NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate (CASRN 117-81-7) Administered in Feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats

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NOTICE

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the NTP Technical Report series are designed and conducted to characterize and evaluate the toxicological potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are derived solely from the results of these NTP studies, and extrapolation of the results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and the Food and Drug Administration Good Laboratory Practice Regulations and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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Explanation of Levels of Evidence of Carcinogenic Activity

The National Toxicology Program (NTP) describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, in as much as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

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

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

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been

adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

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

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

Peer Review

The National Toxicology Program (NTP) convened a virtual external ad hoc panel to peer review the draft *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Di(2-ethylhexyl) Phthalate (CASRN 117-81-7) Administered in Feed to Sprague Dawley Hsd:Sprague Dawley® SD®) Rats* on April 2, 2021. NTP announced the peer-review meeting in the Federal Register (X FR. XXXX. DATE). The public could view the proceedings online and opportunities were provided for submission of written and oral public comments. The selection of panel members and conduct of the peer review were in accordance with federal policies and regulations. The panel was charged to:

(1) Review and evaluate the scientific and technical elements of each study and its presentation.

(2) Determine whether each study's experimental design, conduct, and findings support the NTP's conclusions regarding the conditions of each study.

NTP carefully considered the panel's recommendations in finalizing the report. The peer-review report is provided in Appendix G. Other meeting materials are available on the NTP website (https://ntp.niehs.nih.gov/go/meeting).

Peer Reviewers

[to come]

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Abstract

Di(2-ethylhexyl) phthalate (DEHP) is a member of the phthalate ester chemical class that occurs commonly in the environment and to which humans are widely exposed. Lifetime exposure to DEHP is likely to occur, including during the in utero and early postnatal windows of development. To date, no carcinogenicity assessments of DEHP have used a lifetime exposure paradigm that includes the perinatal period (gestation and lactation). The National Toxicology Program (NTP) tested the hypothesis that exposure during the perinatal period would alter the DEHP carcinogenic response quantitatively (more neoplasms) or qualitatively (different neoplasm types).

Two chronic carcinogenicity assessments of DEHP were conducted in which Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were exposed to dosed feed containing 0, 300, 1,000, 3,000, or 10,000 ppm DEHP for 2 years using different exposure paradigms. In Study 1, groups of 45 F_0 time-mated females were provided dosed feed beginning on gestation day (GD) 6 through lactation. On postnatal day (PND) 21, groups of 50 F_1 rats per sex continued on the study and were provided dosed feed containing the same DEHP concentration as their respective dam for 2 years. In Study 2, groups of 50 rats per sex, aged 5 to 6 weeks at study start, were provided dosed feed containing DEHP for 2 years.

Perinatal and Postweaning Study in Rats (Study 1)

During the perinatal period, lower maternal mean body weight, maternal mean body weight gain, and feed consumption were observed in F_0 dams exposed to 10,000 ppm DEHP relative to control animals. Also in that exposure group, litter size and pup weights on PND 1 were significantly decreased compared to the control group. Male and female pup mean body weight gains were significantly decreased in the 10,000 ppm group during lactation and resulted in significantly decreased pup body weights at weaning when compared to the control group. Pup survival was not affected following gestational and lactational DEHP exposure.

Following perinatal and 2 years of postweaning DEHP exposure, survival of exposed male and female rats to study termination was similar to that of control groups; however, there were decreases in mean body weight in the 10,000 ppm group compared to the control group.

Significant increases in the incidences of hepatocellular adenoma, hepatocellular adenoma or carcinoma (combined), pancreatic acinar adenoma, and pancreatic acinar adenoma or carcinoma (combined) were observed in the 3,000 and 10,000 ppm male rats relative to the control group. Higher incidences of hepatocellular carcinomas (10,000 ppm males) and pancreatic acinar carcinomas (3,000 ppm males) were also observed. In female rats, significant increases in the incidences of liver neoplasms occurred in the 3,000 ppm (hepatocellular adenoma and hepatocellular adenoma or carcinoma [combined]) and 10,000 ppm (hepatocellular carcinoma and hepatocellular adenoma or carcinoma [combined]) groups. Occurrences of pancreatic acinar adenomas were observed in the 3,000 and 10,000 ppm female groups, and a trend of higher incidence of uterine adenocarcinomas with increasing exposure was observed given the incidence in the 10,000 ppm group. Nonneoplastic lesions were observed in the liver (male and female), testis, epididymis, uterus, kidney (male and female), heart (male only), bone marrow (male only), and pituitary gland (male only).

Postweaning-only Study in Rats (Study 2)

Following 2 years of postweaning DEHP exposure, survival of male and female rats was commensurate with or greater than that of control animals, and lower body weights were observed in the 10,000 ppm group. Significant increases in the incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) were observed in male and female rats exposed to 10,000 ppm DEHP relative to the respective control group. In male rats, significantly increased incidences of pancreatic acinar neoplasms were observed in the 3,000 (adenoma) and 10,000 ppm (adenoma and carcinomas) groups. A trend of increasing incidence of testicular interstitial cell adenoma with increasing exposure was observed in male rats given the incidence observed in the 10,000 ppm DEHP group. In female rats, significantly increased incidences of uterine adenocarcinoma and uterine adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) were observed in the 10,000 ppm group compared to the control group. Occurrences of uterine squamous cell papilloma (including multiple) were observed in the 10,000 ppm group. Nonneoplastic lesions were observed in the liver (male and female), pancreas (male and female), testis, epididymis, uterus, heart (male only), bone marrow (male and female), and pituitary gland (male only).

