 09 77 | 08 99 | 10 05 | 10 05 | 10 05 | 08 77 | 10 44 | 10 05 | 09 66 | 10 05 | 10 05 | TISSUES TUMORS |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Sarcoma, NOS | | X | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | 48 |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Thymus | + | – | + | + | + | – | – | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 44 |
| Cystadenoma, NOS | | | | | | | | | | X | | | | | | | | | | | | | | | | 1 |
| Thymoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Hepatocellular carcinoma | | | | | | | X | | | | | | | | | | | | | | | | | | | 1 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | 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 | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | X | | | | | | | | | | | 1 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Pheochromocytoma | | | X | | | | | X | | | | | | | | | | | | X | X | | | | | 6 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | | 49 |
| Follicular-cell adenoma | | | | | | | | | | | | | | | | | | | | X | | | | | | 1 |
| C-cell adenoma | | | X | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | – | + | – | + | + | + | + | + | + | + | – | + | + | | 45 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | X | | | | | | | 1 |
| **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 | X | X | X | 46 |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| 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 | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Meningioma | | | | | | | | | | | | | | | | | | | | | | | | | | 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 | N | N | N | *50 |
| Malig. lymphoma, histiocytic type | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia, mononuclear cell | | | | X | | X | X | | | X | X | | | X | X | | | X | | | X | | | X | | 14 |
| Tail | | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |

*Animals Necropsied

## TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE: VEHICLE CONTROL

| ANIMAL NUMBER | 50101 | 50102 | 50103 | 50104 | 50105 | 50106 | 50107 | 50108 | 50109 | 50110 | 50111 | 50112 | 50113 | 50114 | 50115 | 50116 | 50117 | 50118 | 50119 | 50120 | 50121 | 50122 | 50123 | 50124 | 50125 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10404 | 10404 | 10404 | 09707 | 10404 | 10404 | 10404 | 10404 | 10404 | 09707 | 10405 | 08808 | 10105 | 10005 | 10005 | 10005 | 09707 | 10005 | 10005 | 10005 | 10005 | 10205 | 10005 | 10005 | 10205 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + |
| Fibroma | | | | X | | | | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trachea | + | + | + | + | + | – | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + |
| Thymus | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | – | – | + | |
| Thymoma | X | | | | | | | | | | | | | | | | | | | | | | | | |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Neoplastic nodule | | X | | | | | | | X | | X | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | 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 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Leiomyosarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Large intestine | + | + | – | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | |
| Adenoma, NOS | X | | X | X | X | | | | X | | X | X | | | | X | X | | X | X | X | | | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | X | | | X | | | | | | | | | | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | X | | | | | | | | | | | | | | | | | | | | |
| C-cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | – | + | + | + | + | + | + | + | – | + | + | – | + | + | – | + | + | – | + | – | + | + | – | + | + |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS | | | | | | | | | | X | | | | | | | | | | | | | | | |
| Fibroadenoma | | X | | | | | X | | X | | X | X | | | | | | | | | | X | | | X |
| 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 | N | N | N |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Vagina | 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 |
| Squamous cell papilloma | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endometrial stromal polyp | | | | | | | | | | | | | X | | X | X | | X | | | X | | X | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **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 |
| Leukemia, mononuclear cell | | X | | | | | | | | | | | | | | | X | X | | 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 A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: VEHICLE CONTROL (Continued)

| ANIMAL NUMBER | 5 2 6 | 5 2 7 | 5 2 8 | 5 2 9 | 5 3 0 | 5 3 1 | 5 3 2 | 5 3 3 | 5 3 4 | 5 3 5 | 5 3 6 | 5 3 7 | 5 3 8 | 5 3 9 | 5 4 0 | 5 4 1 | 5 4 2 | 5 4 3 | 5 4 4 | 5 4 5 | 5 4 6 | 5 4 7 | 5 4 8 | 5 4 9 | 5 5 0 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 0 8 9 | 1 0 5 | 0 9 7 | 0 7 6 | 1 0 4 | 1 0 5 | 0 9 8 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | 1 0 5 | TISSUES TUMORS |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | | 47 |
| Spleen | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | | 49 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 49 |
| Thymus | + | + | + | + | + | + | + | + | + | + | – | + | – | – | + | + | + | + | + | + | + | + | + | + | | 43 |
| Thymoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| Neoplastic nodule | | | | | | | | | | | | | | | | | | X | | | X | | | | | 5 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| Gallbladder & common bile duct | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 49 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | | 49 |
| Leiomyosarcoma | | | | | | | | | | | | | | | | | | | | | | X | | | | 1 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | – | + | + | + | - | + | + | + | + | + | + | + | + | + | | 49 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | | 49 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 49 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 49 |
| Adenoma, NOS | X | X | | | X | X | | X | X | | | | | | X | | | X | X | X | | | | X | 23 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | 50 |
| Pheochromocytoma | | X | | | | | | | | | | | | | | | | | | | | X | | | | 4 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | | 48 |
| C-cell adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| C-cell carcinoma | | | | | X | | | | | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | – | + | + | + | – | + | + | + | + | + | – | – | – | + | + | + | + | + | + | + | + | + | | 38 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | + | + | | *50 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroadenoma | X | | | | | | X | X | | | | X | | | X | | | | | | | | | | 12 |
| 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 | N | N | N | *50 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | X | 1 |
| Adenoma, NOS | | | | | | | X | | | | | | | | | | | | | | | | | | | 1 |
| Vagina | 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 | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | | 50 |
| Endometrial stromal polyp | | | | | | | | | | X | | | | | | | | | X | | | X | | | | 9 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | | 50 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | | 49 |
| Astrocytoma | | | | | | | | | | | | 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 | N | N | N | *50 |
| Leukemia, mononuclear cell | | X | X | | | | | | | | X | | X | X | X | | | | X | X | X | X | | | | 15 |

* Animals Necropsied

## TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE: LOW DOSE

| ANIMAL NUMBER | 50101 | 50203 | 50304 | 50405 | 50506 | 50708 | 50809 | 51001 | 51102 | 51203 | 51304 | 51505 | 51606 | 51718 | 51919 | 52001 | 52102 | 52203 | 52304 | 52405 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 09595 | 10404 | 14404 | 04404 | 04404 | 03505 | 01404 | 04505 | 05505 | 05501 | 01505 | 05501 | 05501 | 09431 | 09405 | 07905 | 10105 | 01801 | 04405 | 01001 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + |
| Fibroma | | | | | | | | | | | | | | | | | X | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | | X | | | | | | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | - | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | - | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + |
| Thymus | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | - | + | + | + | + |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Oral cavity | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Squamous cell papilloma | | | | | X | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | - | + | + | + | + |
| Large intestine | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | - | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | |
| Adenoma, NOS | | | X | | | | | | | X | | | | | X | | X | | X | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | X | | | | | | | | | | | | | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Follicular-cell adenoma | | | | | | | | | | | | | | | | | | | | |
| Parathyroid | + | + | + | - | + | + | - | - | + | - | + | + | - | + | + | - | + | + | + | - |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | | |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | N | + | + | + | + | N | + | N | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | X | | | | | |
| Fibroadenoma | | X | X | | | | | | X | | | | | | X | | X | | | X |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Leiomyoma | | | | | | | | | | | | | | X | | | | | | |
| Endometrial stromal polyp | X | | | | | | | | | | X | | X | X | X | | X | | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | |
| **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 |
| Leukemia, mononuclear cell | | | X | | X | | X | | X | X | | | | X | X | | | | | |

+ : Tissue Examined Microscopically
- : Required Tissue Not Examined Microscopically
X : Tumor Incidence
N : Necropsy, No Autolysis, No Microscopic Examination
S : Animal Misexed
: No Tissue Information Submitted
C : Necropsy, No Histology Due To Protocol
A : Autolysis
M : Animal Missing
B : No Necropsy Performed

## TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

| ANIMAL NUMBER | 52 26 | 52 27 | 52 28 | 52 29 | 53 00 | 53 11 | 53 32 | 53 33 | 53 34 | 53 35 | 53 36 | 53 37 | 53 38 | 53 39 | 54 00 | 54 11 | 54 12 | 54 13 | 54 14 | 54 15 | 54 16 | 54 17 | 54 18 | 54 19 | 55 00 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10 05 | 10 05 | 09 04 | 10 05 | 07 03 | 10 05 | 10 05 | 10 05 | 10 05 | 05 04 | 10 11 | 09 11 | 10 05 | 10 11 | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 03 | 10 05 | TISSUES TUMORS |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Adenocarcinoma, NOS, metastatic | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Thymus | + | + | + | + | + | + | – | + | + | – | + | + | + | – | + | – | + | + | + | + | + | + | + | + | + | 44 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| 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 | N | N | N | *50 |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | 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 | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | X | | | | | | | | 1 |
| Adenoma, NOS | X | X | | | | | | | | | | X | X | | | | | | | X | | | X | X | | 12 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | | | | | | X | | | | | | | X | | | | | | | | | | | | | 3 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Follicular-cell adenoma | | | | | | | | | X | | | | | | | | | | | | | | | | | 1 |
| Parathyroid | + | + | + | + | + | + | + | + | + | – | – | + | – | – | + | + | + | + | – | + | + | + | + | + | + | 38 |
| Pancreatic islets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Islet-cell adenoma | | | | | | | | | | | | | | | | | | | X | | | | | | | 1 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Adenoma, NOS | | | | | | | | | | | | | | | X | | | | | | | | | | | 1 |
| Adenocarcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroadenoma | | | | | | | X | X | | | | | | | | X | | X | | | | | X | | | 11 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Leiomyoma | | | | | | | | | | | | | | | | | | | | | | | | | - | 1 |
| Endometrial stromal polyp | | X | X | | | | | | | | | | | | | | | | | | | | | | | 8 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Astrocytoma | | | | | | | | | | | | | | | | | | | | 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 | N | N | N | *50 |
| Leukemia, mononuclear cell | | X | X | | | | | | | X | | X | | | | X | | X | | | X | X | | | | 15 |

* Animals Necropsied

## TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE: HIGH DOSE

| ANIMAL NUMBER | 50101 | 50102 | 50103 | 50104 | 50105 | 50106 | 50107 | 50108 | 50109 | 50110 | 50111 | 50112 | 50113 | 50114 | 50115 | 50116 | 50117 | 50118 | 50119 | 50120 | 50121 | 50122 | 50123 | 50124 | 50125 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10104 | 10104 | 10104 | 10103 | 10104 | 10104 | 10104 | 10104 | 09911 | 10105 | 09905 | 10105 | 10105 | 10105 | 10105 | 10105 | 09909 | 10105 | 09906 | 10105 | 10107 | 09908 | 09902 | 09903 | 10105 |
| **INTEGUMENTARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Fibroma | | | | | | | | | | | | | | | | | | | | | | | | | |
| **RESPIRATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **HEMATOPOIETIC SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | X | | | | | | |
| **CIRCULATORY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **DIGESTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Neoplastic nodule | | | | | | | | | | | X | | | | | | | | | | | | | | |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Gallbladder & common bile duct | 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 |
| Pancreas | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Small intestine | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Large intestine | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **URINARY SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **ENDOCRINE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma, NOS | X | | | | X | | | | | | | X | | | | | | | | | X | | X | | |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma | | X | | | | | | X | | | | | | | | | | | X | | | | | | |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell adenoma | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Parathyroid | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | + | + | + | + | + |
| **REPRODUCTIVE SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + |
| Cystadenoma, NOS | | | | | X | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | X | | | | | | | X | X | | | | | | | | | | | | | | | |
| 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 | N | N | N |
| Carcinoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endometrial stromal polyp | | | | | X | | X | | | | X | | X | | | X | | X | | X | | | | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **NERVOUS SYSTEM** | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| **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 |
| Leukemia, mononuclear cell | | X | X | | X | | | X | | X | X | | | X | X | | | | X | X | | X | 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 A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

| ANIMAL NUMBER | 52 26 | 52 27 | 52 28 | 52 29 | 53 00 | 53 11 | 53 12 | 53 33 | 53 34 | 53 35 | 53 36 | 53 37 | 53 38 | 53 39 | 54 40 | 54 41 | 54 42 | 54 43 | 54 44 | 54 45 | 54 46 | 54 47 | 54 48 | 54 49 | 55 50 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 05 | 10 04 | 07 09 | 10 05 | 09 01 | 09 00 | 10 05 | 10 04 | 00 01 | 07 09 | 09 00 | 10 01 | 09 05 | 10 05 | 09 05 | 09 05 | 09 05 | 09 05 | 10 05 | 10 05 | TISSUES TUMORS |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *50 |
| Fibroma | | | | | | | X | | | | | | | | | | | | | | | | | | | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lungs and bronchi | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Trachea | + | + | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Lymph nodes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Thymus | + | – | + | + | + | + | – | – | – | + | + | + | – | – | – | – | + | + | – | + | + | + | – | – | + | 31 |
| Adenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Neoplastic nodule | | | | | | | | | | | | | X | | | | | | | | | | X | | | 3 |
| Bile duct | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Gallbladder & common bile duct | 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 | *50 |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Stomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Small intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Large intestine | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary | + | + | + | + | + | + | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| Adenoma, NOS | X | | | X | | | | | X | X | X | | X | | | | | X | | | X | X | | X | | 15 |
| Adrenal | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Pheochromocytoma | X | | | X | | | | | | | | | | | | | | | | | | | | | | 5 |
| Thyroid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| C-cell adenoma | | X | | | | | | | | | | | X | | | | | | | | | | | | | 3 |
| Parathyroid | + | + | + | + | + | + | + | + | + | + | + | – | + | + | – | – | + | + | – | + | + | + | + | + | + | 44 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | N | + | + | + | + | + | + | + | + | *50 |
| Cystadenoma, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Fibroadenoma | | | | X | | | | | | | | | X | | | | | | | | | | | | | 5 |
| 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 | N | N | N | *50 |
| Carcinoma, NOS | | | | X | | | | | | | | | | | | | | | | | | | | | | 1 |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| Endometrial stromal polyp | | X | | | | | | | | | | | X | | | | | | | | | | X | | | 10 |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| 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 | *50 |
| Leukemia, mononuclear cell | X | | X | X | | X | | | | | X | | X | | X | X | X | | X | | X | | | | X | 25 |

*Animals Necropsied

# APPENDIX B

# SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE

| | CONTROL (VEH) | 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%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| HEMORRHAGE | | 1 (2%) | |
| ABSCESS, NOS | 1 (2%) | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #TRACHEA | (48) | (49) | (48) |
| INFLAMMATION, SUPPURATIVE | 1 (2%) | | |
| INFLAMMATION, ACUTE | | 1 (2%) | 1 (2%) |
| #LUNG | (50) | (50) | (50) |
| CONGESTION, NOS | | 1 (2%) | 2 (4%) |
| EDEMA, NOS | | | 1 (2%) |
| HEMORRHAGE | | 4 (8%) | 2 (4%) |
| INFLAMMATION, INTERSTITIAL | | 5 (10%) | 3 (6%) |
| INFLAMMATION, GRANULOMATOUS | 3 (6%) | 1 (2%) | |
| PERIVASCULAR CUFFING | | | 4 (8%) |
| FOREIGN MATERIAL, NOS | 1 (2%) | | |
| EPITHELIALIZATION | | | 1 (2%) |
| #LUNG/ALVEOLI | (50) | (50) | (50) |
| HISTIOCYTOSIS | 3 (6%) | 4 (8%) | 6 (12%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| HEMATOPOIESIS | 1 (2%) | | |
| #BONE MARROW | (49) | (50) | (49) |
| ANGIECTASIS | 1 (2%) | | |
| MYELOFIBROSIS | 1 (2%) | | |
| #SPLEEN | (50) | (50) | (50) |
| FIBROSIS | | 2 (4%) | |
| NECROSIS, NOS | | 1 (2%) | |
| #SPLENIC CAPSULE | (50) | (50) | (50) |
| FIBROSIS | | 1 (2%) | |
| #MANDIBULAR L. NODE | (50) | (50) | (49) |
| HEMORRHAGE | | | 1 (2%) |
| DEGENERATION, CYSTIC | 3 (6%) | 3 (6%) | 1 (2%) |
| #MEDIASTINAL L. NODE | (50) | (50) | (49) |
| HEMORRHAGE | 8 (16%) | 4 (8%) | 4 (8%) |
| HEMOSIDEROSIS | | 1 (2%) | |
| LYMPHOID DEPLETION | | 1 (2%) | |
| #HEPATIC LYMPH NODE | (50) | (50) | (49) |
| HEMORRHAGE | | | 1 (2%) |
| DEGENERATION, CYSTIC | | | 1 (2%) |
| PIGMENTATION, NOS | | | 3 (6%) |
| HISTIOCYTOSIS | | | 3 (6%) |
| #PANCREATIC L. NODE | (50) | (50) | (49) |
| HEMORRHAGE | | 1 (2%) | |
| PIGMENTATION, NOS | | | 1 (2%) |
| HISTIOCYTOSIS | | | 1 (2%) |
| #MESENTERIC L. NODE | (50) | (50) | (49) |
| HEMORRHAGE | | 1 (2%) | |
| #RENAL LYMPH NODE | (50) | (50) | (49) |
| HEMORRHAGE | | 1 (2%) | |
| HEMOSIDEROSIS | | 1 (2%) | |
| HISTIOCYTOSIS | | 1 (2%) | |

TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #THYMUS | (42) | (44) | (44) |
| PERSISTENT EMBRYONIC STRUCTURE | 20 (48%) | 21 (48%) | 20 (45%) |
| CYST, NOS | | | 1 (2%) |
| HEMORRHAGE | | | 3 (7%) |
| CIRCULATORY SYSTEM | | | |
| *MEDIASTINUM | (50) | (50) | (50) |
| POLYANGIITIS | | 1 (2%) | |
| #HEART/ATRIUM | (50) | (50) | (50) |
| THROMBOSIS, NOS | | 1 (2%) | |
| #MYOCARDIUM | (50) | (50) | (50) |
| FIBROSIS | 31 (62%) | 35 (70%) | 37 (74%) |
| DEGENERATION, NOS | 3 (6%) | 2 (4%) | 1 (2%) |
| HEMOSIDEROSIS | 1 (2%) | | |
| #CARDIAC VALVE | (50) | (50) | (50) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| *PULMONARY ARTERY | (50) | (50) | (50) |
| MINERALIZATION | 16 (32%) | 19 (38%) | 17 (34%) |
| #LIVER | (50) | (50) | (50) |
| THROMBOSIS, NOS | | 1 (2%) | |
| #PANCREAS | (50) | (50) | (49) |
| POLYANGIITIS | 1 (2%) | 1 (2%) | |
| #KIDNEY | (50) | (50) | (50) |
| EMBOLUS, SEPTIC | | | 1 (2%) |
| #ADRENAL | (50) | (50) | (49) |
| EMBOLUS, SEPTIC | | | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #SALIVARY GLAND | (50) | (50) | (49) |
| ECTOPIA | 1 (2%) | | |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| ATROPHY, NOS | | | 2 (4%) |
| #LIVER | (50) | (50) | (50) |
| CONGESTION, NOS | | 1 (2%) | |
| INFLAMMATION, MULTIFOCAL | 2 (4%) | 2 (4%) | |
| CHOLANGIOFIBROSIS | 1 (2%) | | |
| NECROSIS, NOS | | 1 (2%) | 1 (2%) |
| NECROSIS, COAGULATIVE | | | 1 (2%) |
| METAMORPHOSIS FATTY | 2 (4%) | | |
| PIGMENTATION, NOS | | 1 (2%) | |
| CYTOPLASMIC CHANGE, NOS | 1 (2%) | 6 (12%) | 1 (2%) |
| GROUND-GLASS CYTO CHANGE | 2 (4%) | 1 (2%) | 2 (4%) |
| EOSINOPHILIC CYTO CHANGE | | 2 (4%) | |
| ANGIECTASIS | 2 (4%) | 2 (4%) | 1 (2%) |
| #LIVER/CENTRILOBULAR | (50) | (50) | (50) |
| CONGESTION, NOS | | 1 (2%) | |
| NECROSIS, NOS | | | 1 (2%) |
| METAMORPHOSIS FATTY | | 1 (2%) | 1 (2%) |
| ATROPHY, NOS | | 1 (2%) | |
| #LIVER/PERIPORTAL | (50) | (50) | (50) |
| FIBROSIS | | 3 (6%) | 43 (86%) |
| NECROSIS, NOS | | | 1 (2%) |
| PIGMENTATION, NOS | | 7 (14%) | 45 (90%) |
| #LIVER/HEPATOCYTES | (50) | (50) | (50) |
| ATROPHY, NOS | | | 1 (2%) |
| #BILE DUCT | (50) | (50) | (50) |
| HYPERPLASIA, NOS | 43 (86%) | 19 (38%) | 44 (88%) |
| #PANCREAS | (50) | (50) | (49) |
| FOCAL CELLULAR CHANGE | | 3 (6%) | |
| HYPERPLASIA, FOCAL | | 3 (6%) | |

TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| DIGESTIVE SYSTEM (Continued) | | | |
| #PANCREATIC ACINUS | (50) | (50) | (49) |
| ATROPHY, NOS | 7 (14%) | 10 (20%) | 8 (16%) |
| #ESOPHAGUS | (50) | (49) | (50) |
| INFLAMMATION, SUPPURATIVE | | | 1 (2%) |
| #FORESTOMACH | (50) | (50) | (50) |
| ULCER, NOS | 1 (2%) | 2 (4%) | |
| #GASTRIC FUNDUS | (50) | (50) | (50) |
| EROSION | | | 1 (2%) |
| #DUODENUM | (49) | (50) | (46) |
| HEMORRHAGE | | 1 (2%) | |
| #COLON | (50) | (50) | (49) |
| PARASITISM | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| CYST, NOS | 3 (6%) | | |
| CONGESTION, NOS | | | 1 (2%) |
| HEMORRHAGE | 22 (44%) | 19 (38%) | 19 (38%) |
| PYELONEPHRITIS, NOS | 1 (2%) | | |
| FIBROSIS, DIFFUSE | 1 (2%) | 1 (2%) | |
| NEPHROPATHY | 45 (90%) | 45 (90%) | 46 (92%) |
| DEGENERATION, HYALINE | | | 1 (2%) |
| NECROSIS, NOS | | 1 (2%) | |
| INFARCT, NOS | 1 (2%) | | |
| METAMORPHOSIS FATTY | 1 (2%) | 1 (2%) | |
| HEMOSIDEROSIS | 1 (2%) | 2 (4%) | 1 (2%) |
| #RENAL PAPILLA | (50) | (50) | (50) |
| MINERALIZATION | | | 1 (2%) |
| #URINARY BLADDER | (50) | (50) | (50) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #ANTERIOR PITUITARY | (50) | (49) | (50) |
| CYST, NOS | 2 (4%) | 1 (2%) | 1 (2%) |
| FOCAL CELLULAR CHANGE | 4 (8%) | 7 (14%) | 3 (6%) |
| ANGIECTASIS | 1 (2%) | 1 (2%) | 3 (6%) |
| #ADRENAL | (50) | (50) | (49) |
| CYST, NOS | | 1 (2%) | |
| HEMORRHAGIC CYST | | 1 (2%) | |
| INFLAMMATION, SUPPURATIVE | | | 1 (2%) |
| DEGENERATION, LIPOID | 1 (2%) | 1 (2%) | 1 (2%) |
| NECROSIS, NOS | | 1 (2%) | |
| LIPOIDOSIS | 4 (8%) | | 2 (4%) |
| ANGIECTASIS | 5 (10%) | 2 (4%) | |
| #ADRENAL CORTEX | (50) | (50) | (49) |
| FOCAL CELLULAR CHANGE | 1 (2%) | | |
| HYPERPLASIA, FOCAL | | | 2 (4%) |
| #ADRENAL MEDULLA | (50) | (50) | (49) |
| FOCAL CELLULAR CHANGE | 1 (2%) | | |
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| #THYROID | (49) | (49) | (49) |
| CYSTIC FOLLICLES | | 1 (2%) | |
| HYPERPLASIA, C-CELL | 6 (12%) | 4 (8%) | 3 (6%) |
| #PARATHYROID | (44) | (46) | (45) |
| FOCAL CELLULAR CHANGE | 1 (2%) | | |
| HYPERPLASIA, NOS | | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| GALACTOCELE | | 1 (2%) | |
| HEMOGLOBIN PIGMENT | | 1 (2%) | |

**TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)**

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| REPRODUCTIVE SYSTEM (Continued) | | | |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| DILATATION/DUCTS | 1 (2%) | | |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| HYPERPLASIA, EPITHELIAL | 1 (2%) | | |
| #PROSTATE | (47) | (49) | (49) |
| HEMORRHAGE | | 1 (2%) | |
| INFLAMMATION, SUPPURATIVE | 1 (2%) | | |
| INFLAMMATION, CHRONIC | 5 (11%) | 13 (27%) | 6 (12%) |
| HYPERPLASIA, EPITHELIAL | 13 (28%) | 18 (37%) | 13 (27%) |
| #TESTIS | (50) | (50) | (50) |
| MINERALIZATION | 5 (10%) | 5 (10%) | 8 (16%) |
| HYPOSPERMATOGENESIS | 5 (10%) | 6 (12%) | 3 (6%) |
| HYPERPLASIA, INTERSTITIAL CELL | 8 (16%) | 6 (12%) | 7 (14%) |
| *EPIDIDYMIS | (50) | (50) | (50) |
| INFLAMMATION, CHRONIC | 1 (2%) | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (50) | (50) | (50) |
| HEMORRHAGE | 2 (4%) | | 3 (6%) |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (50) | (50) | (50) |
| HEMORRHAGE | | 1 (2%) | |
| CATARACT | 7 (14%) | 6 (12%) | 2 (4%) |
| *EYE/SCLERA | (50) | (50) | (50) |
| MINERALIZATION | | 4 (8%) | 2 (4%) |
| *EYE/RETINA | (50) | (50) | (50) |
| DEGENERATION, NOS | 6 (12%) | 6 (12%) | 2 (4%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *MANDIBLE | (50) | (50) | (50) |
| EXOSTOSIS | | 1 (2%) | |
| *CARTILAGE, NOS | (50) | (50) | (50) |
| NECROSIS, NOS | 7 (14%) | 4 (8%) | 3 (6%) |
| BODY CAVITIES | | | |
| *THORACIC CAVITY | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | 1 (2%) |
| FOREIGN MATERIAL, NOS | | 1 (2%) | 1 (2%) |
| *MEDIASTINUM | (50) | (50) | (50) |
| HEMORRHAGE | | 1 (2%) | |
| *ABDOMINAL CAVITY | (50) | (50) | (50) |
| NECROSIS, FAT | 2 (4%) | 1 (2%) | 4 (8%) |
| *EPICARDIUM | (50) | (50) | (50) |
| INFLAMMATION, FIBRINOUS | | | 1 (2%) |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| CONGESTION, NOS | 1 (2%) | | |
| HEMORRHAGE | | 1 (2%) | |
| FOOT | | | |
| INFLAMMATION, CHRONIC | 1 | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NONE | | | |

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| ABSCESS, NOS | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| *NASAL CAVITY | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | 1 (2%) |
| #TRACHEA | (48) | (48) | (48) |
| HEMORRHAGE | | 1 (2%) | |
| #LUNG | (50) | (50) | (50) |
| BRONCHIECTASIS | 1 (2%) | | |
| CONGESTION, NOS | 1 (2%) | 1 (2%) | 1 (2%) |
| EDEMA, NOS | | 1 (2%) | |
| HEMORRHAGE | 3 (6%) | 2 (4%) | |
| INFLAMMATION, INTERSTITIAL | | 2 (4%) | 4 (8%) |
| INFLAMMATION, GRANULOMATOUS | 1 (2%) | 1 (2%) | 1 (2%) |
| INFLAMMATION GRANULOMATOUS FOCAL | | | 1 (2%) |
| METAPLASIA, OSSEOUS | 1 (2%) | | |
| #LUNG/ALVEOLI | (50) | (50) | (50) |
| HISTIOCYTOSIS | 14 (28%) | 3 (6%) | 11 (22%) |
| HEMATOPOIETIC SYSTEM | | | |
| #SPLEEN | (49) | (49) | (50) |
| NECROSIS, NOS | | | 1 (2%) |
| NECROSIS, HEMORRHAGIC | | | 1 (2%) |
| HEMOSIDEROSIS | 1 (2%) | 1 (2%) | |
| LYMPHOID DEPLETION | 1 (2%) | | |
| HEMATOPOIESIS | 1 (2%) | | |
| #SPLENIC CAPSULE | (49) | (49) | (50) |
| FIBROSIS | | | 1 (2%) |
| FIBROSIS, FOCAL | 1 (2%) | | |
| #LYMPH NODE | (49) | (49) | (50) |
| HEMORRHAGE | | | 1 (2%) |
| HYPERPLASIA, LYMPHOID | | 1 (2%) | |
| #MANDIBULAR L. NODE | (49) | (49) | (50) |
| DEGENERATION, CYSTIC | 1 (2%) | 3 (6%) | 2 (4%) |
| #MEDIASTINAL L. NODE | (49) | (49) | (50) |
| HEMORRHAGE | 2 (4%) | 4 (8%) | 5 (10%) |
| HEMOSIDEROSIS | | | 2 (4%) |
| #HEPATIC LYMPH NODE | (49) | (49) | (50) |
| DEGENERATION, CYSTIC | | | 1 (2%) |
| PIGMENTATION, NOS | | | 2 (4%) |
| HISTIOCYTOSIS | | 1 (2%) | 3 (6%) |
| #PANCREATIC L. NODE | (49) | (49) | (50) |
| HEMOSIDEROSIS | 1 (2%) | | 2 (4%) |
| HISTIOCYTOSIS | | | 1 (2%) |
| #LUMBAR LYMPH NODE | (49) | (49) | (50) |
| DEGENERATION, CYSTIC | | | 1 (2%) |
| HISTIOCYTOSIS | | 1 (2%) | 1 (2%) |
| #MESENTERIC L. NODE | (49) | (49) | (50) |
| HEMORRHAGE | | 2 (4%) | |
| PIGMENTATION, NOS | | | 2 (4%) |
| HISTIOCYTOSIS | | | 2 (4%) |
| *STERNUM | (50) | (50) | (50) |
| MYELOFIBROSIS | | | 1 (2%) |
| #LUNG | (50) | (50) | (50) |
| HYPERPLASIA, LYMPHOID | 1 (2%) | | |

**TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)**

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| HEMATOPOIETIC SYSTEM (Continued) | | | |
| #LIVER | (50) | (50) | (50) |
| HEMATOPOIESIS | 1 (2%) | | |
| #THYMUS | (43) | (44) | (31) |
| PERSISTENT EMBRYONIC STRUCTURE | 28 (65%) | 29 (66%) | 18 (58%) |
| ECTOPIA | | 1 (2%) | 1 (3%) |
| CYST, NOS | 1 (2%) | | 1 (3%) |
| NECROSIS, NOS | | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| #HEART/ATRIUM | (50) | (50) | (50) |
| THROMBOSIS, NOS | 1 (2%) | | |
| #MYOCARDIUM | (50) | (50) | (50) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| FIBROSIS | 15 (30%) | 7 (14%) | 20 (40%) |
| DEGENERATION, NOS | 3 (6%) | 1 (2%) | 3 (6%) |
| *PULMONARY ARTERY | (50) | (50) | (50) |
| MINERALIZATION | 22 (44%) | 14 (28%) | 12 (24%) |
| #LIVER | (50) | (50) | (50) |
| THROMBOSIS, NOS | 1 (2%) | 1 (2%) | |
| DIGESTIVE SYSTEM | | | |
| #SALIVARY GLAND | (50) | (49) | (50) |
| INFLAMMATION, CHRONIC | | 3 (6%) | |
| FIBROSIS, DIFFUSE | | | 1 (2%) |
| #LIVER | (50) | (50) | (50) |
| ECTOPIA | 1 (2%) | | 2 (4%) |
| DILATATION/DUCTS | | 1 (2%) | |
| LYMPHOCYTIC INFLAMMATORY INFILTR | | 1 (2%) | |
| INFLAMMATION, MULTIFOCAL | 20 (40%) | 13 (26%) | 9 (18%) |
| INFLAMMATION, GRANULOMATOUS | | 1 (2%) | |
| NECROSIS, NOS | 1 (2%) | 1 (2%) | |
| NECROSIS, COAGULATIVE | 1 (2%) | | 1 (2%) |
| METAMORPHOSIS FATTY | 4 (8%) | 1 (2%) | |
| PIGMENTATION, NOS | 4 (8%) | | |
| HEMOSIDEROSIS | | | 2 (4%) |
| BASOPHILIC CYTO CHANGE | | | 1 (2%) |
| GROUND-GLASS CYTO CHANGE | | | 1 (2%) |
| EOSINOPHILIC CYTO CHANGE | 1 (2%) | | |
| ANGIECTASIS | 1 (2%) | 2 (4%) | |
| #LIVER/CENTRILOBULAR | (50) | (50) | (50) |
| NECROSIS, NOS | 1 (2%) | 3 (6%) | 1 (2%) |
| METAMORPHOSIS FATTY | | 1 (2%) | 1 (2%) |
| ATROPHY, NOS | | 1 (2%) | |
| #LIVER/PERIPORTAL | (50) | (50) | (50) |
| FIBROSIS | | | 34 (68%) |
| NECROSIS, NOS | | | 1 (2%) |
| PIGMENTATION, NOS | | 25 (50%) | 46 (92%) |
| #BILE DUCT | (50) | (50) | (50) |
| HYPERPLASIA, NOS | 17 (34%) | 24 (48%) | 47 (94%) |
| #PANCREAS | (49) | (49) | (49) |
| LYMPHOCYTIC INFLAMMATORY INFILTR | | | 2 (4%) |
| #PANCREATIC ACINUS | (49) | (49) | (49) |
| ATROPHY, NOS | 11 (22%) | 6 (12%) | 7 (14%) |
| #ESOPHAGUS | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | 2 (4%) | |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| #FORESTOMACH | (50) | (50) | (49) |
| ULCER, NOS | | | 1 (2%) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| HYPERPLASIA, EPITHELIAL | | | 1 (2%) |

**TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)**

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| DIGESTIVE SYSTEM (Continued) | | | |
| #DUODENUM | (49) | (48) | (49) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| EROSION | | | 1 (2%) |
| #COLON | (49) | (48) | (49) |
| PARASITISM | 1 (2%) | 1 (2%) | |
| URINARY SYSTEM | | | |
| #KIDNEY | (49) | (50) | (50) |
| CALCULUS, UNKNOWN GROSS OR MICRO | | | 1 (2%) |
| CALCULUS, MICROSCOPIC EXAMINATION | 4 (8%) | 1 (2%) | 2 (4%) |
| HYDRONEPHROSIS | | | 1 (2%) |
| HEMORRHAGE | 17 (35%) | 9 (18%) | 14 (28%) |
| PYELONEPHRITIS, NOS | 1 (2%) | | |
| NEPHROPATHY | 26 (53%) | 24 (48%) | 21 (42%) |
| METAMORPHOSIS FATTY | 1 (2%) | | |
| HEMOSIDEROSIS | 3 (6%) | 2 (4%) | 7 (14%) |
| HYPOPLASIA, NOS | 1 (2%) | | |
| HYPERPLASIA, TUBULAR CELL | | | 1 (2%) |
| #RENAL PAPILLA | (49) | (50) | (50) |
| HYPERPLASIA, EPITHELIAL | | | 1 (2%) |
| #KIDNEY/TUBULE | (49) | (50) | (50) |
| CALCULUS, UNKNOWN GROSS OR MICRO | 1 (2%) | | |
| ENDOCRINE SYSTEM | | | |
| #ANTERIOR PITUITARY | (49) | (48) | (49) |
| CYST, NOS | 18 (37%) | 8 (17%) | 13 (27%) |
| HEMORRHAGE | 2 (4%) | 4 (8%) | 1 (2%) |
| HEMORRHAGIC CYST | 1 (2%) | | 1 (2%) |
| HEMOSIDEROSIS | 1 (2%) | | |
| FOCAL CELLULAR CHANGE | 1 (2%) | | 2 (4%) |
| ANGIECTASIS | 3 (6%) | 10 (21%) | 4 (8%) |
| #ADRENAL | (50) | (50) | (50) |
| DEGENERATION, LIPOID | 4 (8%) | 1 (2%) | 1 (2%) |
| LIPOIDOSIS | 1 (2%) | 1 (2%) | 1 (2%) |
| HEMOSIDEROSIS | | | 1 (2%) |
| CYTOPLASMIC VACUOLIZATION | | | 1 (2%) |
| ANGIECTASIS | 6 (12%) | | 1 (2%) |
| #ADRENAL CORTEX | (50) | (50) | (50) |
| FOCAL CELLULAR CHANGE | 1 (2%) | 4 (8%) | 1 (2%) |
| HYPERPLASIA, FOCAL | 3 (6%) | | 3 (6%) |
| #ADRENAL MEDULLA | (50) | (50) | (50) |
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| #THYROID | (48) | (49) | (50) |
| ULTIMOBRANCHIAL CYST | | 1 (2%) | 2 (4%) |
| CYSTIC FOLLICLES | | 1 (2%) | |
| HYPERPLASIA, C-CELL | 3 (6%) | 2 (4%) | 6 (12%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| DILATATION/DUCTS | | 1 (2%) | 1 (2%) |
| GALACTOCELE | 1 (2%) | 1 (2%) | 1 (2%) |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | |
| #UTERUS | (50) | (50) | (50) |
| HEMORRHAGE | 1 (2%) | | |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| DECIDUAL ALTERATION, NOS | 1 (2%) | | |
| #UTERUS/ENDOMETRIUM | (50) | (50) | (50) |
| CYST, NOS | 4 (8%) | 1 (2%) | 2 (4%) |
| HYPERPLASIA, CYSTIC | 1 (2%) | 1 (2%) | |

TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)

| | CONTROL (VEH) | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| REPRODUCTIVE SYSTEM (Continued) | | | |
| #OVARY | (50) | (50) | (50) |
| FOLLICULAR CYST, NOS | 2 (4%) | | |
| PAROVARIAN CYST | 4 (8%) | 4 (8%) | 4 (8%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (49) | (50) | (50) |
| HEMORRHAGE | | | 2 (4%) |
| #BRAIN STEM | (49) | (50) | (50) |
| DISPLACEMENT, NOS | | 2 (4%) | |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (50) | (50) | (50) |
| HEMORRHAGE | 1 (2%) | | |
| CATARACT | 7 (14%) | 6 (12%) | 3 (6%) |
| *EYE/SCLERA | (50) | (50) | (50) |
| MINERALIZATION | 2 (4%) | 1 (2%) | 2 (4%) |
| *EYE/RETINA | (50) | (50) | (50) |
| DEGENERATION, NOS | 6 (12%) | 5 (10%) | 2 (4%) |
| *HARDERIAN GLAND | (50) | (50) | (50) |
| LYMPHOCYTIC INFLAMMATORY INFILTR | | 1 (2%) | |
| MUSCULOSKELETAL SYSTEM | | | |
| *STERNUM | (50) | (50) | (50) |
| OSTEOSCLEROSIS | | 2 (4%) | |
| *CARTILAGE, NOS | (50) | (50) | (50) |
| NECROSIS, NOS | 4 (8%) | 2 (4%) | 1 (2%) |
| BODY CAVITIES | | | |
| *THORACIC CAVITY | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | 6 (12%) | 1 (2%) |
| NECROSIS, FAT | | | 1 (2%) |
| FOREIGN MATERIAL, NOS | | 2 (4%) | |
| *MEDIASTINUM | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | 1 (2%) |
| NECROSIS, FAT | | 2 (4%) | |
| FOREIGN MATERIAL, NOS | | 1 (2%) | |
| *ABDOMINAL CAVITY | (50) | (50) | (50) |
| NECROSIS, FAT | 7 (14%) | 3 (6%) | 3 (6%) |
| *PERICARDIUM | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | |
| *EPICARDIUM | (50) | (50) | (50) |
| INFLAMMATION, FIBRINOUS | | 5 (10%) | 1 (2%) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MINERALIZATION | 1 (2%) | | |
| FOOT | | | |
| INFLAMMATION, CHRONIC | | 1 | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NONE | | | |