Comparative Carcinogenic Benchmark Dose Analyses

Benchmark dose (BMD) levels corresponding to a 10% increased risk of carcinogenic response (BMD_{10}) were estimated for exposure-related carcinogenic responses that were observed in both studies. Generally, the BMDs between studies were within threefold of each other. The lowest estimated BMD_{10} (30.99 mg DEHP/kg body weight/day) corresponded to pancreatic acinar adenoma or carcinoma (combined) in males in the postweaning-only study (Study 2).

Genetic Toxicology

DEHP was tested in a variety of genotoxicity assays in vitro and in vivo; most results were negative. In vitro, negative results were obtained in the following assays: six independent bacterial mutation assays in *Salmonella typhimurium* bacterial strains (TA100, TA1535, TA1537, TA97, and TA98) with and without exogenous metabolic activation systems (S9 mix; induced hamster, rat, and mouse liver S9), a single mouse lymphoma gene mutation assay (with and without induced rat liver S9 mix), and three independent chromosomal aberration assays conducted in Chinese hamster ovary (CHO) cells (with and without rat liver S9). In nine in vitro sister chromatid exchange tests conducted in CHO cells with and without S9, DEHP produced positive responses in four tests, equivocal results in three, and negative results in two.

In vivo, no increases in chromosomal aberrations were observed in bone marrow cells of female B6C3F1 mice following exposure to DEHP in dosed feed for 14 days. DEHP produced mixed results in three independent erythrocyte micronucleus assays: equivocal in female B6C3F1 mice exposed to DEHP in dosed feed for 14 days, equivocal in male TgAC (FVB/N) mice and positive in female TgAC (FVB/N) mice following exposure via dosed feed for 26 weeks, and negative in male and female TgAC (FVB/N) mice following a 26-week dermal exposure. DEHP produced negative results in two independent studies that tested for induction of sex-linked recessive lethal mutations in *Drosophila melanogaster*.

Conclusions

Under the conditions of the perinatal and postweaning feed study (Study 1), there was *clear evidence of carcinogenic activity* of di(2-ethylhexyl) phthalate (DEHP) in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma

(combined) and acinar adenoma or carcinoma (combined) neoplasms (predominately adenomas) of the pancreas. There was *clear evidence of carcinogenic activity* of DEHP in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular adenoma or carcinoma (combined). The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure.

Under the conditions of the postweaning-only feed study (Study 2), there was *clear evidence of carcinogenic activity* of DEHP in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominately adenomas) of the pancreas. The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. There was *clear evidence of carcinogenic activity* of DEHP in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined). The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure.

The BMD analysis shows there was no consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other. However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

In both studies, exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver (male and female), heart (male), pituitary gland (male), testis, and epididymis. In the postweaning-only study (Study 2), DEHP exposure increased nonneoplastic lesions in the pancreas (male and female), bone marrow (male and female), and uterus. Perinatal and postweaning exposure (Study 1) increased gross lesions in the reproductive tract (male), bone marrow (male), and kidney (male and female).

Synonyms: Bis(2-ethylhexyl)phthalate; dioctyl phthalate; phthalic acid di(2-ethylhexyl) ester; bis(2-ethylhexyl) 1,2-benzenedicarboxylate; 1,2-benzenedicarboxylic acid bis(2-ethylhexyl) ester

Trade names: Platinol DOP; Octoil; Silicol 150; Bisoflex 81; Eviplast 80

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of Di(2-ethylhexyl) Phthalate

	Perinatal and Postweaning Study (Study 1)		Postweaning-only Study (Study 2)	
	Male Sprague Dawley Rats	**Female Sprague Dawley Rats**	**Male Sprague Dawley Rats**	**Female Sprague Dawley Rats**
Concentrations in Feed	0, 300, 1,000, 3,000, or 10,000 ppm	0, 300, 1,000, 3,000, or 10,000 ppm	0, 300, 1,000, 3,000, or 10,000 ppm	0, 300, 1,000, 3,000, or 10,000 ppm
Survival Rates	25/50, 33/49, 40/50, 35/50, 29/50	31/50, 32/50, 34/50, 34/50, 27/50	32/50, 35/50, 39/50, 35/50, 42/50	33/50, 34/50, 33/50, 34/50, 32/50
Body Weights	10,000 ppm group 29.7% less than the control group	3,000 ppm group 9.9% less than the control group; 10,000 ppm group 31.7% less than the control group	10,000 ppm group 15.6% less than the control group	10,000 ppm group 21.9% less than the control group
Gross Lesions	Testis: small (2/49, 2/49, 4/50, 2/50, 45/49); enlarged (or swelling) (0/49, 0/49, 0/50, 0/50, 1/49); fluid or blood filled (0/49, 1/49, 1/50, 1/50, 1/49); right or left, abdominal, undescended (1/49, 0/49, 0/50, 0/50, 19/49); right or left, inguinal, undescended (0/49, 1/49, 1/50, 0/50, 4/49); right or left, abdominal or inguinal, undescended (1/49, 1/49, 1/50, 0/50, 23/49); right, not present (0/49, 0/49, 0/49, 0/50, 1/49); cranial suspensory ligament (0/49, 0/49, 0/50, 0/50, 5/49) Epididymis: small (0/49, 0/49, 2/50, 0/50, 14/49); right, cauda, agenesis (0/49, 0/49, 0/49, 0/50, 2/49); right or left, caput, agenesis	Vagina: not patent (0/50, 0/50, 0/50, 0/50, 5/48) Phallus: cleft (0/50, 0/50, 0/50, 2/50, 1/48)	None	None

	Perinatal and Postweaning Study (Study 1)		Postweaning-only Study (Study 2)	
	Male Sprague Dawley Rats	**Female Sprague Dawley Rats**	**Male Sprague Dawley Rats**	**Female Sprague Dawley Rats**
	(0/49, 0/49, 0/50, 0/50, 4/49); right or left, cauda, agenesis (0/49, 0/49, 0/50, 0/50, 2/49); right or left, corpus, agenesis (0/49, 0/49, 0/50, 0/50, 3/49) Levator ani/bulbocavernosus muscle: small (0/50, 0/49, 0/50, 0/50, 2/48) Cowper's glands: left, small (0/50, 0/49, 0/50, 0/50, 1/47); right, small (0/50, 0/49, 0/50, 0/50, 1/47) Prostate glands: small (0/50, 0/49, 0/50, 0/50, 1/47) Seminal vesicles/ coagulating glands: small (1/50, 0/49, 1/50, 1/50, 8/47) Phallus: small (0/50, 0/49, 0/49, 0/50, 3/49); cleft (0/50, 0/49, 0/49, 0/50, 3/49) Prepuce: cleft (0/50, 0/49, 0/50, 0/50, 1/49); incomplete preputial separation (0/50, 0/49, 0/50, 0/50, 7/49) Gubernaculum: right or left, not present (0/47, 0/49, 0/49, 0/50, 18/41); ↑ right length			

	Perinatal and Postweaning Study (Study 1)		Postweaning-only Study (Study 2)	
	Male Sprague Dawley Rats	**Female Sprague Dawley Rats**	**Male Sprague Dawley Rats**	**Female Sprague Dawley Rats**
Nonneoplastic Effects	Liver: hepatocyte, cytoplasmic alteration (0/50, 0/49, 1/50, 28/50, 37/49); hepatocyte, hypertrophy (0/50, 0/49, 0/50, 3/50, 17/49); pigment (0/50, 1/49, 5/50, 40/50, 38/49); necrosis (3/50, 4/49, 1/50, 6/50, 13/49); eosinophilic focus (4/50, 1/49, 7/50, 2/50, 11/49); basophilic focus (1/50, 1/49, 4/50, 4/50, 17/49) Testis: germinal epithelium, degeneration (includes bilateral) (16/49, 25/49, 21/50, 21/50, 44/49); interstitial cell, hyperplasia, focal (includes bilateral) (4/49, 3/49, 6/50, 5/50, 30/49); seminiferous tubule, dysgenesis (includes bilateral) (0/49, 0/49, 0/50, 0/50, 10/49) Epididymis: hypospermia (includes bilateral) (4/49, 5/49, 12/50, 8/50, 43/49) Kidney: papilla, edema (0/50, 0/49, 0/50, 0/50, 39/49); papilla, hemorrhage (0/50, 1/49, 0/50, 2/50, 12/49); epithelium, papilla, hyperplasia (9/50,	Liver: hepatocyte, cytoplasmic alteration (0/49, 4/50, 7/50, 39/50, 39/48); hepatocyte, hypertrophy (0/49, 2/50, 5/50, 9/50, 34/48); pigment (0/49, 6/50, 14/50, 36/50, 40/48); necrosis (3/49, 9/50, 3/50, 7/50, 8/48); eosinophilic focus (3/49, 4/50, 4/50, 7/50, 12/48); basophilic focus (4/49, 5/50, 3/50, 2/50, 10/48); bile duct hyperplasia (9/49, 13/50, 13/50, 21/50, 8/48) Uterus: inflammation, acute (0/50, 0/50, 6/50, 2/50, 0/48) Kidney: papilla, edema (0/50, 0/50, 2/50, 0/50, 38/49); epithelium, papilla, hyperplasia (2/50, 1/50, 2/50, 4/50, 15/49); infarct (0/50, 3/50, 7/50, 5/50, 12/49); renal tubule, cyst (0/50, 0/50, 2/50, 0/50, 7/49); renal tubule, dilation (0/50, 0/50, 0/50, 0/50, 3/49)	Liver: hepatocyte, cytoplasmic alteration (0/50, 1/50, 0/50, 38/50, 49/50); hepatocyte, hypertrophy (0/50, 0/50, 0/50, 2/50, 6/50); pigment (0/50, 0/50, 7/50, 45/50, 50/50); necrosis (0/50, 2/50, 4/50, 7/50, 8/50); eosinophilic focus (1/50, 0/50, 4/50, 2/50, 24/50); clear cell focus (29/50, 31/50, 33/50, 35/50, 39/50) Pancreas: acinus, hyperplasia (7/49, 8/50, 9/50, 24/50, 26/50) Testis: germinal epithelium, degeneration (includes bilateral) (31/50, 25/50, 21/50, 22/50, 50/50); edema (includes bilateral) (27/50, 23/50, 29/50, 24/50, 45/50); interstitial cell, hyperplasia, focal (includes bilateral) (1/50, 1/50, 0/50, 4/50, 4/50) Epididymis: hypospermia (includes bilateral) (4/50, 4/50, 4/50, 3/50, 43/50); duct, exfoliated germ cell (includes bilateral) (2/50, 3/50, 4/50, 4/50, 36/50)	Liver: hepatocyte, cytoplasmic alteration (0/50, 2/50, 15/50, 38/50, 45/49); hepatocyte, hypertrophy (0/50, 0/50, 6/50, 14/50, 28/49); pigment (3/50, 0/50, 18/50, 30/50, 48/49) Pancreas: acinus, hyperplasia (0/50, 1/50, 1/50, 1/50, 5/47) Uterus: inflammation, chronic (2/50, 9/50, 6/50, 8/50, 8/49) Bone marrow: hypercellularity (43/50, 39/50, 43/50, 43/50, 47/50)