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

# APPENDIX C

# ANALYSES OF PRIMARY TUMORS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

TABLE C1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **Skin: Keratoacanthoma** | | | |
| Overall Rates (a) | 5/50 (10%) | 2/49 (4%) | 0/49 (0%) |
| Adjusted Rates (b) | 12.2% | 5.6% | 0.0% |
| Terminal Rates (c) | 5/41 (12%) | 2/36 (6%) | 0/32 (0%) |
| Life Table Tests (d) | P=0.031N | P=0.271N | P=0.058N |
| Incidental Tumor Tests (d) | P=0.031N | P=0.271N | P=0.058N |
| Cochran-Armitage Trend Test (d) | P=0.018N | | |
| Fisher Exact Tests | | P=0.226N | P=0.030N |
| **Subcutaneous Tissue: Fibroma** | | | |
| Overall Rates (a) | 3/50 (6%) | 2/49 (4%) | 1/49 (2%) |
| Adjusted Rates (b) | 7.3% | 4.9% | 3.1% |
| Terminal Rates (c) | 3/41 (7%) | 1/36 (3%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.299N | P=0.547N | P=0.397N |
| Incidental Tumor Tests (d) | P=0.271N | P=0.486N | P=0.397N |
| Cochran-Armitage Trend Test (d) | P=0.229N | | |
| Fisher Exact Tests | | P=0.510N | P=0.316N |
| **Subcutaneous Tissue: Fibroma or Fibrosarcoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 2/49 (4%) | 1/49 (2%) |
| Adjusted Rates (b) | 9.3% | 4.9% | 3.1% |
| Terminal Rates (c) | 3/41 (7%) | 1/36 (3%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.175N | P=0.379N | P=0.257N |
| Incidental Tumor Tests (d) | P=0.135N | P=0.274N | P=0.221N |
| Cochran-Armitage Trend Test (d) | P=0.123N | | |
| Fisher Exact Tests | | P=0.349N | P=0.187N |
| **Hematopoietic System: Mononuclear Cell Leukemia** | | | |
| Overall Rates (a) | 13/50 (26%) | 12/49 (24%) | 14/49 (29%) |
| Adjusted Rates (b) | 27.5% | 28.1% | 33.9% |
| Terminal Rates (c) | 7/41 (17%) | 7/36 (19%) | 6/32 (19%) |
| Life Table Tests (d) | P=0.258 | P=0.583N | P=0.290 |
| Incidental Tumor Tests (d) | P=0.454N | P=0.438N | P=0.461N |
| Cochran-Armitage Trend Test (d) | P=0.431 | | |
| Fisher Exact Tests | | P=0.523N | P=0.475 |
| **Pituitary: Adenoma** | | | |
| Overall Rates (a) | 8/50 (16%) | 6/48 (13%) | 1/49 (2%) |
| Adjusted Rates (b) | 18.9% | 16.7% | 3.1% |
| Terminal Rates (c) | 7/41 (17%) | 6/36 (17%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.037N | P=0.482N | P=0.042N |
| Incidental Tumor Tests (d) | P=0.033N | P=0.443N | P=0.034N |
| Cochran-Armitage Trend Test (d) | P=0.017N | | |
| Fisher Exact Tests | | P=0.419N | P=0.017N |
| **Pituitary: Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 8/50 (16%) | 8/48 (17%) | 1/49 (2%) |
| Adjusted Rates (b) | 18.9% | 21.4% | 3.1% |
| Terminal Rates (c) | 7/41 (17%) | 7/36 (19%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.051N | P=0.509 | P=0.042N |
| Incidental Tumor Tests (d) | P=0.040N | P=0.583 | P=0.034N |
| Cochran-Armitage Trend Test (d) | P=0.022N | | |
| Fisher Exact Tests | | P=0.572 | P=0.017N |
| **Adrenal: Pheochromocytoma** | | | |
| Overall Rates (a) | 13/50 (26%) | 10/49 (20%) | 6/48 (13%) |
| Adjusted Rates (b) | 31.0% | 26.8% | 18.8% |
| Terminal Rates (c) | 12/41 (29%) | 9/36 (25%) | 6/32 (19%) |
| Life Table Tests (d) | P=0.143N | P=0.443N | P=0.169N |
| Incidental Tumor Tests (d) | P=0.122N | P=0.376N | P=0.150N |
| Cochran-Armitage Trend Test (d) | P=0.061N | | |
| Fisher Exact Tests | | P=0.337N | P=0.075N |

TABLE C1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **Thyroid: C-Cell Adenoma** | | | |
| Overall Rates (a) | 2/49 (4%) | 3/48 (6%) | 1/48 (2%) |
| Adjusted Rates (b) | 5.0% | 8.6% | 3.1% |
| Terminal Rates (c) | 2/40 (5%) | 3/35 (9%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.490N | P=0.439 | P=0.578N |
| Incidental Tumor Tests (d) | P=0.490N | P=0.439 | P=0.578N |
| Cochran-Armitage Trend Test (d) | P=0.408N | | |
| Fisher Exact Tests | | P=0.490 | P=0.508N |
| **Thyroid: C-Cell Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 2/49 (4%) | 4/48 (8%) | 1/48 (2%) |
| Adjusted Rates (b) | 5.0% | 10.9% | 3.1% |
| Terminal Rates (c) | 2/40 (5%) | 3/35 (9%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.507N | P=0.285 | P=0.578N |
| Incidental Tumor Tests (d) | P=0.481N | P=0.336 | P=0.578N |
| Cochran-Armitage Trend Test (d) | P=0.415N | | |
| Fisher Exact Tests | | P=0.329 | P=0.508N |
| **Pancreatic Islets: Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 3/50 (6%) | 1/49 (2%) | 1/48 (2%) |
| Adjusted Rates (b) | 7.1% | 2.8% | 3.1% |
| Terminal Rates (c) | 2/41 (5%) | 1/36 (3%) | 1/32 (3%) |
| Life Table Tests (d) | P=0.269N | P=0.342N | P=0.394N |
| Incidental Tumor Tests (d) | P=0.238N | P=0.280N | P=0.342N |
| Cochran-Armitage Trend Test (d) | P=0.212N | | |
| Fisher Exact Tests | | P=0.316N | P=0.324N |
| **Testis: Interstitial Cell Tumor** | | | |
| Overall Rates (a) | 48/50 (96%) | 45/49 (92%) | 46/49 (94%) |
| Adjusted Rates (b) | 96.0% | 97.8% | 100.0% |
| Terminal Rates (c) | 39/41 (95%) | 35/36 (97%) | 32/32 (100%) |
| Life Table Tests (d) | P=0.047 | P=0.414 | P=0.064 |
| Incidental Tumor Tests (d) | P=0.360 | P=0.671 | P=0.479 |
| Cochran-Armitage Trend Test (d) | P=0.407N | | |
| Fisher Exact Tests | | P=0.329N | P=0.490N |

(a) Number of tumor-bearing animals/number of animals examined at the site. One low dose and one high dose male rat that died during week 1 have been excluded.
(b) Kaplan-Meier estimated tumor incidence 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's exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE C2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **Hematopoietic System: Mononuclear Cell Leukemia** | | | |
| Overall Rates (a) | 15/50 (30%) | 15/43 (35%) | 25/49 (51%) |
| Adjusted Rates (b) | 32.2% | 39.6% | 56.0% |
| Terminal Rates (c) | 10/41 (24%) | 10/32 (31%) | 16/35 (46%) |
| Life Table Tests (d) | P=0.013 | P=0.293 | P=0.017 |
| Incidental Tumor Tests (d) | P=0.038 | P=0.513 | P=0.052 |
| Cochran-Armitage Trend Test (d) | P=0.021 | | |
| Fisher Exact Tests | | P=0.389 | P=0.027 |
| **Liver: Neoplastic Nodule** | | | |
| Overall Rates (a) | 5/50 (10%) | 0/43 (0%) | 3/49 (6%) |
| Adjusted Rates (b) | 12.2% | 0.0% | 8.1% |
| Terminal Rates (c) | 5/41 (12%) | 0/32 (0%) | 2/35 (6%) |
| Life Table Tests (d) | P=0.324N | P=0.058N | P=0.446N |
| Incidental Tumor Tests (d) | P=0.325N | P=0.058N | P=0.448N |
| Cochran-Armitage Trend Test (d) | P=0.265N | | |
| Fisher Exact Tests | | P=0.041N | P=0.369N |
| **Pituitary: Adenoma** | | | |
| Overall Rates (a) | 23/49 (47%) | 12/41 (29%) | 14/48 (29%) |
| Adjusted Rates (b) | 50.8% | 32.9% | 37.0% |
| Terminal Rates (c) | 18/40 (45%) | 8/32 (25%) | 11/34 (32%) |
| Life Table Tests (d) | P=0.125N | P=0.122N | P=0.160N |
| Incidental Tumor Tests (d) | P=0.034N | P=0.074N | P=0.061N |
| Cochran-Armitage Trend Test (d) | P=0.042N | | |
| Fisher Exact Tests | | P=0.067N | P=0.055N |
| **Pituitary: Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 23/49 (47%) | 13/41 (32%) | 14/48 (29%) |
| Adjusted Rates (b) | 50.8% | 35.7% | 37.0% |
| Terminal Rates (c) | 18/40 (45%) | 9/32 (28%) | 11/34 (32%) |
| Life Table Tests (d) | P=0.129N | P=0.173N | P=0.160N |
| Incidental Tumor Tests (d) | P=0.036N | P=0.116N | P=0.061N |
| Cochran-Armitage Trend Test (d) | P=0.043N | | |
| Fisher Exact Tests | | P=0.105N | P=0.055N |
| **Adrenal: Pheochromocytoma** | | | |
| Overall Rates (a) | 4/50 (8%) | 3/43 (7%) | 5/49 (10%) |
| Adjusted Rates (b) | 9.8% | 9.4% | 13.7% |
| Terminal Rates (c) | 4/41 (10%) | 3/32 (9%) | 4/35 (11%) |
| Life Table Tests (d) | P=0.336 | P=0.634N | P=0.400 |
| Incidental Tumor Tests (d) | P=0.336 | P=0.634N | P=0.400 |
| Cochran-Armitage Trend Test (d) | P=0.416 | | |
| Fisher Exact Tests | | P=0.585N | P=0.487 |
| **Thyroid: C-Cell Adenoma** | | | |
| Overall Rates (a) | 1/48 (2%) | 0/43 (0%) | 3/49 (6%) |
| Adjusted Rates (b) | 2.4% | 0.0% | 8.6% |
| Terminal Rates (c) | 1/41 (2%) | 0/32 (0%) | 3/35 (9%) |
| Life Table Tests (d) | P=0.148 | P=0.549N | P=0.250 |
| Incidental Tumor Tests (d) | P=0.148 | P=0.549N | P=0.250 |
| Cochran-Armitage Trend Test (d) | P=0.185 | | |
| Fisher Exact Tests | | P=0.527N | P=0.316 |
| **Thyroid: C-Cell Adenoma or Carcinoma** | | | |
| Overall Rates (a) | 2/48 (4%) | 0/43 (0%) | 3/49 (6%) |
| Adjusted Rates (b) | 4.9% | 0.0% | 8.6% |
| Terminal Rates (c) | 2/41 (5%) | 0/32 (0%) | 3/35 (9%) |
| Life Table Tests (d) | P=0.336 | P=0.294N | P=0.428 |
| Incidental Tumor Tests (d) | P=0.336 | P=0.294N | P=0.428 |
| Cochran-Armitage Trend Test (d) | P=0.400 | | |
| Fisher Exact Tests | | P=0.275N | P=0.510 |

TABLE C2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF DIALLYLPHTHALATE (Continued)

| | Vehicle Control | 50 mg/kg | 100 mg/kg |
|---|---|---|---|
| **Mammary Gland: Fibroadenoma** | | | |
| Overall Rates (a) | 12/50 (24%) | 11/43 (26%) | 5/49 (10%) |
| Adjusted Rates (b) | 27.7% | 31.1% | 13.5% |
| Terminal Rates (c) | 10/41 (24%) | 8/32 (25%) | 4/35 (11%) |
| Life Table Tests (d) | P=0.109N | P=0.425 | P=0.109N |
| Incidental Tumor Tests (d) | P=0.059N | P=0.491 | P=0.059N |
| Cochran-Armitage Trend Test (d) | P=0.056N | | |
| Fisher Exact Tests | | P=0.525 | P=0.059N |
| **Uterus: Endometrial Stromal Polyp** | | | |
| Overall Rates (a) | 9/50 (18%) | 8/43 (19%) | 10/49 (20%) |
| Adjusted Rates (b) | 22.0% | 22.5% | 27.6% |
| Terminal Rates (c) | 9/41 (22%) | 5/32 (16%) | 9/35 (26%) |
| Life Table Tests (d) | P=0.306 | P=0.485 | P=0.348 |
| Incidental Tumor Tests (d) | P=0.300 | P=0.500 | P=0.347 |
| Cochran-Armitage Trend Test (d) | P=0.430 | | |
| Fisher Exact Tests | | P=0.575 | P=0.480 |

(a) Number of tumor-bearing animals/number of animals examined at the site. Seven low dose and one high dose female rats that died during week 1 have been excluded.
(b) Kaplan-Meier estimated tumor incidence 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's exact tests compare directly the overall incidencerates. A negative trend or lower incidence in a dosed group is indicated by (N).

# APPENDIX D

# HISTORICAL INCIDENCES OF TUMORS IN F344/N RATS ADMINISTERED CORN OIL BY GAVAGE

TABLE D1. HISTORICAL INCIDENCE OF LEUKEMIAS IN FEMALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| | Leukemia (All Types) |
|---|---|
| **Historical Incidence at Litton Bionetics, Inc.** | |
| Diallylphthalate | 15/50 |
| Tris(2-ethylhexyl)phosphate | 8/50 |
| 2,4-Toluene diisocyanate | 21/50 |
| TOTAL | 44/150 (29.3%) |
| SD (b) | 13.01% |
| Range (c) | |
| High | 21/50 |
| Low | 8/50 |
| **Overall Historical Incidence** | |
| TOTAL | 185/1,147 (16.1%) |
| SD (b) | 8.9% |
| Range (c) | |
| High | 21/50 (d) |
| Low | 1/49 |

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Second highest: 15/50; third highest: 14/50

TABLE D2. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)

| | Adenoma (b) | Carcinoma (c) | Adenoma or Carcinoma |
|---|---|---|---|
| **Historical Incidence at Litton Bionetics, Inc.** | | | |
| Diallylphthalate | 8/50 | 0/50 | 8/50 |
| Tris(2-ethylhexyl)phosphate | 7/49 | 2/49 | 9/49 |
| 2,4-Toluene diisocyanate | 5/50 | 1/50 | 6/50 |
| TOTAL | 20/149 (13.4%) | 3/149 (2.0%) | 23/149 (15.4%) |
| SD (d) | 3.09% | 2.04% | 3.22% |
| Range (e) | | | |
| High | 8/50 | 2/49 | 9/49 |
| Low | 5/50 | 0/50 | 6/50 |
| **Overall Historical Incidence** | | | |
| TOTAL | 270/1,106 (24.4%) (f) | 22/1,106 (2.0%) (g) | 292/1,106 (26.4%) (f) |
| SD (d) | 10.87% | 2.83% | 10.19% |
| Range (e) | | | |
| High | 23/48 | 5/47 | 23/48 |
| Low | 3/47 | 0/52 | 6/50 |

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Includes all adenomas designated NOS, chromophobe, or acidophil
(c) Includes carcinoma, NOS, and chromophobe carcinoma
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) Total includes one group of 17/50 designated as adenoma, NOS of the anterior pituitary.
(g) One adenocarcinoma, NOS of the anterior pituitary was also observed.

**TABLE D3. HISTORICAL INCIDENCE OF KERATOACANTHOMAS IN MALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)**

| | Skin Keratoacanthoma | Subcutaneous Keratoacanthoma | Total Integumentary Keratoacanthoma (skin or subcutaneous) |
|---|---|---|---|
| **Historical Incidence at Litton Bionetics, Inc.** | | | |
| Diallylphthalate | 5/50 | 0/50 | 5/50 |
| Tris (2-ethylhexyl)phosphate | 1/50 | 0/50 | 1/50 |
| 2,4-Toluene diisocyanate | 1/50 | 0/50 | 1/50 |
| TOTAL | 7/150 (4.7%) | 0/150 (0%) | 7/150 (4.7%) |
| SD (b) | 4.62% | 0% | 4.62% |
| Range (c) | | | |
| High | 5/50 | 0/50 | 5/50 |
| Low | 1/50 | 0/50 | 1/50 |
| **Overall Historical Incidence** | | | |
| TOTAL | 24/1,146 (2.1%) | 2/1,146 (0.2%) | 26/1,146 (2.3%) |
| SD (b) | 2.37% | 0.58% | 2.36% |
| Range (c) | | | |
| High | 5/50 | 1/49 | 5/50 |
| Low | 0/52 | 0/52 | 0/52 |

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

**TABLE D4. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN FEMALE F344/N RATS ADMINISTERED CORN OIL BY GAVAGE (a)**

| | Adenoma (b) | Adenocarcinoma or Carcinoma (c) | Adenoma, Carcinoma, or Adenocarcinoma |
|---|---|---|---|
| **Historical Incidence at Litton Bionetics, Inc.** | | | |
| Diallylphthalate | 23/49 | 0/49 | 23/49 |
| Tris(2-ethylhexyl)phosphate | 18/50 | 1/50 | 19/50 |
| 2,4-Toluene diisocyanate | 25/50 | 2/50 | 27/50 |
| Total | 66/149 (44.3%) | 3/149 (2.0%) | 69/149 (46.3%) |
| SD (d) | 7.36% | 2.0% | 8.02% |
| Range (e) | | | |
| High | 25/50 | 2/50 | 27/50 |
| Low | 18/50 | 0/49 | 19/50 |
| **Overall Historical Incidence** | | | |
| Total | 410/1,092 (37.5%) (f) | 37/1,092 (3.4%) (g) | 447/1,092 (40.9%) (f) |
| SD (d) | 10.22% | 3.72% | 9.64% |
| Range (e) | | | |
| High | 28/50 | 6/46 | 30/50 |
| Low | 8/46 | 0/50 | 13/48 |

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Includes all adenomas designated NOS, chromophobe, acidophil, or basophil
(c) Includes carcinoma, NOS, adenocarcinoma, NOS, and chromophobe carcinoma
(d) Standard deviation
(e) Range and SD are presented for groups of 35 or more animals.
(f) Total includes one group of 16/50 designated adenoma, NOS of the anterior pituitary.
(g) Two adenocarcinomas, NOS of the anterior pituitary were also observed.

# APPENDIX E

# GENETIC TOXICOLOGY OF DIALLYLPHTHALATE AND RELATED COMPOUNDS

TABLE E1. MUTAGENICITY OF DIALLYLPHTHALATE IN SALMONELLA

| Strain | Dose (µg/plate) | Revertants/Plate (a) −S9 | +S9 (rat) | +S9 (hamster) |
|---|---|---|---|---|
| TA100 | 0 | 113 ± 5.6 | 161 ± 7.0 | 156 ± 11.1 |
| | 1 | -- | 167 ± 6.2 | 161 ± 12.9 |
| | 3.3 | -- | 158 ± 8.5 | 137 ± 0.7 |
| | 10 | 121 ± 14.2 | 152 ± 4.0 | 137 ± 3.0 |
| | 33 | 130 ± 6.1 | 136 ± 5.8 | 119 ± 11.9 |
| | 100 | 127 ± 4.5 | 100 ± 2.4 | 89 ± 0.7 |
| | 333 | 112 ± 8.7 | -- | -- |
| | 1,000 | 124 ± 5.8 | -- | -- |
| TA1535 | 0 | 18 ± 3.4 | 13 ± 2.3 | 14 ± 2.1 |
| | 1 | -- | 15 ± 2.1 | 12 ± 1.0 |
| | 3.3 | -- | 11 ± 2.0 | 13 ± 1.5 |
| | 10 | 14 ± 2.3 | 12 ± 0.9 | 8 ± 0.7 |
| | 33 | 18 ± 4.0 | 8 ± 1.5 | 11 ± 1.5 |
| | 100 | 21 ± 3.2 | 11 ± 0.9 | 9 ± 1.0 |
| | 333 | 14 ± 2.1 | -- | -- |
| | 1,000 | 24 ± 2.8 | -- | -- |
| TA1537 | 0 | 4 ± 1.7 | 11 ± 2.0 | 8 ± 1.7 |
| | 1 | -- | 7 ± 1.5 | 8 ± 1.7 |
| | 3.3 | -- | 9 ± 0.0 | 7 ± 0.7 |
| | 10 | 6 ± 1.2 | 11 ± 2.7 | 6 ± 1.2 |
| | 33 | 6 ± 0.9 | 8 ± 0.5 | 6 ± 1.5 |
| | 100 | 6 ± 0.7 | 7 ± 0.0 | 9 ± 3.4 |
| | 333 | 4 ± 1.5 | -- | -- |
| | 1,000 | 6 ± 1.2 | -- | -- |
| TA98 | 0 | 10 ± 1.9 | 27 ± 2.2 | 28 ± 1.8 |
| | 1 | -- | 28 ± 0.9 | 32 ± 2.3 |
| | 3.3 | -- | 33 ± 0.3 | 30 ± 3.2 |
| | 10 | 19 ± 2.0 | 32 ± 3.8 | 29 ± 2.6 |
| | 33 | 16 ± 2.6 | 23 ± 3.0 | 25 ± 4.9 |
| | 100 | 19 ± 2.2 | 19 ± 1.8 | 23 ± 3.2 |
| | 333 | 14 ± 2.2 | -- | -- |
| | 1,000 | 17 ± 3.2 | -- | -- |

(a) The S9 fractions were prepared from the livers of Aroclor 1254-induced animals (male Sprague-Dawley rats and male Syrian hamsters). Cells and test compound or solvent (DMSO) were incubated for 20 min at 37° C in the presence of either S9 or buffer (Yahagi et al., 1975). After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 h (Ames et al., 1975). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.