	Perinatal and Postweaning Study (Study 1)		Postweaning-only Study (Study 2)	
	Male Sprague Dawley Rats	**Female Sprague Dawley Rats**	**Male Sprague Dawley Rats**	**Female Sprague Dawley Rats**
	4/49, 4/50, 3/50, 17/49); infarct (2/50, 10/49, 9/50, 7/50, 17/49) Heart: valve, fibrosis (0/50, 2/49, 1/50, 3/50, 11/49); valve, thrombus (0/50, 0/49, 0/50, 0/50, 6/49) Bone marrow: hypercellularity (21/50, 17/49, 29/50, 34/50, 36/50) Pituitary gland: pars distalis, hypertrophy (3/50, 7/49, 5/50, 15/50, 37/49)		Heart: valve, fibrosis (2/50, 0/50, 0/50, 1/50, 9/50); valve, thrombus (0/50, 0/50, 0/50, 2/50, 6/50) Bone marrow: hypercellularity (18/50, 22/50, 30/50, 25/50, 34/50) Pituitary gland: pars distalis, hypertrophy (8/50, 10/50, 11/50, 14/50, 37/50)	
Neoplastic Effects	Liver: hepatocellular adenoma (0/50, 1/49, 0/50, 3/50, 8/49); hepatocellular carcinoma (1/50, 0/49, 0/50, 0/50, 3/49); hepatocellular adenoma or carcinoma (combined) (1/50, 1/49, 0/50, 3/50, 11/49) Pancreas: acinar adenoma (10/50, 7/49, 8/50, 36/50, 22/49); acinar carcinoma (0/50, 0/49, 0/50, 3/50, 1/49); acinar adenoma or carcinoma (combined) (10/50, 7/49, 8/50, 38/50, 22/49)	Liver: hepatocellular adenoma (1/49, 0/50, 5/50, 9/50, 5/48); hepatocellular carcinoma (0/49, 0/50, 0/50, 0/50, 8/48); hepatocellular adenoma or carcinoma (combined) (1/49, 0/50, 5/50, 9/50, 13/48) Pancreas: acinar adenoma or carcinoma (combined) (0/49, 0/50, 0/50, 2/50, 1/48)	Liver: hepatocellular adenoma (0/50, 2/50, 0/50, 1/50, 6/50); hepatocellular carcinoma (0/50, 0/50, 0/50, 0/50, 6/50); hepatocellular adenoma or carcinoma (combined) (0/50, 2/50, 0/50, 1/50, 12/50) Pancreas: acinar adenoma (1/49, 4/50, 5/50, 23/50, 30/50); acinar carcinoma (0/49, 1/50, 0/50, 1/50, 5/50); acinar adenoma or carcinoma (combined) (1/49, 5/50, 5/50, 23/50, 33/50)	Liver: hepatocellular adenoma (0/50, 0/50, 1/50, 1/50, 13/49); hepatocellular carcinoma (0/50, 0/50, 0/50, 0/50, 2/49); hepatocellular adenoma or carcinoma (combined) (0/50, 0/50, 1/50, 1/50, 14/49) Pancreas: acinar adenoma or carcinoma (combined) (0/50, 0/50, 0/50, 1/50, 2/47) Uterus: adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) (2/50,

	Perinatal and Postweaning Study (Study 1)		Postweaning-only Study (Study 2)	
	Male Sprague Dawley Rats	**Female Sprague Dawley Rats**	**Male Sprague Dawley Rats**	**Female Sprague Dawley Rats**
				4/50, 1/50, 6/50, 13/50)
Equivocal Findings	None	Uterus: adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) (3/50, 1/50, 1/50, 3/50, 7/50)	Testis: interstitial cell, adenoma (7/50, 3/50, 3/50, 6/50, 15/50)	None
Level of Evidence of Carcinogenic Activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

Genetic Toxicology

Bacterial gene mutations: Negative in *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA97, and TA98, with and without S9

Mouse lymphoma L5178Y $tk^{+/-}$ cells: Negative with and without S9

In vitro CHO cell chromosomal aberration test: Negative with and without S9

In vitro CHO cell sister chromatid exchange test:

Without rat liver S9: Positive or equivocal in 7 out of 9 studies

With rat liver S9: Negative in 9 out of 9 studies

In vivo chromosome aberration test: Negative in female B6C3F1 mice exposed via dosed feed for 14 days

In vivo micronucleus test in mice:

B6C3F1 mice: Equivocal in females exposed via dosed feed for 14 days

TgAC (FVB/N) mice: Equivocal in males and positive in females exposed via dosed feed for 26 weeks

TgAC (FVB/N) mice: Negative in males and females exposed dermally for 26 weeks

Drosophila melanogaster sex-linked recessive lethal test:

Adult injection: Negative

Larval feeding: Negative

Overview

Phthalates are plasticizers used to provide flexibility in products composed of polyvinyl chloride plastic or vinyl chloride resins. Studies have shown that in utero and early life phthalate exposure can result in adverse reproductive, developmental, and potentially carcinogenic effects. The National Toxicology Program therefore initiated a broad-based program of work to provide toxicity data and a cancer hazard assessment for lifetime exposure to environmental phthalates. Data generated from this program are intended to facilitate cumulative and aggregate risk characterization efforts for multiple phthalates, including di(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl phthalate, and di-isobutyl phthalate.

Previous carcinogenicity assessments of phthalates did not include perinatal exposure (gestation and lactation), whereas human exposure studies indicate that there is lifetime exposure to some phthalates. Thus, whether developmental exposure would alter lifetime DEHP-associated carcinogenic risk is unknown. Exposure during these critical periods of development and growth might be relevant for the evaluation of lifetime toxicological and carcinogenic risk.

The two 2-year toxicity and carcinogenicity studies described in this technical report were conducted to assess whether perinatal exposure would alter lifetime DEHP-associated carcinogenic risk. Specifically, the goal of the studies was to evaluate whether exposure to DEHP during the perinatal period would influence the pattern, dose response, incidence, or severity of the carcinogenic or noncarcinogenic response in rats relative to chronic exposure that does not include this critical period of development and growth.