**TABLE E2. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY DIALLYLPHTHALATE**

| Route of Exposure | Dose (ppm) | No. of Lethals/No. of X Chromosomes Tested (a) | | | |
|---|---|---|---|---|---|
| | | Mating 1 | Mating 2 | Mating 3 | Total (percent) |
| Injection | 0 | 2/2,071 | 3/1,897 | 1/1,581 | 6/5,549 (0.11) |
| | 500 | 0/1,975 | 2/1,903 | 1/1,712 | 3/5,590 (0.05) |
| Feeding | 0 | 0/1,142 | 3/1,130 | 1/1,109 | 4/3,381 (0.12) |
| | 140 | 2/888 | 1/868 | 0/857 | 3/2,613 (0.11) |

(a) The sex-linked recessive lethal assay was performed essentially as described by Abrahamson and Lewis (1971). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the test chemical dissolved in 5% sucrose. Exposure by injection was done by injecting 72-hour-old adult males at the base of the halteres with enough of the test chemical dissolved in 0.7% sodium chloride to distend the abdomen (approximately 0.3 µl). Injected flies were allowed to recover for 24 hours before being mated. 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, after which the parents were discarded. $F_1$ heterozygous females were crossed to their siblings and placed in individual vials. $F_1$ daughters from the same parental males were kept together to identify clusters; none were found. After 17 days, presumptive lethals were identified as vials containing no wild-type males; these were retested.

TABLE E3. MUTAGENICITY OF ACROLEIN IN SALMONELLA

| Strain | Dose (µg/plate) | Revertants/Plate (a) −S9 | +S9 (rat) | +S9 (hamster) |
|---|---|---|---|---|
| TA100 | 0 | 125 ± 10.3 | 137 ± 7.0 | 129 ± 6.1 |
| | 0.03 | 142 ± 3.0 | -- | -- |
| | 0.1 | 136 ± 2.3 | -- | -- |
| | 0.3 | 131 ± 4.6 | -- | -- |
| | 1.0 | 137 ± 5.5 | -- | -- |
| | 3.3 | 129 ± 5.2 | -- | -- |
| | 10 | -- | 150 ± 3.2 | 136 ± 4.4 |
| | 15 | -- | 138 ± 4.6 | 137 ± 13.4 |
| | 20 | -- | 133 ± 8.2 | 149 ± 7.0 |
| | 25 | -- | 136 ± 3.2 | 133 ± 2.2 |
| | 33 | -- | 171 ± 8.3 | 138 ± 6.4 |
| | 40 | -- | 197 ± 7.0 | 148 ± 7.4 |
| | 50 | -- | 245 ± 4.8 | 173 ± 1.3 |
| | 100 | | 44 ± 5.6 | 42 ± 5.9 |
| TA1535 | 0 | 27 ± 3.2 | 14 ± 2.3 | 11 ± 0.7 |
| | 0.1 | 27 ± 2.6 | -- | -- |
| | 0.3 | 20 ± 2.3 | -- | -- |
| | 1.0 | 24 ± 2.1 | 11 ± 2.3 | 8 ± 0.9 |
| | 3.3 | 19 ± 3.7 | 7 ± 0.3 | 10 ± 0.7 |
| | 10.0 | Toxic | 12 ± 2.3 | 10 ± 2.7 |
| | 33.0 | -- | 15 ± 0.0 | 19 ± 2.3 |
| | 100.0 | -- | Toxic | Toxic |
| TA1537 | 0 | 7 ± 2.3 | 7 ± 1.3 | 9 ± 2.1 |
| | 0.1 | 7 ± 0.6 | -- | -- |
| | 0.3 | 6 ± 1.9 | -- | -- |
| | 1.0 | 12 ± 1.2 | 6 ± 2.5 | 8 ± 1.5 |
| | 3.3 | 5 ± 1.0 | 6 ± 1.7 | 6 ± 2.4 |
| | 10.0 | Toxic | 11 ± 1.0 | 9 ± 3.1 |
| | 33.0 | -- | 10 ± 1.2 | 5 ± 1.2 |
| | 100.0 | -- | Toxic | Toxic |
| TA98 | 0 | 22 ± 3.8 | 25 ± 1.8 | 29 ± 2.2 |
| | 0.1 | 24 ± 3.2 | -- | -- |
| | 0.3 | 23 ± 1.5 | -- | -- |
| | 1.0 | 20 ± 2.9 | 24 ± 3.3 | 29 ± 4.0 |
| | 3.3 | Toxic | 24 ± 4.3 | 29 ± 0.3 |
| | 10.0 | Toxic | 21 ± 1.8 | 22 ± 1.0 |
| | 33.0 | -- | 30 ± 3.4 | 25 ± 4.2 |
| | 100.0 | -- | Toxic | 10 ± 0.5 |

(a) The S9 fractions were prepared from the livers of Aroclor 1254-induced animals (male Sprague-Dawley rats and male Syrian hamsters). Cells and test compound or solvent (water) were incubated for 20 minutes at 37° C in the presence of either S9 or buffer (Yahagi et al., 1975). After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 hours (Ames et al., 1975). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.

TABLE E4. INDUCTION OF SISTER-CHROMATID EXCHANGES (SCE'S) IN CHINESE HAMSTER OVARY (CHO) CELLS BY ACROLEIN

| −S9 (a) | | +S9 (b) | |
|---|---|---|---|
| Dose (μg/ml) | SCE/Cell | Dose (μg/ml) | SCE/Cell |
| $H_2O$ (100 μl) | 8.08 | $H_2O$ (100 μl) | 8.60 |
| 0.1 | 9.28 | 0.1 | 10.22 |
| 0.3 | 9.64 | 0.3 | 9.16 |
| 1.0 | 10.38 | 1.0 | 9.52 |
| Triethylenemelamine (0.015) | 29.88 | Cyclophosphamide (1) | 22.50 |

(a) In the absence of S9, CHO cells were incubated with test compound or solvent for 2 hours at 37° C. Then BrdU was added, and incubation continued for 24 hours. Cells were washed, fresh medium containing BrdU (10 μM) and colcemid (0.1 μg/ml) was added, and incubation was continued for 2-3 hours. Cells then were collected by mitotic shake-off, treated for 3 minutes with potassium chloride (75 mM), washed twice with fixative, and dropped onto slides and air-dried. Staining was by a modified technique (after Perry and Wolff, 1974; Goto et al., 1978).
(b) In the presence of S9, cells were incubated with test compound or solvent for 2 hours at 37° C. Then cells were washed, and medium containing 10 μM BrdU was added. Cells were incubated 26 hours longer, with colcemid (0.1 μg/ml) present for the final 2-3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

TABLE E5. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS BY ACROLEIN

| −S9 (a) | | +S9 (b) | |
|---|---|---|---|
| Dose (μg/ml) | Abs/100 Cells (percent cells w/abs) | Dose (μg/ml) | Abs/100 Cells (percent cells w/abs) |
| $H_2O$ (100 μl) | 1 (1) | $H_2O$ (100 μl) | 0 (0) |
| 0.1 | 2 (2) | 0.1 | 2 (2) |
| 0.3 | 2 (2) | 0.3 | 3 (2) |
| 1.0 | 5 (5) | 1.0 | 5 (3) |
| Triethylenemelamine (0.015) | 32 (27) | Cyclophosphamide (15) | 47 (33) |

(a) In the absence of S9, CHO cells were incubated with test compound or solvent for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid (0.1 μg/ml) was added. After another 2-3 hours of incubation, cells were harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the presence of S9, cells were incubated with test compound or solvent for 2 hours at 37° C. Cells then were washed, medium was added, and incubation continued for 8-10 hours. Colcemid (0.1 μg/ml) was added for the last 2-3 hours of incubation; then cells were harvested and fixed as above. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

## Conclusions:

Sister Chromatid Exchange: Weakly positive with or without S9

Chromosome Aberrations: Positive with or without S9

TABLE E6. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY ACROLEIN

| Route of Exposure | Dose (ppm) | No. of Lethals/No. of X Chromosomes Tested (a) | | | |
|---|---|---|---|---|---|
| | | Mating 1 | Mating 2 | Mating 3 | Total (percent) |
| Feeding | 0 | 2/2,103 | 1/1,945 | 0/1,850 | 3/5,898 (0.05) |
| | 3,000 | 3/2,644 | 2/2,377 | 0/2,019 | 5/7,040 (0.07) |
| Injection | 0 | 1/2,025 | 2/1,960 | 1/1,857 | 4/5,842 (0.07) |
| | 200 | 3/2,032 | 0/1,947 | 0/1,852 | 3/5,831 (0.05) |

(a) The sex-linked recessive lethal assay was performed essentially as described by Abrahamson and Lewis (1971). Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the test chemical dissolved in 5% sucrose. Exposure by injection was done by injecting 72-hour-old adult males at the base of the halteres with enough of the test chemical dissolved in 0.7% sodium chloride to distend the abdomen (approximately 0.3 µl). Injected flies were allowed to recover for 24 hours before being mated. 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, after which the parents were discarded. $F_1$ heterozygous females were crossed to their siblings and placed in individual vials. $F_1$ daughters from the same parental males were kept together to identify clusters; none were found. After 17 days, presumptive lethals were identified as vials containing no wild-type males; these were retested.

TABLE E7. MUTAGENICITY OF GLYCIDALDEHYDE IN SALMONELLA

| Strain | Dose (µg/plate) | Revertants/Plate (a) −S9 | +S9 (rat) | +S9 (hamster) |
|---|---|---|---|---|
| TA100 | 0 | 121 ± 5.8 | 139 ± 3.5 | 151 ± 2.2 |
| | 0.1 | 123 ± 16.6 | 128 ± 7.8 | 128 ± 8.8 |
| | 0.3 | 205 ± 9.9 | 176 ± 6.8 | 161 ± 7.5 |
| | 1.0 | 382 ± 12.4 | 295 ± 4.3 | 148 ± 9.1 |
| | 3.3 | 695 ± 20.1 | 622 ± 6.5 | 173 ± 6.7 |
| | 10.0 | 1,123 ± 22.8 | 1,158 ± 37.9 | 223 ± 24.5 |
| | 33.3 | 1,700 ± 30.6 | 1,793 ± 45.1 | 595 ± 56.4 |
| | 100.0 | 1,977 ± 50.9 | 2,335 ± 83.8 | 1,537 ± 35.0 |
| TA1535 | 0 | 25 ± 3.2 | 19 ± 4.4 | 12 ± 2.7 |
| | 0.1 | 35 ± 2.8 | 34 ± 3.8 | 13 ± 2.9 |
| | 0.3 | 69 ± 4.8 | 61 ± 2.0 | 9 ± 1.5 |
| | 1.0 | 192 ± 7.2 | 196 ± 2.9 | 17 ± 3.6 |
| | 3.3 | 472 ± 11.4 | 482 ± 21.5 | 15 ± 3.0 |
| | 10.0 | 942 ± 24.7 | 1,070 ± 9.6 | 56 ± 4.6 |
| | 33.3 | 1,434 ± 15.0 | 1,726 ± 27.9 | 332 ± 29.2 |
| | 100.0 | 1,490 ± 63.8 | 1,967 ± 45.3 | 1,415 ± 43.5 |
| TA1537 | 0 | 9 ± 0.9 | 6 ± 0.9 | 9 ± 3.0 |
| | 0.3 | -- | 5 ± 0.7 | -- |
| | 1.0 | 6 ± 0.7 | 5 ± 0.9 | 13 ± 3.1 |
| | 3.3 | 6 ± 1.8 | 4 ± 1.3 | 6 ± 1.2 |
| | 10.0 | 7 ± 1.5 | 4 ± 1.5 | 14 ± 0.3 |
| | 33.3 | 11 ± 2.5 | 11 ± 2.3 | 10 ± 0.6 |
| | 100.0 | 29 ± 4.9 | 23 ± 1.2 | 28 ± 0.7 |
| TA98 | 0 | 15 ± 1.8 | 19 ± 4.1 | 51 ± 1.2 |
| | 0.3 | 15 ± 0.7 | 16 ± 1.0 | 45 ± 0.6 |
| | 1.0 | 11 ± 2.7 | 18 ± 3.2 | 95 ± 45.4 |
| | 3.3 | 10 ± 3.5 | 17 ± 1.5 | 89 ± 28.2 |
| | 10.0 | 13 ± 2.6 | 24 ± 2.0 | 405 ± 101.8 |
| | 33.0 | 27 ± 3.4 | 30 ± 4.5 | 445 ± 13.2 |
| | 100.0 | 31 ± 5.6 | 41 ± 4.6 | 742 ± 100.1 |

(a) The S9 fractions were prepared from the livers of Aroclor 1254-induced animals (male Sprague-Dawley rats and male Syrian hamsters). Cells and test compound or solvent (DMSO) were incubated for 20 minutes at 37° C in the presence of either S9 or buffer (Yahagi et al., 1975). After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 hours (Ames et al., 1975). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.

TABLE E8. MUTAGENICITY OF GLYCIDOL IN SALMONELLA

| Strain | Dose (μg/plate) | Revertants/Plate (a) −S9 | +S9 (rat) | +S9 (hamster) |
|---|---|---|---|---|
| TA100 | 0 | 127 ± 6.4 | 151 ± 14.2 | 136 ± 15.0 |
| | 1 | 132 ± 7.9 | 126 ± 8.1 | 137 ± 6.7 |
| | 3 | 138 ± 8.7 | 139 ± 0.3 | 157 ± 18.3 |
| | 10 | 180 ± 1.0 | 222 ± 8.4 | 211 ± 11.2 |
| | 33 | 253 ± 9.5 | 303 ± 3.2 | 296 ± 6.7 |
| | 100 | 410 ± 11.3 | 533 ± 3.5 | 486 ± 18.3 |
| TA1535 | 0 | 20 ± 3.0 | 7 ± 0.9 | 10 ± 2.2 |
| | 1 | 33 ± 3.2 | 19 ± 3.3 | 14 ± 2.7 |
| | 3 | 53 ± 2.6 | 33 ± 4.0 | 33 ± 5.7 |
| | 10 | 98 ± 6.7 | 90 ± 1.2 | 92 ± 9.8 |
| | 33 | 194 ± 4.8 | 218 ± 4.2 | 221 ± 14.7 |
| | 100 | 459 ± 17.7 | 470 ± 8.1 | 478 ± 11.0 |
| TA1537 | 0 | 5 ± 0.9 | 4 ± 0.3 | 6 ± 0.6 |
| | 333 | 7 ± 1.2 | 7 ± 0.7 | 8 ± 1.9 |
| | 1,000 | 12 ± 2.0 | 15 ± 1.5 | 7 ± 0.3 |
| | 1,666 | 13 ± 2.3 | 19 ± 5.4 | 12 ± 3.3 |
| | 333 | 15 ± 1.9 | 23 ± 1.8 | 22 ± 3.8 |
| | 6,666 | 16 ± 3.4 | 13 ± 3.0 | 22 ± 3.8 |
| TA98 | 0 | 16 ± 1.2 | 21 ± 2.7 | 21 ± 1.5 |
| | 333 | 22 ± 2.7 | 30 ± 1.9 | 32 ± 4.2 |
| | 1,000 | 25 ± 3.7 | 43 ± 7.8 | 40 ± 6.4 |
| | 1,666 | 30 ± 3.5 | 34 ± 3.1 | 44 ± 5.3 |
| | 3,333 | 34 ± 3.2 | 42 ± 5.5 | 58 ± 2.0 |
| | 6,666 | 42 ± 1.5 | 47 ± 2.5 | 57 ± 2.6 |

(a) The S9 fractions were prepared from the livers of Aroclor 1254-induced animals (male Sprague-Dawley rats and male Syrian hamsters). Cells and test compound or solvent (water) were incubated for 20 minutes at 37° C in the presence of either S9 or buffer (Yahagi et al., 1975). After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 hours (Ames et al., 1975). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.

# APPENDIX F

# CHEMICAL CHARACTERIZATION OF DIALLYLPHTHALATE

## I. Identity and Purity Determinations of Lot No. 25-121 Performed by the Analytical Chemistry Laboratory

### A. Physical Properties

| | | |
|---|---|---|
| **1. Appearance:** | Pale yellow liquid | |
| **2. Boiling Point:** | **Determined** | **Literature Values** |
| | 160°-161° C/5 mm Hg (macro distillation) | 165°-167° C/5mm Hg (Beilstein) |
| **3. Index of Refraction:** | **Determined** | **Literature Values** |
| | $n_D^{20}$ 1.584 | $n_D^{20}$ 1.5203 (Beilstein) |

### B. Spectral Data

| | | |
|---|---|---|
| **1. Infrared** | **Determined** | **Literature Values** |
| **a. Instrument:** | Beckman IR-12 | |
| **b. Cell:** | Neat liquid between sodium chloride plates | |
| **c. Results:** | See Figure 4 | Consistent with literature spectrum (Sadtler Standard Spectra) |

| | | | | |
|---|---|---|---|---|
| **2. Ultraviolet/Visible** | **Determined** | | **Literature Values** | |
| **a. Instrument:** | Cary 118 | | | |
| **b. Solvent:** | 95% Ethanol | | | |
| **c. Results:** | $\lambda_{max}$ (nm) | $\varepsilon \times 10^{-3}$ | $\lambda_{max}$ (nm) | $\varepsilon \times 10^{-3}$ |
| | 225 | 8.3 ± 0.5 (6) | 225 | 8.5 |
| | 275 | 1.2 ± 0.1 (6) | 275 | 1.3 |
| | No maximum observed between 350 nm and 800 nm at 0.15 mg/ml | | Calculated from literature spectrum (Sadtler Standard Spectra) | |

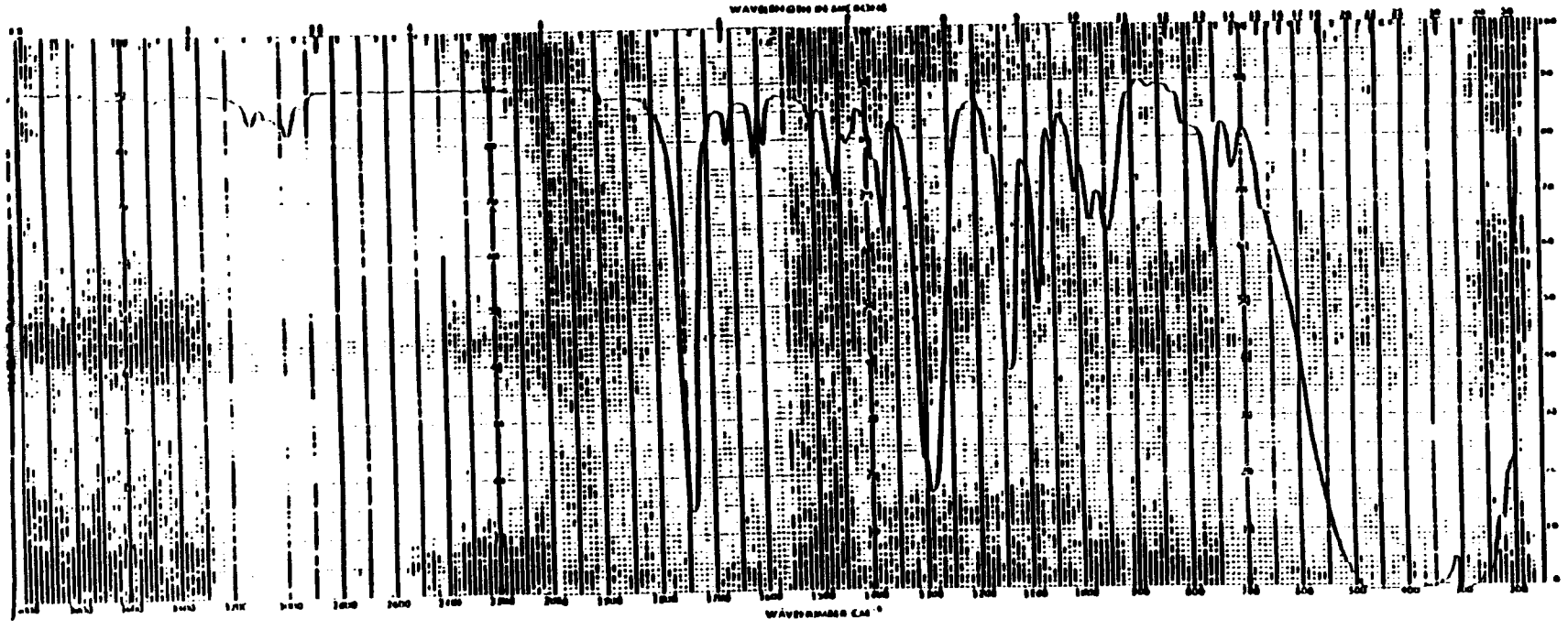

FIGURE 4. INFRARED ABSORPTION SPECTRUM OF DIALLYLPHTHALATE (LOT NO. 25-121)