Introduction

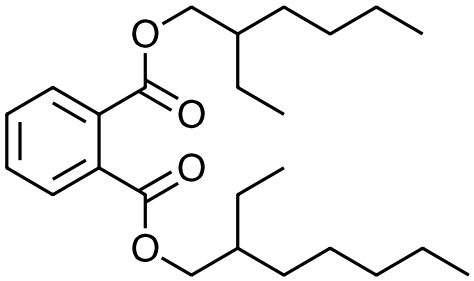


Figure 1. Di(2-ethylhexyl) Phthalate (CASRN 117-81-7; Chemical Formula: $C_{24}H_{38}O_4$; Molecular Weight: 390.6).

Synonyms: Bis(2-ethylhexyl)phthalate; dioctyl phthalate; phthalic acid di(2-ethylhexyl) ester; bis(2-ethylhexyl) 1,2-benzenedicarboxylate; 1,2-benzenedicarboxylic acid bis(2-ethylhexyl) ester.

Trade names: Platinol DOP; Octoil; Silicol 150; Bisoflex 81; Eviplast 80.

Chemical and Physical Properties

Di(2-ethylhexyl) phthalate (DEHP) is the diester of phthalic acid and the branched-chain 2-ethylhexanol. DEHP is a pale-yellow to colorless viscous liquid at room temperature and can have a slight odor. DEHP has a boiling point of 384°C, a melting point of −55°C, and a flash point of 215°C.[1] At 25°C, DEHP has a limited water solubility of approximately 0.3 mg/L and a vapor pressure ranging from 1.42×10^{-7} to 9.75×10^{-6} mm Hg.[2] DEHP has an estimated log K_{ow} of 7.6[3] and is miscible in organic solvents, such as hexane.

Production, Use, and Human Exposure

DEHP is a widely used member of the phthalate ester chemical class. Phthalates are employed predominantly as plasticizers to provide flexibility in products composed of polyvinyl chloride (PVC) plastic or vinyl chloride resins. DEHP is produced by the esterification of phthalic anhydride with 2-ethylhexanol in the presence of an acid catalyst, such as sulfuric acid or para-toluenesulfonic acid.[4] DEHP is considered a high-production volume chemical with an estimated 10 to 50 million pounds produced in the United States in 2015, as reported to the U.S. Environmental Protection Agency (EPA),[5] a production level consistent with annual production reports from 1986 to 2014, indicating that DEHP use remained consistent.

Globally, between 90% and 95% of DEHP is used as a plasticizer in the manufacture of PVC polymers and corresponding products.[6; 7] DEHP is used in a variety of plastic consumer products, including construction materials, shower curtains, garden hoses, floor tiles, automobile upholstery, and food packaging materials. Plastics may contain 1% to 40% DEHP by weight, with materials that exhibit increased softness or flexibility likely containing higher levels of DEHP or other phthalates. DEHP is used inthe production of medical devices, such as blood bags, enteral/parenteral nutrition bags, peritoneal dialysis bags, and medical tubing.[8-10] Because DEHP is not covalently bonded to the PVC polymer, potential exists for DEHP to leach into contact media. Migration from PVC storage bags into collected blood, blood products, and other biological products is likely associated with the lipophilic nature of DEHP.

Exposure to DEHP can occur via numerous pathways, such as contact with DEHP-containing plastic products, consumption of foods packaged in plastics, drinking of well water near waste sites, workplace/indoor inhalation of aerosols or particulates containing DEHP, or exposure during certain medical procedures.[4] The most common exposure pathway through ingestion of food contaminated with DEHP, which typically occurs because of contact with plastic packaging materials. Migration efficiency of DEHP into foodstuffs from packaging materials is likely associated with the lipophilic nature of DEHP and the contact surface area with the packing materials. In the United States, average daily DEHP exposure from food is estimated to be 0.3 mg/day with a maximum of 2.0 mg/day per individual.[11] Higher DEHP concentrations (≥300 μg/kg) have been noted in poultry, cooking oil, and cream-based dairy products relative to other assessed foodstuffs.[12] In water, DEHP exhibits low solubility, suggesting a lower relative contribution of drinking water to estimated total daily exposure.[13; 14] Additionally, the low vapor pressure of DEHP indicates a limited capacity for DEHP to volatilize into the air; however, it can readily adsorb to dust particles that can then be respired or ingested. Fromm et al. measured concentrations of DEHP in indoor air and vacuum cleaner dust samples.[15] The median indoor air DEHP concentration was 156 ng/m^3 (95th percentile, 390 ng/m^3) and 703.4 mg/kg (95th percentile, 1,542 mg/kg) in dust samples.

Measurable urinary DEHP metabolite concentrations from participants in the National Health and Nutrition Examination Survey (NHANES) indicate widespread exposure to DEHP in the U.S. population, but have been declining over the years.[16] Urinary concentration (50th percentile) of a DEHP metabolite, mono(2-ethylhexyl) phthalate (MEHP) (2015–2016) was 1.24 μg/g of creatinine (95th percentile, 5.93 μg/g of creatinine).[16] Using the NHANES data, researchers estimated a median cumulative DEHP exposure of 0.17 μg DEHP/kg body weight/day (μg/kg/day) (95th percentile, 12.0 μg/kg/day).[17] Urinary concentrations of DEHP and its metabolites are higher in exposed workers relative to unexposed workers and are detected at higher concentrations in postshift relative to preshift samples.[15; 18; 19] High exposures have been documented in workers in countries other than the United States and observed in various industries.[20-23] Newborns and infants may be at risk for higher DEHP exposure relative to the general population due to differences in metabolic capacity, increased food, water, and air intake per unit body weight, and behaviors such as crawling and mouthing, which can increase exposure to contaminants present in soil, house dust, and consumer products.[24] Additionally, DEHP and its metabolites have been detected in breast milk and baby formula. Average DEHP exposure in nursing infants has been estimated at between 6 and 24 μg/kg/day.[25; 26] Multiple DEHP metabolites have also been measured in human amniotic fluid samples, indicating exposure can occur in utero.[27]

DEHP exposure has been associated with certain medical procedures that use PVC plastic bags and tubing is thought to be much higher than from other anticipated environmental exposures. Parenteral exposure to DEHP can occur in patients undergoing medical procedures, such as intravenous administration of drugs, total parenteral nutrition, transfusion of blood or blood products, cardiopulmonary bypass, and extracorporeal membrane oxygenation.[8; 28-30]

Regulatory Status

Numerous regulatory statutes and guidelines are concerned with DEHP levels in consumer products, allowable environmental levels, and limits of occupational exposure. In the Consumer

Product and Safety Improvement Act of 2008, issued by Congress, and in a final ruling by the U.S. Consumer Product Safety Commission in 2017, any children's toy or childcare articles are prohibited from containing concentrations of more than 0.1% of eight designated phthalates, including DEHP.[31; 32] FDA regulates the use of DEHP as an indirect food additive used in food-contact materials. DEHP can be used in semi-rigid and rigid acrylic plastic materials at levels up to 3% by weight.[33] Additionally, DEHP can be a component of cellophane food packaging materials if DEHP levels alone or in combination with other phthalates do not exceed 5% by weight.[34] EPA established a maximum contaminant level for DEHP in drinking water at 6 μg/L and an oral reference dose of 0.02 mg/kg/day on the basis of increased relative liver weights in exposed guinea pigs.[35-37] Due to the potential for increased exposure via inhalation in occupational settings, the Occupational Safety and Health Administration (OSHA) has set an 8-hour time-weighted average permissible exposure limit of 5 mg/m^3, which is equivalent to the limits recommended by the American Conference of Governmental Industrial Hygienists and the National Institute for Occupational Safety and Health.[38] The short-term (15-minute) exposure limit allowable by OSHA is 10 mg/m^3. The Agency for Toxic Substances and Disease Registry developed DEHP minimal risk levels of 0.1 and 0.06 mg/kg/day via an oral exposure route for intermediate and chronic exposure durations, respectively.[4]

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Numerous studies have evaluated the absorption, distribution, metabolism, and excretion (ADME) properties of DEHP. High levels of hydrolase activity present in the intestinal tract of various mammalian species hydrolyze DEHP to its monoester form, MEHP, and 2-ethylhexanol. Endogenous hydrolytic activity has been shown to vary between species.[39-42] In general, investigators believed that most of consumed DEHP is efficiently hydrolyzed to its monoester form prior to absorption in the intestinal tract, and that absorption of the diester form is associated with high-exposure levels that exceed the hydrolytic capacity of the intestinal pancreatic lipases. Albro et al.[41] found no DEHP in the livers of rats after oral administration of DEHP at low doses (<0.4 g/kg), but did find detectable levels after administration of higher doses (>0.5 g/kg). Comparative studies in which male Sprague Dawley rats were administered DEHP by intraperitoneal injection (4 g/kg) or oral gavage (2 g/kg) revealed that approximately 80% of the oral dose undergoes mono-de-esterification compared to only 1% of the parenteral dose.[43] Co-administration of a pancreatic lipase inhibitor (*S*,*S*,*S*-tributylphosphorothionate) resulted in a marked inhibition of DEHP intestinal absorption, suggesting MEHP is more readily absorbed than its parent molecule, DEHP.

In adult Wistar rats following a single oral administration of [^{14}C]-DEHP (2.9 mg/kg), the dose was excreted primarily in the urine (42%) and feces (57%) by 7 days postadministration, with an estimated absorbed dose of 50% from the gastrointestinal tract.[44] Dermal absorption efficiency of [^{14}C]-DEHP is limited. Only an estimated 6.5% of a single 30–40 mg/kg dose in ethanol was absorbed by 7 days postapplication on exposed skin of male Fischer 344 (F344) rats.[45] Numerous studies report little retention of radiolabeled DEHP or its metabolites in isolated tissues.[39; 46; 47] However, elevated concentrations have been detected in rodent liver, adipose tissue, kidney, bladder, testis, and lungs; these findings may be associated with variables of study design such as the administered dose, route of exposure, or duration of exposure prior to necropsy.[48-50] A

comparative study in adult male Sprague Dawley rats, male dogs (beagles), and male miniature pigs (Hormel strain) reported differential DEHP excretion profiles following dietary exposure to 50 mg/kg/day for 21–28 days before administration of a single dose of [^{14}C]-DEHP (50 mg/kg).[47] Excretion of radioactivity in urine and feces by 24 hours postadministration was 27% and 57% (rats), 12% and 56% (dogs), and 37% and 0.1% (pigs), respectively; and after 4 days was 37% and 53% (rats), 21% and 75% (dogs), and 79% and 26% (mini-pigs), respectively. Overall elimination of radioactivity was complete by postadministration day 4 in all species and was most rapid in rats, followed by dogs, and least rapid in mini-pigs. Sjöberg et al.[51] investigated the kinetics of DEHP and MEHP in Sprague Dawley rats following a single oral gavage of 1,000 mg/kg DEHP. In blood samples collected at 1, 3, 7, 9, 12, 15, 24, and 30 hours after dosing, DEHP was only detectable within the first 7 hours after dosing. The maximal plasma concentration (C_{max}) of MEHP occurred within 1 hour of dosing (C_{max} of 0.093 μg/mL), and a plasma elimination half-life of approximately 2.8–3.9 hours was determined. In another study, plasma C_{max} of DEHP (8.8 μg/mL) and MEHP (63.2 μg/mL plasma) were reached within 6 hours of a single oral administration of DEHP in male Wistar rats (2.8 g/kg).[52] In the same study, daily dosing for a week resulted in no accumulation of DEHP or MEHP in plasma.