3. Nuclear Magnetic Resonance

| | Determined | Literature Values |
|---|---|---|
| a. Instrument: | Varian HA-100 | |
| b. Solvent: | Neat liquid with added tetramethylsilane | |
| c. Assignments: | See Figure 5 | Consistent with literature spectrum (Sadtler Standard Spectra) |

d. Chemical Shift (δ):

a -- 4.72 ppm
b -- 5.12 ppm
c -- 5.26 ppm
d -- 5.73-6.15 ppm
e -- 7.35-7.51 ppm
f -- 7.57-7.73ppm

e. Coupling Constants:

$J_{ab} = 1.5$ Hz, $J_{ad} = 5$ Hz
$J_{bc} = 2$ Hz, $J_{bd} = 10$ Hz
$J_{cd} = 18$ Hz

f. Integration Ratios:

a -- 3.82
b -- 1.84
c -- 2.18
d -- 2.08
e -- 2.01
f -- 2.08

C. Water Analysis (Karl Fischer): 0.144% ± 0.004 (δ) %

D. Elemental Analysis:

| Element | C | H |
|---|---|---|
| Theory (T) | 68.28 | 5.73 |
| Determined (D) | 68.38<br>68.22 | 5.69<br>5.80 |
| Percent D/T | 99.97 | 100.35 |

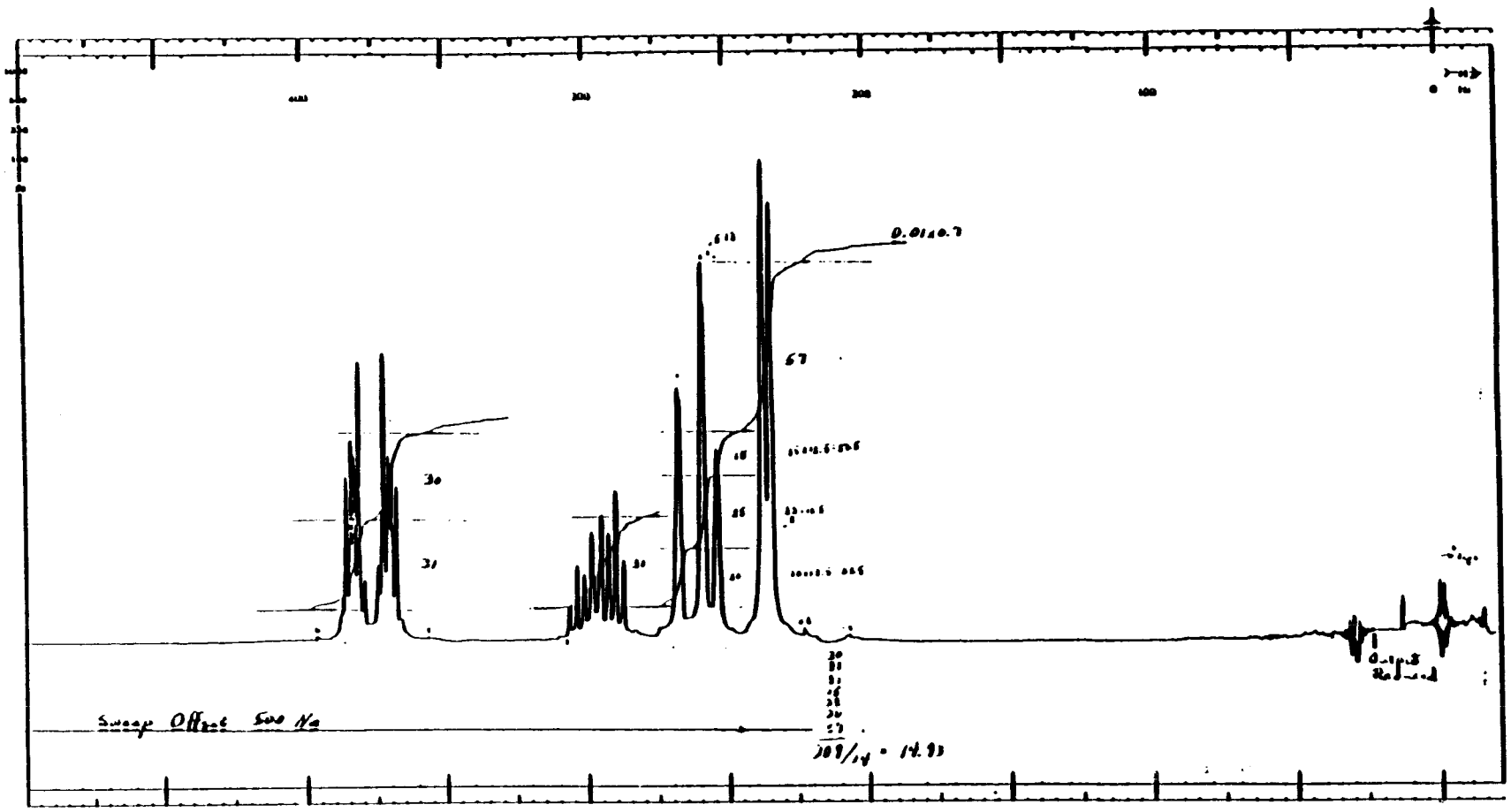

FIGURE 5. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF DIALLYLPHTHALATE (LOT NO. 25-121)

E. Chromatographic Analyses

1. Thin-Layer Chromatography

**Plates:** Silica gel F-254
**Amount Spotted:** 100 and 300 µg
**Ref. Standard:** Dimethyl terephthalate
**Detection Systems:** Ultraviolet (254 nm) and iodine vapor

a. System 1:

(1) Methanol (100%)
(2) $R_f$: 0.85
(3) $R_{st}$: 1.04

b. System 2:

(1) Benzene (100%)
(2) $R_f$: 0.25
(3) $R_{st}$: 1.18

2. Gas Chromatography

**Instrument:** Tracor MT 220
**Detector:** Flame ionization
**Column:** 3 % OV-17, 1.8 m × 4 mm I.D.
**Oven temperature program:** 100° C, 5 min; 100°-235° C at 10° C/min
**Results:** Major peak and eight impurities

| Peak | Retention Time (min) | Retention Time Relative to Diallylphthalate | Area (percent of major peak) |
|---|---|---|---|
| 1 | 8.8 | 0.56 | 0.106 |
| 2 | 10.6 | 0.67 | 0.046 |
| 3 | 11.2 | 0.71 | 0.064 |
| 4 | 13.0 | 0.82 | trace |
| 5 | 13.2 | 0.84 | trace |
| 6 | 14.8 | 0.94 | 0.045 |
| 7 | 15.8 | 1.00 | 100 |
| 8 | 17.4 | 1.10 | 0.074 |
| 9 | 18.0 | 1.14 | 0.731 |

**F. Conclusions:** Results of elemental analyses agreed with the theoretical values. Thin-layer chromatography indicated only the spot for the major component. Gas chromatography indicated eight impurities, totaling about 1% of the major peak. The infrared and nuclear magnetic resonance spectra were consistent with the structure.

## II. Test Chemical Stability Study Performed by the Analytical Chemistry Laboratory

**A. Sample Storage:** Four samples of diallylphthalate were stored for 2 weeks, one at each of the following temperatures: −20°, 5°, 25°, and 60° C. Analysis was carried out by gas chromatography as described below.

**B. Analytical Method**

1. **Instrument:** Tracor MT-220
2. **Detector:** Flame ionization
3. **Column:** 3% OV-1 on Supelcoport, 80/100 mesh, 4 mm × 1.8 m, glass, silanized
4. **Retention time:** 3.20 min
5. **Oven temperature:** 150° C, isothermal

**C. Results:**

| Storage Temperature (° C) | Percent Diallylphthalate |
|---|---|
| − 20 | 102.3 ± 4.8 |
| + 5 | 107.7 ± 4.8 |
| + 25 | 104.8 ± 4.8 |
| + 60 | 103.8 ± 4.8 |

**D. Conclusion:** Diallylphthalate is stable under conditions of storage for 2 weeks at temperatures up to 60° C.

## III. Test Chemical Stability Study at the Testing Laboratory

**A. Storage Conditions:** Upon receipt of the bulk chemical, the testing laboratory removed a standard reference sample that was stored at −20° C before analyses were performed. This reference sample was analyzed in tandem with the bulk test chemical during each bulk chemical reanalysis. The bulk chemical was stored at 4° C.

### B. Analytical Methods

#### 1. Purity Determination, Gas Chromatography

**a. Instrument:** Hewlett Packard® 5880 with 7672A Liquid Sampler
**b. Column:** 3% OV-17 on 80/100 mesh Supelcoport 1.8 m × 2 mm (ID), silanized glass
**c. Detector:** Flame ionization
**d. Detector temperature:** 275° C
**e. Inlet temperature:** 225° C
**f. Temperature program:** 100° C for 5 min; 100° to 250° C at 10° C/min; 250° C for 5 min
**g. Carrier gas:** Nitrogen
**h. Carrier flow rate:** 40 ml/min
**i. Sample injections:** 3 µl each of 20%, 1.0% and 0.5% diallylphthalate in methanol to quantitate the major peak area and to check for column and/or detector overloading.

**2. Identity Determination:** The infrared absorption spectra of the samples were obtained as potassium bromide disks using a Perkin-Elmer® Model 398 spectrophotometer.

### C. Results

#### 1. Purity:

| Date | Percent Purity | | Relative Percent Purity |
|---|---|---|---|
| | Bulk | Reference | |
| 1/78 | 98.9 | -- | -- |
| 5/78 | 98.2 | 98.5 | 99.7 |
| 10/78 | 99.5 | 99.5 | 100.0 |
| 7/79 | 99.0 | 98.6 | 100.4 |
| 11/79 | 99.2 | -- | -- |
| 3/80 | 99.4 | 99.4 | 100.0 |
| 7/80 | 99.5 | 99.5 | 100.0 |
| 11/80 | 99.5 | 99.4 | 100.1 |
| 3/81 | 99.2 | 99.2 | 100.0 |
| 6/81 | 99.0 | 99.3 | 99.7 |
| 9/81 | 99.2 | 99.3 | 99.9 |
| 1/82 | 99.1 | 99.1 | 100.0 |

Mean relative percent purity: 99.9%
Coefficient of variation: 0.2%

**2. Identity:** All spectra were consistent with the original spectra supplied by the analytical chemistry laboratory.

**D. Conclusion:** No notable degradation was observed in the bulk chemical throughout the studies.

# APPENDIX G

# PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

# APPENDIX G. PREPARATION AND CHARACTERIZATION

## I. Studies Conducted at the Analytical Chemistry Laboratory

**A. Sample Preparation and Storage:** One milliliter of corn oil was sealed (septum and aluminum crimp-seal, Wheaton Scientific) in an 8-ml septum vial. The vial was weighed, and approximately 80 µl of diallylphthalate was injected by micro syringe. The vial was reweighed to determine the actual amount of diallylphthalate added. Twenty-four samples were prepared in this manner and thoroughly shaken to mix the vial contents. Twelve of the samples were stored at room temperature (25° C) with no attempt to protect them from the light. The other 12 samples were stored in a refrigerator at 5° C. Two samples from each storage temperature were analyzed at various intervals over a 15-day period.

**B. Extraction and Analysis:** The diallylphthalate was extracted from the corn oil by injecting 4 ml of methanol into the septum vials, followed by 15 seconds of vigorous manual shaking of the vials. When the methanol and corn oil layers had separated (after approximately 15 minutes), 5-µl aliquots of the methanol solution were injected directly into a gas chromatograph for analysis.

1. **System 1:**

**Instrument:** Bendix 2500 or Tracor MT-220
**Column:** 3% OV-1 on Supelcoport DMCS, 80/100 mesh, 4 mm × 1.8 m, glass
**Detector:** Flame ionization
**Temperatures:**
Inlet, 205° C
Oven, 170° C, isothermal
Detector, 225° C

2. **System 2:**

**Instrument:** Bendix 2500 or Tracor MT-220
**Column:** 3% DEGS on Gas Chrom P, 80/100 mesh, 4 mm × 1.8, glass
**Detector:** Flame ionization
**Temperatures:**
Inlet, 205° C
Oven, 195° C, isothermal
Detector, 225° C

Systems 1 and 2 were used interchangeably depending on instrument availability during the study.

**C. Stability Results:**

| Storage Time (days) | Column | Average Percent Chemical Found in Chemical/Vehicle Mixture (a) at 25° C | at 5° C |
|---|---|---|---|
| 1 | OV-1 | -- | 9.27 ± 0.28 |
| 2 | OV-1 | 9.11 ± 0.31 | 9.22 ± 0.28 |
| 5 | OV-1 | 8.57 ± 0.44 | 8.85 ± 0.43 |
| 8 | OV-1 | 8.67 ± 0.40 | 8.83 ± 0.46 |
| 10 | DEGS | 9.37 ± 0.38 | 9.02 ± 0.40 |
| 15 | DEGS | 9.07 ± 0.29 | 9.16 ± 0.40 |

(a) Corrected for a spike recovery yield: 84.2% ± 2.5% of theoretical. Average theoretical (actual) compound added to corn oil: 9.01%.

**D. Conclusion:** All of the corrected analytical values were within ±0.45 units of the mean value, 9.02%. This represents a mean percentage recovery of 84.2 ± 4.2, which compares very well with the spiked recovery yield of 84.2% ± 2.5%. Therefore, diallylphthalate mixed with corn oil at the 9.0% (w/v) level is stable for 15 days at temperatures of 25° C or below.

**E. Storage Conditions for Mixtures in Corn Oil:** Diallylphthalate mixed with corn oil at the 9.0% (w/v) level may be stored in closed containers in the presence of light for a period of 2 weeks at room temperature (25° C) or for at least 8 weeks at 5° C. This latter storage time is extrapolated from the above stability study at 25° C. Further conclusions regarding the stability of diallylphthalate mixed with corn oil cannot be drawn from this study.

**II. Stability Studies Conducted at the Testing Laboratory**

**A. Procedure:** One milliliter of the corn oil solution was diluted to 10 ml with carbon disulfide. This solution was further diluted with carbon disulfide to a suitable volume for analysis. Content of the test compound was determined by gas chromatography with the following system:

**Instrument:** Packard Model 7400
**Column:** 3% OV-17 on 80/100 mesh Supelcoport, 1.8 m × 4 mm ID, glass
**Detector:** Flame ionization
**Column temperature:** 230° C
**Carrier:** Nitrogen
**Flow Rate:** 35 ml/min

Concentration of the test compound was determined by reference to a calibration curve prepared by analysis of standard solutions of diallylphthalate in carbon disulfide.

**B. Results:**

| Date Analyzed | Concentration (mg/ml) Target | Determined |
|---|---|---|
| 8/17/77 | 15.0 | 14.3 |
| | 30.0 | 30.9 |
| 8/24/77 | 15.0 | 14.4 |
| | 30.0 | 32.7 |
| 8/31/77 | 15.0 | 15.1 |
| | 30.0 | 30.2 |

**C. Conclusion:** Diallylphthalate/corn oil mixtures are stable for up to 15 days.

# APPENDIX H

# ANALYSIS OF DOSE MIXTURES: METHODS

## I. Testing Laboratory

**A. Carbon Disulfide Extraction Method for the Thirteen-Week Studies:** An aliquot of the corn oil dose mixture was diluted with carbon disulfide. The solution was mixed well. Analysis was performed by gas chromatography. The concentration of the test compound was determined by reference to a calibration curve prepared by analysis of a standard solution of diallylphthalate.

A stock standard solution of diallylphthalate was prepared by dissolving 300 mg of the compound in carbon disulfide to a final volume of 50 ml.

Three milliliters of carbon disulfide was added to samples of diallylphthalate/corn oil mixture containing approximately 15 mg diallylphthalate and mixed by shaking on an automatic shaker box for 10 minutes.

Approximately 2 ml of each sample was transferred with a disposable syringe into septa vials suitable for the automatic sampler.

**Instrument:** Hewlett-Packard® 5840A
**Column:** 3% OV-17 on 80/100 mesh Supelcoport, 1.8 m × 2 mm ID, glass
**Detector:** Flame ionization
**Temperatures**
  **Column:** 210° C
  **Injector:** 225° C
  **Detector:** 250° C
**Carrier:** Nitrogen
**Carrier flow rate:** 20 ml/min
**Method of calibration:** External standard

**B. Methanol Extraction Method for the Two-Year Studies:** An aliquot of the corn oil dose mixture was extracted with methanol, containing triphenylmethane as an internal standard. The concentration of the test compound was determined by reference to a calibration curve prepared by analysis of a set of diallylphthalate working standards.

Twenty milliliters of the internal standard solution was added to 2.0 ml of diallylphthalate/corn oil solutions. Samples were mixed on a Vortex mixer for 30 seconds, sonicated for 30 seconds, and centrifuged for 3 minutes at 1,200 rpm.

Approximately 1 ml of the solvent layer was transferred via disposable pipettes into septa vials for the automatic sampler.

**Instrument:** Hewlett-Packard® 5840A
**Column:** 3% OV-17 on 80/100 mesh Supelcoport, 1.8 m × 7 mm ID, glass, silanized
**Detector:** Flame ionization
**Temperatures**
  **Column:** 200° C
  **Injector:** 250° C
  **Detector:** 270° C
**Carrier:** Nitrogen
**Carrier flow rate:** 30 ml/min
**Injector volume:** 3 µl

## II. Analytical Chemistry Laboratory

### A. Procedure

**1. Preparation of Standard Spiked Corn Oil:** Two working standard solutions of diallylphthalate in methanol were prepared independently. These solutions were diluted with methanol to bracket the concentration range of the dose mixtures. Twenty-milliliter aliquots of the six standard solutions were pipetted into individual 35-ml septum vials containing 2 g of undosed corn oil to make spiked corn oil standards bracketing the specified dose range of the referee sample. One 35-ml septum vial containing 2 g of undosed corn oil was treated with 20 ml of methanol for use as a blank. After the vials were sealed, the spiked corn oil samples and the corn oil blank were analyzed immediately according to the procedure described below.

**2. Preparation of the Referee Sample:** Three portions (approximately 2 g each) of the referee corn oil sample were transferred to individually tared 35-ml septum vials and were weighed to the nearest 0.001 g. Methanol (20 ml) was pipetted into each vial. Then the vials were sealed and analyzed immediately according to the procedure described below.

**3. Analysis:** Vials with samples, standards, and the blank were vigorously agitated for 15 seconds on a Vortex mixer, then shaken for 15 minutes at maximum stroke on a Burrell Model 75 Wrist-Action® shaker. After the samples were centrifuged for 3 minutes, a 5-ml aliquot of the methanol layer from each vial was combined with 5 ml of internal standard solution (triphenylmethane in methanol, 3 mg/ml) and the solutions were thoroughly mixed. The diallylphthalate content of the samples was then determined by the following gas chromatography system.