Additional studies suggest that differential ADME properties during gestational and juvenile development could increase exposure in these sensitive subgroups. DEHP is able to cross the placental barrier,[53; 54] and maternal transfer of DEHP and its metabolites can occur via lactation.[55] Increased intestinal tissue surface area relative to body weight and higher relative blood flow to the intestines may contribute to higher absorption rates in neonate/juvenile animals than in adults.[56]

Following hydrolysis of DEHP to MEHP by pancreatic lipases in the intestinal tract, MEHP can be further metabolized through oxidation to additional products and undergo subsequent conjugation with glucuronic acid. Interspecies variation in metabolic competencies can lead to distinct urinary metabolite profiles. Oral gavage of DEHP or MEHP to Sprague Dawley or F344 rats resulted in identification of over 20 distinct urinary metabolites.[40; 41] Phthalic diacids typically constitute most metabolites identified in rat urine.[41] Rats differ from other tested species in that they display extensive oxidative metabolism of DEHP, but little capacity to conjugate these metabolites. In mice, exposure to MEHP resulted in detectable concentrations of MEHP and metabolite glucuronide conjugates in urine.[57; 58] Primates generally display reduced pancreatic lipase activity in the intestinal tract compared with rodents, leading to reduced conversion of DEHP to MEHP.[59] Additionally, primates exhibit a reduced capacity to oxidize DEHP metabolites, but an increased capacity to conjugate (glucuronidate) MEHP metabolites.[59] Therefore, primates predominately excrete glucuronides of MEHP and metabolites with hydroxyl side chains that require limited oxidative metabolism.[41]

Humans

Studies have been conducted investigating DEHP toxicokinetic properties in humans. Similar to laboratory mammals, humans hydrolyze DEHP to MEHP by pancreatic lipases in the lumen of the intestinal tract, generate further oxidative metabolites, and conjugate these metabolites for excretion in urine and feces. In a study by Koch et al., urinary and serum concentrations of DEHP metabolites were determined from a human male volunteer following a single oral dose (0.64 mg/kg) of deuterium-labeled DEHP.[60] Peak concentrations of three DEHP metabolites [MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-oxohexyl)

phthalate (MEOHP)] were reached in serum after 2 hours and in urine after 4 hours. In serum, DEHP metabolites were unconjugated and contained high concentrations of MEHP relative to MEHP oxidation products. Urine samples contained higher concentrations of polar MEHP oxidation products than MEHP. Estimated serum elimination half-lives were <2 hours for the three measured DEHP metabolites. In a follow-up study, five DEHP urinary metabolites were identified [MEHP, MEHHP, MEOHP, mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and mono(2-[carboxymethyl]hexyl) phthalate (MCMHP)] that could be used as biomarkers for more accurate estimations of DEHP exposure (Figure 2).[61] An additional three oxidative metabolites—mono(2-ethyl-3-carboxypropyl) phthalate (MECPrP), mono-2-(1-oxoethylhexyl) phthalate (MOEHP), and mono(2-ethyl-4-carboxybutyl) phthalate (MECBP)—were reported in human biomonitoring studies (Figure 2).[62] Most of these metabolites undergo phase 2 metabolism to form glucuronide conjugates. Urinary concentrations of MEHHP and MEOHP have been detected at 10-fold higher concentrations than MEHP in 127 paired human samples, suggesting these metabolites may be more sensitive measures of DEHP exposure in the general population.[63] Given these findings, DEHP exposure could be significantly underestimated in studies that measure only MEHP concentrations to predict human exposure. Other numerous oxidative metabolites of DEHP have also been proposed.[62]

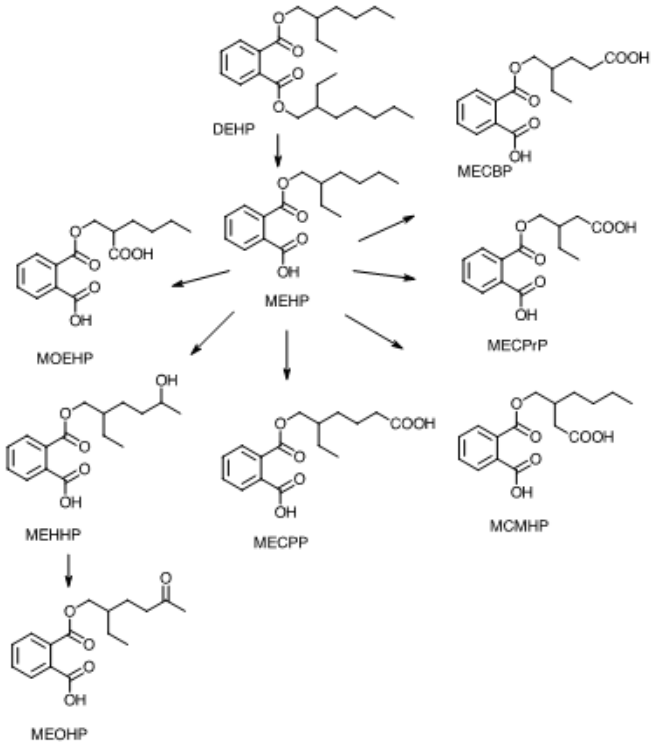


Figure 2. Di(2-ethylhexyl) Phthalate (DEHP) and Metabolites Used to Quantify DEHP Exposure