- **a. Instrument:** Varian 3700 with Autosampler and Varian CDS 111-C integrator
- **b. Column:** 3% OV-17 on 80/100 mesh Supelcoport, 1.8 m × 2 mm ID, glass, silanized
- **c. Detector:** Flame ionization
- **d. Detector temperature:** 280° C
- **e. Inlet temperature:** 230° C
- **f. Temperature program:** 200° C, isothermal
- **g. Carrier gas:** Nitrogen
- **h. Flow rate:** 30 ml/min
- **i. Volume of solution injected:** 3 µl
- **j. Retention times:**
  - (1) Diallylphthalate: 5.0 min
  - (2) Triphenylmethane internal standard: 13.1 min

The total amount of diallylphthalate in the referee corn oil samples was determined by using a linear regression equation calculated from the standard data and relating the ratio of the peak area of each spiked corn oil sample and the peak area of the internal standard to the amount of chemical in the respective spiked corn oil sample.

**B. Quality Assurance Measures:** The referee corn oil sample was analyzed in triplicate (Table I3), and the corn oil blank sample was analyzed once. Individually spiked portions of undosed corn oil (six levels) prepared from two independently weighed standards were used to obtain standard data. Triplicate injections of each standard and sample were made into the gas chromatograph in a randomized order.

# APPENDIX I

# ANALYSES OF DOSE MIXTURES: DATA

# APPENDIX I. ANALYSES: DATA

**I. Thirteen-Week Studies:** Table I1 contains results of analysis of dose mixtures used in the 13-week studies. The results ranged from 94.8% to 103.3% of the target concentration.

**II. Two-Year Studies:** To estimate the accuracy of the dose preparation during the studies, samples of the preparations were periodically analyzed. It is assumed that the number of remixes required reflects the number of mixes not within the specified ±10% of the target concentrations. It can be estimated that the 15 mg/ml and the 30 mg/ml mixes were out of specification 6% of the time.

Split sample analyses were performed by the testing and analytical (referee) laboratories to verify analytical procedures. The analyses by both laboratories were within ±10% of each other (Tables I2 and I3).

TABLE I1. ANALYSES OF CORN OIL MIXTURES IN THE THIRTEEN-WEEK GAVAGE STUDIES OF DIALLYLPHTHALATE

| | Concentrations of Diallylphthalate in Corn Oil (mg/ml) | | | | |
|---|---|---|---|---|---|
| **Target** | 7.5 | 15.0 | 30.0 | 60.0 | 120.0 |
| **Determined** | 7.3 | 14.3 | 30.9 | 62.0 | 113.8 |

TABLE I2. ANALYSES OF CORN OIL MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE (a)

| Date Mixed | Concentration of Diallylphthalate in Corn Oil for Target Concentrations | |
|---|---|---|
| | 15 mg/ml | 30 mg/ml |
| 3/4/80 | 14.6 | 29.6 |
| 4/1/80 | 16.0 | |
| 4/29/80 | 14.4 | 28.4 |
| | | 29.2 |
| 6/24/80 | 15.0 | 29.6 |
| | | 30.0 |
| 7/22/80 | 15.3 | |
| 8/19/80 | 15.0 | 29.4 |
| | | 29.3 |
| 10/14/80 | 15.0 | 29.9 |
| 12/10/80 | 15.6 | 30.7 |
| 2/3/81 | 14.3 | 29.7 |
| | 14.7 | 30.3 |
| 3/21/81 | 13.4 | (b) 15.8 |
| 4/3/81 | (c) 15.1 | (c) 29.7 |
| 5/26/81 | 14.7 | 31.5 |
| 7/21/81 | 14.8 | 29.0 |
| 9/15/81 | 14.2 | 28.4 |
| 11/10/81 | 14.7 | 29.2 |
| 1/5/82 | 14.9 | 29.2 |
| Mean (mg/ml) | 14.8 | (d) 28.8 |
| Standard deviation | 0.59 | 3.43 |
| Coefficient of variation (%) | 4.0 | 11.9 |
| Range (mg/ml) | 13.4-16.0 | 15.8-31.5 |
| Number of samples | 16 | 17 |

(a) The data presented are the results of duplicate analyses.
(b) Out of specifications. Not used in study, but included in the mean determination.
(c) Remix. Not included in mean determination.
(d) The statistical analyses of the data reflect only the initial mixes (not remixes). These should reflect the doses given to the animals throughout the 2-year studies.

TABLE I3. REFEREE SAMPLE DATA IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

| Date Mixed | Target Concentration (mg/ml) | Testing Laboratory | Analytical Chemistry Laboratory |
|---|---|---|---|
| 6/24/80 | 15.0 | 15.0 | 14.4 |
| 12/10/80 | 30.0 | 30.7 | 29.7 |
| 5/26/81 | 30.0 | 31.5 | 30.8 |
| 11/10/81 | 15.0 | 14.7 | 15.1 |
| 1/05/82 | 15.0 | 14.9 | 14.9 |

# APPENDIX J

# SENTINEL ANIMAL PROGRAM

# APPENDIX J. SENTINEL ANIMAL PROGRAM

## I. METHODS

Rodents used in the NTP Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect test 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 in the program is monitored via viral serology on sera from extra (sentinel) animals in the test rooms. These animals are untreated, and they and the test 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 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 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 viral antibody titers. The following tests are performed:

| Hemagglutination Inhibition | Complement Fixation |
|---|---|
| PVM (pneumonia virus of mice) | RCV (rat coronavirus) |
| Sendai | |
| KRV (Kilham rat virus) | |
| H-1 (Toolan's H-1 virus) | |

## II. RESULTS

TABLE J1. MURINE VIRUS ANTIBODY DETERMINATIONS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF DIALLYLPHTHALATE

| Interval (months) | Number | Positive Serologic Reaction for |
|---|---|---|
| 6 | 10/10 | RCV |
| 12 | 1/10 | RCV |
| 18 | 10/10 | RCV |
| 24 | 1/10 | RCV |

# APPENDIX K

# PHARMACOKINETIC STUDIES OF DIALLYLPHTHALATE IN RATS AND MICE

# APPENDIX K. PHARMACOKINETIC STUDIES

These studies were performed under NTP contract no. NO1-ES-8-2130 at the University of Arizona, Tucson. Principal Investigators: Dr. I. G. Sipes, Dr. D.E. Carter; Project Investigator: Dr. D.A. Eigenberg.

## I. Methodology

### A. Preliminary Study

Mildly anesthetized (ether) rats and mice were intubated with approximately 0.2 ml or 0.05 ml, respectively, of one of three doses of $^{14}$C-diallylphthalate in water:ethanol:emulphor (3:2:1). Rats received 30 µCi/kg and mice received 120 µCi/kg of $^{14}$C-diallylphthalate labelled in the side chain.

1. **Route of exposure:** Oral gavage

2. **Doses:** Low dose--1.0 mg/kg, mid dose--10 mg/kg, high dose--100 mg/kg

The oral $LD_{50}$ value for diallylphthalate in rats and mice is reported to be 892 and 1,050 mg/kg, respectively.

3. **Excreta studies**

Rats and mice were placed in metabolism cages and $^{14}CO_2$, volatile metabolites, urine, and feces were collected for 24 hours. Volatile metabolites were trapped in ethanol (−15° C) and $^{14}CO_2$ was trapped in Carbosorb:ethanol (2:1).

4. **Disposition studies**

Animals were killed at the end of 24 hours and total $^{14}$C in the tissues was determined.

### B. Intravenous Distribution/Excretion Study

Approximately 0.2 ml per rat or 0.05 ml per mouse of diallylphthalate in water:ethanol:emulphor (3:2:1) was injected intravenously.

1. **Dose:** 10 mg/kg (approximately 30 µCi/kg for rats and 120 µCi/kg for mice).

2. **Distribution study**

a. *Determination of diallylphthalate kinetics in blood*

Rats--Animals were anesthetized with pentobarbital (60 mg/kg) and the femoral artery was cannulated with PE50 tubing. Blood samples were collected at various times after intravenous administration of $^{14}$C-diallylphthalate. These samples were then immediately extracted four times with 1 ml of acetonitrile to quantitate diallylphthalate and monoallylphthalate. The blood extracts were analyzed by high-performance liquid chromatography (Section C2).

Mice-- Animals were killed 5, 10, 15, or 30 minutes after intravenous administration of diallylphthalate. Blood samples were immediately extracted four times with 1 ml of acetonitrile to quantitate diallylphthalate and monoallylphthalate. The blood extracts were analyzed by high-performance liquid chromatography (Section C2).

b. Animals were killed at the following post-exposure times: 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours. Three animals were used per time point.

c. All standard tissues, injection site, excreta, and intestinal contents were analyzed for $^{14}C$.

**3. Metabolism studies**

a. *Selection of tissues for analysis*

Only those tissues that contained greater than 1% of the dose at 30 minutes were chosen for parent and metabolite analysis. Tissues chosen for analysis were blood, liver, kidney, small intestine, skin, and muscle. Urine was also analyzed for parent and metabolites.

b. *Analysis of diallylphthalate and monoallylphthalate in tissues*

Tissue samples (0.5-1.0 g) were homogenized in 3-4 ml of acetonitrile and then extracted three more times with 2 ml of acetonitrile. To homogenize skin, homogenizing tubes were placed in a dry ice/methanol bath. After drying under a stream of nitrogen, the residues were dissolved in 1.0 ml of high-performance liquid chromatography solvent (35% acetonitrile:64% water:1% acetic acid), and chromatographed by high-performance liquid chromatography ($C_8$ column). To quantitate diallylphthalate, an acetonitrile:water (60:40) solvent system was used (retention time--4.5 minutes) and for monoallylphthalate, an acetonitrile:water:acetic acid (35:64:1) solvent system was used (retention time--5.5 min).

c. *Extraction of metabolites from urine*

Ethyl acetate extracts of urine (pH = 1.0) were chromatographed on thin-layer chromatography silica gel plates. Monoallylphthalate, allyl alcohol, 3-hydroxypropylmercapturic acid, and unidentified metabolites were found, but no parent compound was present. Monoallylphthalate and allyl alcohol were identified by gas chromatography-mass spectrometry. The following procedure was used to quantitate monoallylphthalate, allyl alcohol, and unidentified metabolites. Two-milliliter aliquots of urine were added to 1.0 ml of boric acid/sodium borate buffer (pH 9.0). Samples were extracted four times with 2 ml of ethyl acetate to extract allyl alcohol. Sixty microliters of 6N hydrochloric acid were added to urine samples, and the samples were extracted four times with 2 ml of toluene to extract monoallylphthalate. The urine was saturated with sodium chloride and extracted four times with 2 ml of acetonitrile. The organic extracts were counted in a liquid scintillation counter.

d. *Verification of selective extraction of metabolites from urine*

To determine that monoallylphthalate was not carried over during the ethyl acetate extraction of allyl alcohol, monoallylphthalate was added to 1 ml of blank urine (pH 9.0) and extracted three times with 2 ml of ethyl acetate. The organic solvent was counted in a liquid scintillation counter; only 0.4% of the $^{14}C$ was extracted, indicating minimal, if any, co-extraction of monoallylphthalate with allyl alcohol. Extraction of monoallylphthalate was determined by extracting aliquots of urine samples (pH 1.0) with toluene (post ethyl acetate extraction) and analyzing the extracts on silica gel thin-layer chromatography by an ethyl acetate:dichloromethane:acetic acid (30:69:1) solvent system ($R_f = 0.71$). The extraction efficiencies of $^{14}C$-allyl alcohol and $^{14}C$-monoallylphthalate were greater than 95% and 99%, respectively.

e. *Determination of the conjugates*

Urine samples were incubated with 480 units of β-glucuronidase (pH 6.8) or water for 3 hours at 35° C. After treatment, the samples were acidified by adding 20 µl of 6M hydrochloric acid and extracted four times with 2 ml of toluene. The percent of $^{14}C$ in the organic and aqueous phases of control and β-glucuronidase-treated samples were compared to determine if a glucuronide conjugate was present and to quantitate any glucuronide that was present.

## II. Distribution and Pharmacokinetics Following Intravenous Administration of 10 mg/kg Diallylphthalate

### A. Results

The following tissues contained greater than 1% of the dose of diallylphthalate 30 minutes after dosing: blood (rat--4.6%, mouse--2.1%); liver (rat--6.6%, mouse--3.8%); kidney (rat--2.6%, mouse--3.6%); small intestine (rat--8.1%, mouse--13.6%); muscle (rat--11.4%, mouse--7.6%) and skin (rat--5.1%, mouse--4.6%) (Table K1). Twenty-four hours after dosing, 6%-7% of the dose remained in tissues of rats and 1%-3% in mice. Table K2 summarizes the total $^{14}C$ (percent of administered dose) found in tissues over time.

### B. Pharmacokinetic Equations

1. Pharmacokinetic parameters were calculated using methods described by Levy and Gibaldi (1975). The following equations were used to determine rate constants and half-lives: $\alpha = -\text{slope} \times 2.303$; $t_{\frac{1}{2}}\alpha = 0.693/\alpha$; $\beta = -\text{slope} \times 2.303$; $t_{\frac{1}{2}}\beta = 0.693/\beta$.

2. Decay curves for $^{14}C$-diallylphthalate and $^{14}C$-monoallylphthalate were described by a monoexponential ($C = C_0e^{-kt}$) or biexponential ($C = Ae^{-\alpha t} + Be^{-\beta t}$) decay curve (Table K3).

3. Diallylphthalate was rapidly cleared from the blood of rats and mice; $t_{\frac{1}{2}}$ in blood was 2 minutes (Table K4). No diallylphthalate was found in blood, liver, kidney, muscle, skin, or small intestine 30 minutes after intravenous administration of diallylphthalate (Table K3).

4. Monoallylphthalate was rapidly cleared from all tissues in the body (Tables K1 and K3). The half-life of monoallylphthalate in blood was 32 minutes in the rat and 9.3 minutes in the mouse. Within 4 hours after dosing rats and 2 hours after dosing mice with diallylphthalate, no monoallylphthalate was detected in blood, liver, kidney, skin, muscle, or small intestine (Table K2).

5. The decay of total $^{14}C$ in blood is best described by a biexponential decay curve: $C = Ae^{-\alpha t} + Be^{-\beta t}$, with $t_{\frac{1}{2}}\alpha$ = 21 minutes and 22 minutes, $t_{\frac{1}{2}}\beta$ = 9.3 hours and 12.8 hours in rats and mice, respectively (Tables K5 and K6).

The area under the curve for total $^{14}C$ for the tissues containing the majority of the administered diallylphthalate (liver, blood, muscle, skin, and small intestine) is larger in the rat than mouse.

TABLE K1. SUMMARY OF TOTAL $^{14}C$ AND MONOALLYLPHTHALATE IN TISSUES OF RATS AND MICE AFTER INTRAVENOUS ADMINISTRATION OF 10 MG/KG DIALLYLPHTHALATE (a)

| Species | Tissue | Number of Hours after Exposure | | | |
|---|---|---|---|---|---|
| | | 0.5 | 2 | 8 | 24 |
| RAT | Blood | 4.6 ± 0.7 (1.7 ± 0.6) | 2.4 ± 0.1 (0.21 ± 0.04) | 0.72 ± 0.02 (b) | 0.4 ± 0.1 (b) |
| | Liver | 6.6 ± 0.6 (0.47 ± 0.09) | 3.7 ± 0.3 (0.07 ± 0.01) | 1.3 ± 0.1 (b) | 0.65 ± 0.03 (b) |
| | Kidney | 2.6 ± 0.6 (0.83 ± 0.35) | 0.88 ± 0.05 (0.09 ± 0.02) | 0.23 ± 0.00 (b) | 0.12 ± 0.00 (b) |
| | Small intestine | 8.1 ± 2.7 (0.91 ± 0.30) | 2.7 ± 0.2 (0.38 ± 0.07) | 0.79 ± 0.06 (b) | 0.39 ± 0.05 (b) |
| | Skin | 5.1 ± 0.7 (1.3 ± 0.3) | 3.8 ± 0.5 (0.09 ± 0.03) | 1.6 ± 0.3 (b) | 1.2 ± 0.2 (b) |
| | Muscle | 4.4 ± 0.6 (2.9 ± 0.7) | 11.3 ± 0.7 (0.32 ± 0.09) | 2.6 ± 0.21 (b) | 1.93 ± 0.05 (b) |
| MOUSE (c) | Blood | 2.1 ± 0.2 (0.45 ± 0.08) | 0.55 ± 0.02 (b) | 0.25 ± 0.03 (b) | 0.4 ± 0.00 (b) |
| | Liver | 3.8 ± 0.4 (0.20 ± 0.07) | 1.1 ± 0.1 (b) | 0.30 ± 0.02 (b) | 0.17 ± 0.02 (b) |
| | Kidney | 3.6 ± 0.5 (0.35 ± 0.07) | 0.49 ± 0.01 (0.03 ± 0.02) | 0.17 ± 0.02 (b) | 0.07 ± 0.01 (b) |
| | Small intestine | 15.22 ± 4.2 (0.16 ± 0.06) | 1.0 ± 0.1 (b) | 0.31 ± 0.03 (b) | 0.14 ± 0.01 (b) |
| | Skin | 4.6 ± 0.7 (1.7 ± 0.5) | 0.8 ± 0.09 (b) | 0.33 ± 0.01 (b) | 0.25 ± 0.11 (b) |
| | Muscle | 7.6 ± 0.8 (1.4 ± 0.3) | 1.7 ± 0.1 (b) | 0.64 ± 0.06 (b) | 0.55 ± 0.22 (b) |

(a) Data are presented as a percent of the administered dose, mean ± standard error of the mean (SEM) of three animals. Total $^{14}C$ activity is provided initially, with monoallylphthalate in parentheses. Diallylphthalate was not detected after 0.5 hours.
(b) Monoallylphthalate not detected
(c) All tissues of six mice were examined after 0.5 hours; blood of one mouse was examined after 24 hours.

TABLE K2. SUMMARY OF THE PERCENT OF $^{14}C$-EQUIVALENTS IN TISSUES AND EXCRETA VERSUS TIME IN RATS AND MICE AFTER INTRAVENOUS ADMINISTRATION OF 10 MG/KG DIALLYLPHTHALATE

| Tissue/Excreta | 30 Minutes | 1 Hour | 2 Hours | 4 Hours | 8 Hours | 12 Hours | 24 Hours |
|---|---|---|---|---|---|---|---|
| **RATS (a)** | | | | | | | |
| Blood | 4.6 ± 0.7 | 2.8 ± 0.1 | 2.4 ± 0.12 | 1.5 ± 0.03 | 0.72 ± 0.02 | 0.63 ± 0.07 | 0.4 ± 0.06 |
| Brain | 0.16 ± 0.01 | 0.25 ± 0.4 | 0.24 ± 0.01 | 0.19 ± 0.01 | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.04 ± 0.01 |
| Lung | 0.23 ± 0.03 | 0.20 ± 0.02 | 0.14 ± 0.01 | 0.10 ± 0.01 | 0.07 ± 0.00 | 0.06 ± 0.00 | 0.09 ± 0.00 |
| Liver | 6.6 ± 0.6 | 5.0 ± 0.1 | 3.7 ± 0.3 | 3.0 ± 0.2 | 1.3 ± 0.09 | 0.90 ± 0.01 | 0.65 ± 0.03 |
| Kidney | 2.6 ± 0.6 | 1.4 ± 0.1 | 0.88 ± 0.05 | 0.58 ± 0.07 | 0.23 ± 0.00 | 0.16 ± 0.01 | 0.12 ± 0.00 |
| Spleen | 0.09 ± 0.00 | 0.34 ± 0.02 | 0.09 ± 0.1 | 0.04 ± 0.1 | 0.04 ± 0.01 | 0.04 ± 0.00 | 0.02 ± 0.00 |
| Testes | 0.62 ± 0.06 | 0.47 ± 0.03 | 0.42 ± 0.02 | 0.31 ± 0.03 | 0.12 ± 0.00 | 0.10 ± 0.01 | 0.08 ± 0.01 |
| Small intestine | 8.1 ± 2.7 | 7.9 ± 1.4 | 2.7 ± 0.2 | 1.7 ± 0.53 | 0.79 ± 0.06 | 0.68 ± 0.02 | 0.39 ± 0.05 |
| Muscle | 11.4 ± 0.61 | 10.8 ± 0.6 | 11.3 ± 0.7 | 6.4 ± 0.33 | 2.6 ± 0.21 | 2.4 ± 0.4 | 1.93 ± 0.05 |
| Skin | 5.1 ± 0.70 | 5.3 ± 0.2 | 3.8 ± 0.5 | 3.4 ± 0.18 | 1.6 ± 0.30 | 1.8 ± 0.1 | 1.2 ± 0.23 |
| Fat | 0.60 ± 0.12 | 1.1 ± 0.1 | 0.53 ± 0.08 | 0.64 ± 0.15 | 0.72 ± 0.12 | 0.66 ± 0.13 | 0.67 ± 0.12 |
| Intestinal contents | 4.7 ± 0.6 | 5.7 ± 2.8 | 6.6 ± 2.6 | 1.03 ± 0.42 | 0.23 ± 0.04 | 0.12 ± 0.05 | 1.8 ± 0.15 |
| Urine | 9.9 ± 8.7 | 29.7 ± 5.8 | 37.1 ± 5.1 | 54.2 ± 3.5 | 54.1 ± 1.3 | 64.8 ± 9.0 | 70.0 ± 3.6 |
| **MICE (a)** | | | | | | | |
| Blood | (b) 2.1 ± 0.2 | (b) 1.1 ± 0.1 | 0.55 ± 0.02 | 0.33 ± 0.02 | 0.25 ± 0.03 | 0.21 ± 0.01 | 0.11 ± 0.01 |
| Brain | 0.12 ± 0.01 | 0.10 ± 0.02 | 0.07 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 |
| Lung | 0.21 ± 0.04 | 0.12 ± 0.01 | 0.11 ± 0.02 | 0.07 ± 0.01 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 |
| Liver | (b) 3.8 ± 0.4 | (b) 1.7 ± 0.1 | 1.1 ± 0.1 | 0.57 ± 0.02 | 0.30 ± 0.02 | 0.29 ± 0.04 | 0.17 ± 0.02 |
| Kidney | (b) 3.6 ± 0.5 | (b) 1.8 ± 0.2 | 0.49 ± 0.01 | 0.23 ± 0.02 | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.07 ± 0.01 |
| Spleen | 0.08 ± 0.01 | 0.09 ± 0.01 | 0.05 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 |
| Testes | 0.24 ± 0.12 | 0.10 ± 0.02 | 0.06 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| Small intestine | (b) 13.55 ± 4.64 | (b) 4.6 ± 1.1 | 1.0 ± 0.1 | 1.4 ± 0.6 | 0.31 ± 0.03 | 0.38 ± 0.15 | 0.14 ± 0.01 |
| Muscle | (b) 7.6 ± 0.8 | (c) 5.5 ± 0.8 | 1.7 ± 0.1 | 0.93 ± 0.09 | 0.64 ± 0.06 | 0.55 ± 0.02 | 0.55 ± 0.22 |
| Skin | (b) 4.60 ± 0.74 | (b) 3.6 ± 0.09 | 0.80 ± 0.09 | 0.75 ± 0.27 | 0.33 ± 0.01 | 0.48 ± 0.04 | 0.25 ± 0.11 |
| Fat | 0.61 ± 0.14 | 0.51 ± 0.16 | 0.22 ± 0.04 | 0.12 ± 0.00 | 0.12 ± 0.00 | 0.12 ± 0.01 | 0.28 ± 0.21 |
| Bile | 0.71 ± 0.2 | (d) 0.6 ± 0.12 | (d) 0.11 ± 0.02 | (d) 0.54 ± 0.09 | 0.15 ± 0.07 | 0.05 ± 0.02 | --- |
| Urine | 36.3 ± 10.3 | 62.1 ± 1.0 | 72.7 ± 4.1 | 78.7 ± 3.3 | 70.2 ± 13.5 | 83.4 ± 3.6 | 90.9 ± 1.1 |

(a) Tissue/excreta from three animals were examined except where noted.
(b) Tissue/excreta from six animals were examined.
(c) Tissue/excreta from five animals were examined.
(d) Tissue/excreta from two animals were examined.

TABLE K3. TOTAL $^{14}C$-DIALLYLPHTHALATE AND $^{14}C$-MONOALLYLPHTHALATE IN THE BLOOD OF MICE AND RATS AFTER INTRAVENOUS ADMINISTRATION OF 10 MG/KG DIALLYLPHTHALATE (a)

| Time (min) | Animal | Total $^{14}C$ | Diallylphthalate | Monoallylphthalate |
|---|---|---|---|---|
| 5 | Mouse (b) | 8.8 ± 0.8 | 0.17 ± 0.05 | 2.7 ± 0.6 |
| | Rat (c) | 16.1 ± 1.1 | 0.45 ± 0.16 | 7.6 ± 0.30 |
| 10 | Mouse | 4.9 ± 0.6 | 0.23 ± 0.04 | 2.9 ± 0.4 |
| | Rat | 13.8 ± 0.7 | 0.06 ± 0.03 | 6.7 ± 0.3 |
| 15 | Mouse | 5.3 ± 0.2 | 0.01 ± 0.01 | 2.2 ± 0.2 |
| | Rat | 13.8 ± 0.7 | 0.06 ± 0.03 | 7.4 ± 0.6 |
| 20 | Rat | 12.0 ± 1.0 | -- | 6.1 ± 0.6 |
| 30 | Mouse | 2.1 ± 0.2 | -- | 0.45 ± 0.08 |
| | Rat | 11.2 ± 1.4 | -- | 4.3 ± 0.8 |

(a) Values are calculated as a percent of the dose of $^{14}C$ administered.
(b) Three mice were used for each time point.
(c) Serial blood samples were taken from three rats anesthetized with pentobarbital (60 mg/kg).

TABLE K4. ELIMINATION CONSTANTS FOR DIALLYLPHTHALATE AND MONOALLYLPHTHALATE FROM BLOOD

| Species | Compound | A | Alpha ($min^{-1}$) | n (a) | r (b) | $t_{\frac{1}{2}}$ (min) |
|---|---|---|---|---|---|---|
| Rat (c) | Diallylphthalate | 3.4 | 0.403 | 2 | 1.00 | 1.7 |
| | Monoallylphthalate | 8.9 | 0.022 | 5 | 0.91 | 32 |
| Mouse (d) | Diallylphthalate | 1.2 | 0.283 | 3 | 0.82 | 2.4 |
| | Monoallylphthalate | 5.1 | 0.075 | 5 | 0.99 | 9.3 |

(a) The number of points used to determine the terminal phase of the decay curve
(b) Correlation coefficient for the terminal phase of the decay curve
(c) The values for these calculations are from serial blood samples taken from three rats that were anesthetized with pentobarbital (60 mg/kg).
(d) Three mice were used for each time point.

TABLE K5. ELIMINATION CONSTANTS FOR DIALLYLPHTHALATE (AS $^{14}C$-EQUIVALENTS) FROM TISSUES OF RATS

| Tissue | A | B | Alpha ($hour^{-1}$) | Beta ($hour^{-1}$) | n (a) | r (b) | $t_{\frac{1}{2}}$ (hour) alpha | $t_{\frac{1}{2}}$ (hour) beta |
|---|---|---|---|---|---|---|---|---|
| Blood | 7.3 | 1.9 | 1.98 | 0.0744 | 5 | 0.90 | 0.35 | 9.3 |
| Liver | 6.0 | 3.4 | 1.146 | 0.0786 | 5 | 0.91 | 0.37 | 8.8 |
| Kidney | 4.9 | 0.698 | 1.86 | 0.0858 | 5 | 0.88 | 0.37 | 8.1 |
| Muscle | 1.3 | 3.01 | 0.202 | 0.0185 | 3 | 1.00 | 3.4 | 37 |
| Skin | 4.1 | (c) | 0.593 | (c) | 6 | 0.88 | 11.7 | (c) |
| Small intestine | 12.2 | 1.7 | 0.912 | 0.0654 | 4 | 0.93 | 0.76 | 10.6 |
| Brain | 0.19 | 0.09 | 0.129 | 0.0347 | 3 | 0.92 | 5.4 | 20 |
| Testes | (d) | 0.39 | (d) | 0.0798 | 6 | 0.88 | (d) | 8.7 |

(a) The number of points used to determine the terminal phase of the decay curve
(b) Correlation coefficient for the terminal phase of the decay curve
(c) The data are best described by a monoexponential equation.
(d) Insufficient data to calculate the alpha elimination phase

TABLE K6. ELIMINATION CONSTANTS FOR DIALLYLPHTHALATE (AS $^{14}C$-EQUIVALENTS) FROM TISSUES OF MICE

| Tissue | A | B | Alpha ($hour^{-1}$) | Beta ($hour^{-1}$) | n (a) | r (b) | $t_{\frac{1}{2}}$ (hour) alpha | $t_{\frac{1}{2}}$ (hour) beta |
|---|---|---|---|---|---|---|---|---|
| Blood | 6.6 | 0.4 | 1.89 | 0.0539 | 4 | 1.00 | 0.37 | 12.8 |
| Liver | 4.6 | 0.57 | 1.08 | 0.0533 | 4 | 0.93 | 0.64 | 13 |
| Kidney | 8.4 | 0.29 | 1.79 | 0.0583 | 4 | 0.99 | 0.39 | 11.9 |
| Muscle | 15.5 | 0.84 | 1.40 | 0.0215 | 4 | 0.84 | 0.50 | 32 |
| Skin | 17.3 | 0.71 | 2.29 | 0.0442 | 4 | 0.71 | 0.30 | 15.7 |
| Small intestine | 56 | 1.2 | 2.77 | 0.096 | 5 | 0.90 | 0.25 | 7.2 |
| Brain | 0.11 | 0.04 | 0.66 | 0.0320 | 4 | 0.82 | 1.1 | 21.7 |
| Testes | 0.99 | 0.02 | 1.30 | 0.0334 | 3 | 0.69 | 0.53 | 21 |
| Lung | (c) | 0.096 | (c) | 0.0612 | 6 | 0.84 | (c) | 11.3 |

(a) The number of points used to determine the terminal phase of the decay curve
(b) Correlation coefficient for the terminal phase of the decay curve
(c) Insufficient data to calculate the alpha elimination phase

## III. Disposition of Diallylphthalate ($^{14}C$-Equivalents) 24 Hours After Oral or Intravenous Administration of Diallylphthalate to Rats and Mice

### Elimination of Diallylphthalate ($^{14}C$-Equivalents) in Urine and as Carbon Dioxide ($CO_2$)

**A.** Following oral (1, 10, or 100 mg/kg) or intravenous (10 mg/kg) administration of diallylphthalate to mice and rats, the major routes of elimination of $^{14}C$ equivalents are via the urine and as $^{14}CO_2$ (Tables K7 and K8). In rats approximately 60% of the dose is excreted in the urine and 30% is eliminated as $^{14}CO_2$; in mice, approximately 91% of the dose is excreted in the urine and 8% is eliminated as $^{14}CO_2$ in 24 hours. Six to seven percent and 1%-3% of the dose was found in tissues of rats and mice, respectively, 24 hours after oral or intravenous administration of diallylphthalate.

TABLE K7. DISPOSITION OF $^{14}C$-DIALLYLPHTHALATE IN RATS AND MICE TWENTY-FOUR HOURS AFTER EXPOSURE (a)

| Species | Route | Dose (mg/kg) | Total Recovery of $^{14}C$ (percent) | $^{14}C$ Eliminated as $CO_2$ (percent) | $^{14}C$ Excreted via Urine (percent) |
|---|---|---|---|---|---|
| Rat | Oral gavage | 1 | 87.2 ± 0.6 | 27.3 ± 0.0 | 50.7 ± 0.8 |
| | | 10 | 102.1 ± 1.3 | 29.6 ± 1.5 | 60.0 ± 5.3 |
| | | 100 | 96.1 ± 2.0 | 25.7 ± 2.0 | 58.6 ± 0.8 |
| | Intravenous injection | 10 | 104.5 ± 3.6 | 25.8 ± 1.2 | 70.0 ± 3.6 |
| Mouse | Oral gavage | 1 | 100.6 ± 1.8 | 7.2 ± 1.0 | 92.0 ± 2.9 |
| | | 10 | 101.4 ± 1.8 | 8.3 ± 0.3 | 91.5 ± 2.0 |
| | | 100 | 102.1 ± 3.6 | 12.1 ± 0.8 | 87.6 ± 3.5 |
| | Intravenous injection | 10 | 99.8 ± 1.2 | 6.3 ± 0.29 | 90.9 ± 1.1 |

(a) Values are means for three animals ± SEM, calculated as a percentage.

TABLE K8. CUMULATIVE EXCRETION OF $^{14}CO_2$ IN RATS AND MICE AFTER EXPOSURE TO $^{14}C$-DIALLYLPHTHALATE (a)

| Species | Route | Dose (mg/kg) | Hours after Exposure 1 | 3 | 6 | 12 | 24 |
|---|---|---|---|---|---|---|---|
| Rat | | | | | | | |
| | Oral gavage | 1 | 2.8 ± 0.8 | 9.4 ± 0.8 | 16.7 ± 0.7 | 25.0 ± 0.2 | 27.3 ± 0.0 |
| | | 10 | 4.0 ± 2.0 | 13.6 ± 3.3 | 20.9 ± 1.7 | 27.4 ± 1.1 | 29.6 ± 1.5 |
| | | 100 | 0.6 ± 0.2 | 4.0 ± 1.8 | 9.9 ± 2.3 | 19.3 ± 2.3 | 25.7 ± 2.0 |
| | Intravenous injection | 10 | 8.4 ± 0.4 | 17.9 ± 1.0 | 21.9 ± 1.3 | 24.4 ± 1.3 | 25.8 ± 1.2 |
| Mouse | | | | | | | |
| | Oral gavage | 1 | 0.6 ± 0.1 | 3.0 ± 0.4 | 5.5 ± 0.8 | 6.7 ± 1.0 | 7.2 ± 1.0 |
| | | 10 | 0.6 ± 0.1 | 3.5 ± 0.5 | 6.0 ± 0.3 | 7.2 ± 0.2 | 8.3 ± 0.3 |
| | | 100 | 0.5 ± 0.1 | 3.3 ± 0.1 | 7.5 ± 0.2 | 11.2 ± 0.9 | 12.1 ± 0.8 |
| | Intravenous injection | 10 | 1.2 ± 0.1 | 3.1 ± 0.4 | 4.7 ± 0.3 | 5.6 ± 0.2 | 6.3 ± 0.3 |

(a) Values are means for three animals ± SEM, calculated as a percentage.

B. The route of elimination of diallylphthalate as a percent of the dose administered is not significantly different following oral doses of 1 to 100 mg/kg to rats. In mice, there was a significant increase in the quantity of $^{14}CO_2$ excreted at the 100 mg/kg dose (Table K9).

C. Approximately 90% of diallylphthalate ($^{14}C$-equivalents) was cleared from the bodies of rats and essentially 100% from the bodies of mice in 24 hours (Table K9).

**TABLE K9. SUMMARY OF THE PERCENT OF $^{14}C$-EQUIVALENTS IN TISSUES AND EXCRETA FROM RATS AND MICE TWENTY-FOUR HOURS AFTER ORAL ADMINISTRATION OF DIALLYLPHTHALATE (a)**

| Tissue/Excreta | Percent of Administered Dose | | |
|---|---|---|---|
| | 1 mg/kg | 10 mg/kg | 100 mg/kg |
| **RATS** | | | |
| Blood | 0.62 ± 0.05 | 0.99 ± 0.62 | 0.72 ± 0.09 |
| Brain | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 |
| Lung | 0.06 ± 0.00 | 0.07 ± 0.01 | 0.06 ± 0.01 |
| Liver | 0.81 ± 0.05 | 0.93 ± 0.09 | 0.06 ± 0.10 |
| Kidney | 0.15 ± 0.00 | 0.17 ± 0.01 | 0.19 ± 0.02 |
| Spleen | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 |
| Testes | 0.08 ± 0.00 | 0.08 ± 0.01 | 0.09 ± 0.01 |
| Small intestine | 0.57 ± 0.04 | 0.61 ± 0.04 | 0.70 ± 0.15 |
| Muscle | 1.88 ± 0.05 | 2.3 ± 0.23 | 2.2 ± 0.2 |
| Skin | 0.91 ± 0.18 | 1.2 ± 0.3 | 1.0 ± 0.07 |
| Fat | 0.42 ± 0.08 | 0.90 ± 0.55 | 0.33 ± 0.04 |
| Feces | 3.07 ± 0.35 | 4.38 ± 2.29 | 5.2 ± 1.1 |
| Urine | 50.70 ± 0.8 | 60.0 ± 5.3 | 58.6 ± 0.8 |
| Carbon dioxide | 27.3 ± 0.0 | 29.6 ± 1.5 | 25.7 ± 2.0 |
| TOTAL | 87.2 ± 0.6 | 102.1 ± 1.3 | 96.1 ± 2.0 |
| **MICE** | | | |
| Blood | 0.09 ± 0.01 | 0.09 ± 0.06 | 0.19 ± 0.02 |
| Brain | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.04 ± 0.00 |
| Lung | 0.02 ± 0.00 | 0.04 ± 0.01 | 0.02 ± 0.00 |
| Liver | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.24 ± 0.02 |
| Kidney | 0.04 ± 0.01 | 0.14 ± 0.10 | 0.07 ± 0.00 |
| Spleen | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.00 |
| Testes | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.00 |
| Small intestine | 0.19 ± 0.04 | 0.21 ± 0.01 | 0.38 ± 0.03 |
| Muscle | 0.37 ± 0.10 | 0.43 ± 0.06 | 0.77 ± 0.06 |
| Skin | 0.15 ± 0.02 | 0.23 ± 0.03 | 0.31 ± 0.12 |
| Fat | 0.37 ± 0.14 | 0.26 ± 0.2 | 0.42 ± 0.09 |
| Feces | 0.48 ± 0.36 | 0.79 ± 0.49 | 0.40 ± 0.14 |
| Urine | 92.0 ± 2.9 | 91.5 ± 2.0 | 87.6 ± 3.5 |
| Carbon dioxide | 7.2 ± 1.0 | 8.3 ± 0.3 | 12.1 ± 0.8 |
| TOTAL | 100.6 ± 1.8 | 101.4 ± 1.8 | 102.1 ± 3.6 |

(a) Values are means for three animals ± SEM, calculated as a percentage of the administered dose.

D. Monoallylphthalate, allyl alcohol, 3-hydroxypropylmercapturic acid, and an unidentified polar metabolite were found in the urine of rats and mice (Table K10). There was no difference between the species in the quantity of allyl alcohol excreted, but mice excreted more monoallylphthalate, (39% vs 33%), 3-hydroxypropylmercapturic acid (28% vs 17%), and unidentified polar metabolites (20% vs 8%) than did rats.

TABLE K10. DIALLYLPHTHALATE METABOLITES IN RAT AND MOUSE URINE (a, b)

| Species | Route | Dose (mg/kg) | Allyl alcohol | Monoallyl-phthalate | 3-Hydroxypropyl-mercapturic acid | Nonacetonitrile extracted metabolite |
|---|---|---|---|---|---|---|
| Rat | Oral gavage | 1 | 2.3 ± 0.2 | 29.0 ± 0.7 | 13.2 ± 0.4 | 6.6 ± 0.4 |
| | | 10 | 3.0 ± 1.6 | 32.5 ± 1.7 | 18.4 ± 5.9 | 7.8 ± 1.6 |
| | | 100 | 3.1 ± 2.3 | 32.2 ± 0.6 | 16.5 ± 1.2 | 7.5 ± 0.7 |
| | Intravenous injection | 10 | 2.9 ± 2.0 | 38.0 ± 1.0 | 20.6 ± 1.1 | 8.3 ± 1.1 |
| Mouse | Oral gavage | 1 | 3.6 ± 1.1 | 39.2 ± 1.9 | 27.0 ± 1.0 | 19.1 ± 1.7 |
| | | 10 | 4.8 ± 2.3 | 37.7 ± 4.2 | 29.7 ± 0.2 | 19.0 ± 1.2 |
| | | 100 | 2.3 ± 0.6 | 44.7 ± 1.9 | 21.1 ± 1.9 | 19.4 ± 1.4 |
| | Intravenous injection | 10 | 7.5 ± 5.2 | 31.5 ± 5.8 | 34.4 ± 2.4 | 20.8 ± 1.5 |

(a) Values are calculated as a percent of the administered dose of $^{14}C$.
(b) Values are the mean of three animals ± SEM.

## IV. Summary

The major routes of elimination of diallylphthalate labelled in the allyl moiety are via the urine and as $^{14}CO_2$. In rats, approximately 60% of the dose (in $^{14}C$-equivalents) is excreted in the urine and approximately 30% as $CO_2$; in mice, approximately 91% of the dose is excreted in the urine and 8% as $^{14}CO_2$ in 24 hours.

The percentages of diallylphthalate eliminated were not significantly different following oral doses of 1-100 mg/kg to rats. In mice, there was a significant difference in the quantity of $^{14}CO_2$ excreted at the 100 mg/kg dose ($P < 0.005$; ANOVA).

Monoallylphthalate, allyl alcohol, 3-hydroxypropylmercapturic acid, and an unidentified polar metabolite were found in the urine of rats and mice. There was no difference in the quantity of allyl alcohol excreted, but mice excreted more monoallylphthalate, 3-hydroxypropylmercapturic acid, and unidentified polar metabolites than did rats.

The production of $^{14}CO_2$ from the total oxidative metabolism of allyl alcohol is directly related to the formation of acrolein and is an indication of the amount of acrolein converted to acrylic acid. Rats produced three times more $^{14}CO_2$ than did mice, indicating that rats either produced more acrolein or oxidized acrolein to acrylic acid to a greater extent than did mice.

Diallylphthalate is rapidly cleared from the bodies of rats and mice; $t_{\frac{1}{2}}$ in blood was 2 minutes. No diallylphthalate was found in blood, liver, kidney, muscle, skin, or small intestine 30 minutes after intravenous administration of diallylphthalate.

Monoallylphthalate is rapidly cleared from rats and mice; $t_{\frac{1}{2}}$ in blood was 32 minutes in the rat and 9.3 minutes in the mouse. Within 8 hours after rats and mice were given intravenous injections of diallylphthalate, no monoallylphthalate was detected in the blood, liver, kidney, skin, muscle, or small intestine.

The decay of total $^{14}CO_2$ in blood is best described by a biexponential decay curve: $C = Ae^{-\alpha t} + Be^{-\beta t}$; $t_{\frac{1}{2}}\alpha$ = 21 minutes and 22 minutes, $t_{\frac{1}{2}}\beta$ = 9.3 hours and 12.8 hours in rats and mice, respectively.

# APPENDIX L

# SELECTED SECTIONS [a] OF THE NATIONAL TOXICOLOGY PROGRAM'S TWO-YEAR TOXICOLOGY AND CARCINOGENESIS STUDIES OF DIALLYL PHTHALATE IN MICE [b]

(a) Abstract, III. Results, IV. Discussion

(b) A complete Technical Report is available: *Carcinogenesis Bioassay of Diallyl Phthalate (CAS No. 131-17-9) in B6C3F$_1$ Mice (Gavage Study)*, Technical Report Series No. 242, National Toxicology Program, P.O. Box 12233 Research Triangle Park, NC 27709.

# APPENDIX L. DIALLYLPHTHALATE STUDIES IN MICE

## ABSTRACT

A carcinogenesis bioassay of diallyl phthalate (99% pure) was conducted by administering 0 (vehicle control), 150, or 300 mg/kg diallyl phthalate in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 male and 50 female $B6C3F_1$ mice. Survival rates and mean body weights of dosed mice were not different from those of controls, and pathological lesions unrelated to proliferative changes were not observed. Therefore, a maximally tolerated dose for the purposes of carcinogenicity testing may not have been achieved.

The incidences of lymphoma and either lymphoma or leukemia in dosed male mice were not significantly greater than those in the controls according to pairwise comparisons ($P=0.051$ to $P=0.096$), but the trend tests were statistically significant by either life table or incidental tumor analyses ($P=0.031$ to $P=0.045$). The incidence of lymphomas in the high-dose male mice was 12/50 (24%) in comparison with 6/50 (12%) in the controls. Recent historical incidences at the performing laboratory and in the NTP Bioassay Program were 18/120 (15%) and 71/661 (11%), respectively. Since the incidence of high-dose male mice with leukemia was not significantly greater than that of concurrent or historical controls at the performing laboratory by pairwise comparisons, this marginal increase was considered only to be equivocally related to diallyl phthalate administration.

Increased incidences of squamous cell papillomas, hyperplasia, and inflammatory lesions of the forestomach were observed in diallyl phthalate-dosed mice of both sexes in a dose-related manner. Papillomas of the forestomach were observed in 0%, 2%, and 4% of the control, low-dose, and high-dose mice of both sexes. The recent historical incidence of this tumor in gavage control mice from both the performing laboratory and other laboratories within the Bioassay Program was less than 1%. Forestomach hyperplasia was diagnosed in 0%, 15%, and 18%, and in 8%, 2%, and 29% of the control, low-dose, and high-dose male and female mice, respectively; chronic inflammation of the forestomach was diagnosed in 0%, 9%, and 16% and in 4%, 2%, and 18% of the control, low-dose, and high-dose male and female mice, respectively. Because of the numerical elevation of forestomach papillomas in high-dose mice of both sexes, the concomitant observation of dose-related forestomach hyperplasia, and the rarity of this tumor in corn oil (gavage) control $B6C3F_1$ mice, the development of squamous cell papillomas of the forestomach may have been related to diallyl phthalate administration.

Under the conditions of this bioassay, the development of chronic inflammation and hyperplasia of the forestomach in both male and female $B6C3F_1$ mice was considered to be related to the administration of diallyl phthalate. The development of squamous cell papillomas of the forestomach may also have been related to chemical administration, but the available data are insufficient to indicate a clear cause and effect relationship. An increase in the incidence of male mice with lymphomas was observed, but this increase was considered only to be equivocally related to diallyl phthalate administration. The results of this bioassay, therefore, do not indicate that diallyl phthalate is carcinogenic in $B6C3F_1$ mice, although a maximal tolerated dose may not have been achieved.

## RESULTS: TWO-YEAR STUDIES

### Pathology and Statistical Analyses of Results

*Hematopoietic System:* Statistically significant positive trends ($P<0.05$, life table and incidental tumor tests) were observed in the incidence of male mice with lymphomas (overall rates of 12%, 10%, and 24% in control, low-, and high-dose groups) and in the combined incidence of male mice with lymphomas or leukemias (overall rates of 12%, 12%, and 24% in control, low-, and high-dose groups). No significant differences were observed, however, in pairwise comparisons between incidences in male control and dosed groups. The incidences of female mice with hematopoietic system tumors were not statistically significant (Table L2).

*Liver:* Statistically significant ($P<0.05$) positive trends were observed in the incidences of male mice with hepatocellular adenomas (overall rates of 0%, 0%, and 6% in control, low-, and high-dose groups). No significant differences, however, were observed in pairwise comparisons between control and dosed groups of male mice, nor was the incidence of hepatocellular adenomas or carcinomas increased sigificantly in male or female mice.

*Uterus:* Statistically significant ($P<0.05$) negative trends were observed in the incidences of female mice with endometrial stromal polyps (overall rates of 8%, 4%, and 0% in control, low-, and high-dose groups). No significant differences, however, were observed in pairwise comparisons between control and dosed groups of female mice.

*Forestomach:* The incidences of dosed male and female mice with papillomas, hyperplasia, or inflammatory lesions were increased relative to those for the controls (Table L3). The papillomas consisted of frond-like proliferations of squamous cells supported by a stalk of fibrovascular stroma. Hyperplasia consisted of more diffuse squamous cell proliferations, with no stalk formation and minimal protrusion into the gastric lumen. Hyperplasia often was associated with chronic inflammation of the underlying submucosa and surrounding mucosa. Chronic inflammation of the forestomach also was observed as a separate entity.

The incidences of both male and female mice with papillomas of the forestomach were 0%, 2%, and 4% for control, low-, and high-dose groups, respectively (Table L3). All papillomas were diagnosed at terminal kill, producing incidences of 0/38 (0%), 1/38 (3%), 2/32 (6%) and 0/38 (0%), 1/35 (3%), and 2/39 (5%) in control, low-, and high-dose male and female mice, respectively. Statistical comparisons with concurrent controls are performed only when overall incidences of 5% or more are observed, because of the insensitivity of the statistical tests for detecting effects at low incidences with the limited number of animals utilized in a chronic bioassay.

Of the six mice with forestomach papillomas, the one male mouse and the one female mouse from the low-dose group, and one of the two female mice from the high-dose group had neither forestomach hyperplasia nor chronic forestomach inflammation. One of the two male mice with forestomach papillomas from the high-dose group also had forestomach hyperplasia, while the other exhibited chronic forestomach inflammation. The remaining female mouse with forestomach papillomas in the high-dose group had both chronic inflammation and hyperplasia of the forestomach. A correlation between papillomas and inflammation or hyperplasia could not be proved because of the low incidence of forestomach papillomas. Hyperplasia occurred more frequently in mice with chronic forestomach inflammation than in those without inflammation in both the low- and high-dose male groups and in the control and high-dose female groups (Table L4).

TABLE [L1]. ANALYSES OF MALE MICE WITH PRIMARY TUMORS (a)

| | Vehicle Control | Low Dose | High Dose |
|---|---|---|---|
| **Hematopoietic System: Lymphoma** | | | |
| Tumor Rates | | | |
| Overall (b) | 6/50 (12%) | 5/50 (10%) | 12/50 (24%) |
| Adjusted (c) | 15.8% | 13.2% | 32.7% |
| Terminal (d) | 6/38 (16%) | 5/38 (13%) | 8/32 (25%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.031 | P=0.500N | P=0.051 |
| Incidental Tumor Test | P=0.037 | P=0.500N | P=0.058 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.063 | P=0.500N | P=0.096 |
| **Hematopoietic System: Lymphoma or Leukemia** | | | |
| Tumor Rates | | | |
| Overall (b) | 6/50 (12%) | 6/50 (12%) | 12/50 (24%) |
| Adjusted (c) | 15.8% | 15.4% | 32.7% |
| Terminal (d) | 6/38 (16%) | 5/38 (13%) | 8/32 (25%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.034 | P=0.620 | P=0.051 |
| Incidental Tumor Test | P=0.045 | P=0.608 | P=0.058 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.067 | P=0.620 | P=0.096 |
| **Liver: Adenoma** | | | |
| Tumor Rates | | | |
| Overall (b) | 0/50 (0%) | 0/49 (0%) | 3/50 (6%) |
| Adjusted (c) | 0.0% | 0.0% | 9.4% |
| Terminal (d) | 0/38 (0%) | 0/38 (0%) | 3/32 (9%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.026 | (f) | P=0.092 |
| Incidental Tumor Test | P=0.026 | (f) | P=0.092 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.038 | (f) | P=0.121 |
| **Liver: Carcinoma** | | | |
| Tumor Rates | | | |
| Overall (b) | 7/50 (14%) | 5/49 (10%) | 4/50 (8%) |
| Adjusted (c) | 15.5% | 12.7% | 12.5% |
| Terminal (d) | 2/38 (5%) | 4/38 (11%) | 4/32 (13%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.286N | P=0.405N | P=0.347N |
| Incidental Tumor Test | P=0.233N | P=0.507N | P=0.312N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.210N | P=0.394N | P=0.262 |
| **Liver: Adenoma or Carcinoma** | | | |
| Tumor Rates | | | |
| Overall (b) | 7/50 (14%) | 5/49 (10%) | 7/50 (14%) |
| Adjusted (c) | 15.5% | 12.7% | 21.9% |
| Terminal (d) | 2/38 (5%) | 4/38 (11%) | 7/32 (22%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.455 | P=0.405N | P=0.502 |
| Incidental Tumor Test | P=0.510 | P=0.507N | P=0.524 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.560 | P=0.394N | P=0.613 |

(a) Dosed groups received 150 or 300 mg/kg of diallyl phthalate by gavage.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the control incidence are the P-values associated witht the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the 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 non-fatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend or lower incidence is indicated by (N).
(f) Not significant; no tumors in low-dose or vehicle control group.

**TABLE [L2]. ANALYSES OF FEMALE MICE WITH PRIMARY TUMORS (a)**

| | Vehicle Control | Low Dose | High Dose |
|---|---|---|---|
| **Hematopoietic System: Lymphoma or Leukemia** | | | |
| Tumor Rates | | | |
| Overall (b) | 16/50 (32%) | 14/50 (28%) | 18/49 (37%) |
| Adjusted (c) | 36.8% | 34.7% | 42.3% |
| Terminal (d) | 11/38 (29%) | 9/35 (26%) | 15/39 (38%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.406 | P=0.536N | P=0.440 |
| Incidental Tumor Test | P=0.292 | P=0.543 | P=0.331 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.348 | P=0.414N | P=0.388 |
| **Liver: Adenoma or Carcinoma** | | | |
| Tumor Rates | | | |
| Overall (b) | 1/50 (2%) | 2/49 (4%) | 3/49 (6%) |
| Adjusted (c) | 2.3% | 5.1% | 7.7% |
| Terminal (d) | 0/38 (0%) | 1/35 (3%) | 3/39 (8%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.234 | P=0.467 | P=0.316 |
| Incidental Tumor Test | P=0.177 | P=0.731 | P=0.254 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.216 | P=0.492 | P=0.301 |

(a) Dosed groups received 150 or 300 mg/kg of diallyl phthalate by gavage.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the control incidence are the P-values associated witht the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the 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 non-fatal. The Cochran-Armitage and Fisher's exact tests compare directly the overall incidence rates. A negative trend or lower incidence is indicated by (N).

TABLE [L3]. INCIDENCES OF MICE WITH LESIONS OF THE FORESTOMACH (a)

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | Vehicle Control | Low Dose | High Dose | Vehicle Control | Low Dose | High Dose |
| No. of Mice Examined | 49 | 47 | 49 | 48 | 47 | 49 |
| Papilloma | 0 | 1 | 2 | 0 | 1 | 2 |
| Hyperplasia | 0 | 7(b) | 9(b) | 4 | 1 | 14(b) |
| Inflammation, Chronic | 0 | 4 | 8(b) | 2 | 1 | 9(c) |
| Inflammation, Suppurative | 1 | 0 | 1 | 0 | 0 | 2 |
| Ulcer | 0 | 2 | 1 | 1 | 1 | 0 |
| Erosion | 0 | 0 | 1 | 0 | 0 | 0 |

(a) The number of animals exhibiting the lesions is shown.
(b) Significantly greater than control (by Fischer's exact test), $P<0.01$.
(c) Significantly greater than control (by Fischer's exact test), $P<0.05$.

TABLE [L4]. COMPARATIVE INCIDENCES OF FORESTOMACH HYPERPLASIA AND CHRONIC INFLAMMATION OF THE FORESTOMACH IN MICE

| Sex | Chronic Inflammation | Incidence of Hyperplasia (%) | | |
|---|---|---|---|---|
| | | Vehicle Control | Low Dose | High Dose |
| Male | Present | 0/0 (0%) | 3/4 (75%)(a) | 5/8 (62%)(b) |
| | Absent | 0/49 (0%) | 4/43 (9%) | 4/41 (10%) |
| Female | Present | 2/2 (100%)(a) | 0/1 (0%) | 9/9 (100%)(c) |
| | Absent | 2/46 (4%) | 1/46 (2%) | 5/40 (12%) |

(a) Significantly greater incidence of hyperplasia in mice with chronic inflammation (present) than in mice without (absent), $P<0.01$.
(b) Same as a, $P<0.005$.
(c) Same as a, $P<0.001$.

## DISCUSSION AND CONCLUSIONS

The doses of diallyl phthalate used in the 2-year study, 150 and 300 mg/kg, were selected because deaths occurred in groups of mice receiving 400 mg/kg or 600 mg/kg diallyl phthalate for 14 days. A dose of 400 mg/kg for 13 weeks, however, produced neither chemically-induced deaths nor pathological lesions and did not cause a clear depression of body weight gain. Since body weight gain and survival in the 2-year study were not different in dosed and control mice and since pathological lesions other than chronic forestomach inflammation, hyperplasia, and papillomas were not observed, a maximally tolerated dose for purposes of carcinogenicity testing may not have been administered. Numerous deaths at 600 mg/kg in the 14-day study and a marked increase of chronic inflammation of the forestomach in the chronic study, however, indicate that 300 mg/kg, the highest dose in the 2-year study, was at least near a theoretical maximally tolerated dose.

The incidence of male mice with lymphomas and the incidence of male mice with either lymphomas or leukemia were statistically significant ($P<0.05$) in the trend tests, but not in pairwise comparisons between dosed and control groups. The combined incidence of control male mice with lymphomas or leukemia from this study is within the range for controls at this and other laboratories in the Program, indicating that the rates in control male mice were not abnormal. A pairwise comparison (using Fisher's exact test) between the incidences in the high-dose group of male mice from this study (12/50, 24%) and the historical rate at Litton Bionetics for control male mice receiving corn oil by gavage (19/120, 16%) is not statistically significant, although a comparison with the overall historical rate from the Bioassay Program (80/661) is statistically significant ($P=0.019$). Since the incidence of high-dose male mice with either leukemia or lymphoma was not significantly greater than that of concurrent or historical controls at the performing laboratory by pairwise comparisons, this marginal increase was considered only to be equivocally related to diallyl phthalate administration.

The incidence of male mice with hepatocellular adenomas was increased by diallylphthalate administration, but the data were considered to be of little or no toxicological significance because the incidence in control animals was abnormally low and because the incidences of animals with hepatocellular carcinomas or adenomas or carcinomas (combined) were not increased.

The incidences of both male and female mice with squamous-cell papillomas of the forestomach were greater than those for control $B6C3F_1$ mice in other studies conducted at Litton Bionetics or at other laboratories within the Bioassay Program. Because of the low overall tumor incidences (less than 5%), the sample sizes used in this study may have been too small to permit detection of a statistically significant increase in the incidence of a rare tumor type (such as papillomas of the forestomach). Because the historical incidence of this tumor type in control (corn oil-gavaged) mice is generally less than 1%, a pairwise comparison (by Fisher's exact test, one-tailed) was made between the incidences in the high-dose male (2/49) and female (2/49) groups in this study and the historical (corn oil-gavaged) control incidences for male and female mice in the Bioassay Program. Statistically significant values were observed ($P=0.043$, males; $P=0.041$, females). Therefore, the data in this study are insufficient to indicate that diallyl phthalate caused forestomach papillomas, but results of a comparison of the high incidences of forestomach papillomas in high-dose mice with both concurrent and historical control rates suggest a cause and effect relationship.

The correlation between the occurrence of chronic forestomach inflammation and forestomach hyperplasia (Table L4) suggests that an irritating effect of diallyl phthalate on the gastric epithelium may have predisposed this tissue to the hyperplasia. The incidence of squamous cell papillomas of the forestomach, however, was too low for valid comparisons to be made between their occurrence and the presence of chronic inflammation or hyperplasia. The data are insufficient to indicate whether or not the forestomach papillomas were secondary to the irritation, but the correlation between animals with chemically-induced inflammation and those with hyperplasia supports such a speculation.

*Conclusions: Under the conditions of this bioassay, the development of chronic inflammation and hyperplasia of the forestomach in both male and female B6C3F$_1$ mice was considered to be related to the administration of diallyl phthalate. The development of squamous cell papillomas of the forestomach may also have been related to chemical administration, but the available data are insufficient to indicate a clear cause and effect relationship. An increase in the incidence of male mice with lymphomas was observed, but this increase was considered only to be equivocally related to diallyl phthalate administration. The results of this bioassay, therefore, do not indicate that diallyl phthalate is carcinogenic in B6C3F$_1$ mice, although a maximally tolerated dose may not have been achieved.*

# APPENDIX M

# DATA AUDIT SUMMARY

# APPENDIX M. DATA AUDIT SUMMARY

The experimental data and draft tables of the NTP Technical Report on the toxicology and carcinogenesis studies of diallylphthalate in F344/N rats were examined on December 5-9, 1983, for Good Laboratory Practice compliance and scientific procedures by the following persons: National Toxicology Program--Ms. C. Davies, Ms. A. Grant, Dr. W. Kluwe, Dr. C. Lingeman, Dr. B. Schwetz, Dr. C. Whitmire, and Dr. M. Wolfe; Argus Research Laboratories, Inc. --Mr. P. Ference, Dr. J. Goeke, Dr. A. Hoberman, Ms. C. Sunier, and Dr. D. Willigan. These 2-year studies in rats were conducted between February 1980 and February 1982.

The full report of the audit of studies on diallylphthalate is on file at the National Toxicology Program, NIEHS. The audit revealed no significant problems with the execution of the study or with the collection or reporting of the experimental data. Ten animals from the diallylphthalate-dosed groups died during the first 2 weeks of the studies from gavage-related trauma. This problem did not persist throughout the rest of the studies. Neither these deaths during the first 2 weeks of the studies nor trauma related to the gavage process were considered to influence the final interpretation of the studies. Other minor problems not mentioned here were likewise considered not to affect the outcome of the studies. In conclusion, no data discrepancies were found that influenced the final interpretation of this experiment.

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