Toxicity

Experimental Animals

Extensive literature exists on the toxicity of DEHP in numerous animal models. Acute oral median lethal dose (LD_{50}) values for DEHP range from 9,800 to >40,000 mg/kg in rats,[4; 64-66] and 9,860 to >31,360 mg/kg in mice,[4; 64; 67] LD_{50} values of 33,900 mg/kg in rabbits[65] and 26,300 mg/kg in guinea pigs have also been reported.[64; 68] Neonatal and young animals may be more sensitive, however, to the acute effects of DEHP. Mortality was observed in 6- to 21-day-old male Sprague Dawley rats administered five daily oral doses of 1,000 or 2,000 mg DEHP/kg, whereas no mortality occurred in rats age 6 weeks or older when administered the same five daily doses.[69]

NTP has reported findings from studies investigating the acute, subchronic, and chronic toxicities of DEHP in rodent models.[70] No effect on survival was observed in F344 rats or B6C3F1 mice during a 14-day observation period following a single administration of DEHP by oral gavage (800 to 20,000 mg/kg for rats; 1,252 mg/kg for mice). In 13-week feeding studies, F344 rats were administered a diet containing 0, 1,600, 3,100, 6,300, 12,500, or 25,000 ppm DEHP. Significantly reduced mean body weight gains were observed in male and female rats exposed to 25,000 ppm, and testicular atrophy was observed in males exposed to dietary concentrations of 12,500 ppm or higher. B6C3F1 mice exposed to 0, 800, 1,600, 3,100, 6,300, or 12,500 ppm DEHP in the diet for 13 weeks showed similar effects on body weight at the higher concentrations. Decreased mean body weight gains (≥10% relative to the control groups) were noted in male mice exposed to 3,100 ppm DEHP or higher and in all DEHP-exposed female mice, except the 1,600 ppm group. In a 2-year study, F344 rats were exposed to 0, 6,000, or 12,000 ppm DEHP in the diet, resulting in mean daily chemical intakes of 322 and 674 mg/kg body weight (mg/kg) for males, respectively; and 394 and 774 mg/kg for females, respectively. At the end of the 2-year study, mean body weights of exposed rats were up to 20% lower in the high-exposure groups compared to the control groups. In a companion 2-year study, B6C3F1 mice were exposed to 0, 3,000, or 6,000 ppm DEHP in the diet, resulting in mean daily chemical intakes of 672 and 1,325 mg/kg for males, respectively, and 799 and 1,821 mg/kg for females, respectively. At the end of the 2-year study, mean body weights were 7% and 10% lower in the 3,000 and 6,000 mg/kg male groups, and 21% and 33% lower in the 3,000 and 6,000 mg/kg female groups, respectively, relative to the control groups. The incidence of testicular tubule degeneration or atrophy was significantly elevated in high-exposure group male rats (approximately 90%) and male mice (approximately 14%) relative to the control groups.

Numerous laboratory animal studies have reported reductions in body weight and body weight gain following repeated exposures to DEHP, and common target organs of DEHP toxicity include the testis, kidney, and liver. Toxic effects of phthalates on the male reproductive tract are well characterized and are addressed in a subsequent section of this Introduction. DEHP effects on the kidney include reduced creatinine clearance, increased absolute and relative kidney weights, increased incidence and/or severity of mineralization of renal papilla, increased incidence and/or severity of tubule cell pigment, and increased incidence and/or severity of chronic progressive nephropathy.[71-73] Liver enlargement due to both hepatocyte hyperplasia and hypertrophy, with associated morphological changes such as increased size and number of peroxisomes and corresponding increases in fatty-acid metabolism, are known hallmarks of DEHP toxicity in rodents. Activation of the peroxisome proliferator-activated receptor alpha

(PPARα) in hepatocytes is recognized as a key molecular initiating event by which DEHP induces adverse effects in the liver.[74] PPARα-deficient mice did not exhibit characteristic liver toxicity following 24 weeks of DEHP exposure but did exhibit moderate kidney and testicular toxicity.[75] These findings suggest that while DEHP-induced liver toxicity is associated with PPARα status, renal and testicular toxicities likely manifest via alternative mechanisms.

Decreased severity of hepatic effects in nonrodent species may be related to interspecies differences in PPARα expression, binding, localization, and downstream molecular signaling pathways.[42] DEHP metabolites such as MEHP have been reported to be more potent activators of human and mouse PPARα relative to its parent molecule.[76] Therefore, interspecies differences in pancreatic lipase activity, which converts DEHP to MEHP, may influence observed DEHP toxicities. Additionally, routes of administration that bypass first pass metabolism in the intestinal tract and liver (intravenous), reducing hydrolysis of DEHP to its metabolites, could influence subsequent toxicity.

Humans

The health effects of DEHP have been evaluated extensively in animal models, but data that address the relationship between human health effects or adverse outcomes and exposure to DEHP are limited. Shaffer et al.[65] presented a case report in which two male subjects had ingested single DEHP doses of 5 g and 10 g, respectively.[65] The individual who consumed the 10 g dose presented with symptoms of mild gastric disturbance and moderate diarrhea, whereas no effects were observed at the lower dose.

Reproductive and Developmental Toxicity

Experimental Animals

Studies with laboratory rodents demonstrate that DEHP exposure can cause adverse effects on reproduction and development. In adult rats, oral DEHP exposure is associated with numerous deleterious effects on the male reproductive tract, including decreased weights of the testes, prostate, seminal vesicles, and epididymis; degeneration and atrophy of the seminiferous tubules; altered sperm parameters; and reduced fertility.[4; 14] The testes are considered a primary target tissue of DEHP toxicity. Decreased testicular weight and increased incidence of tubular atrophy have been observed in numerous rodent studies in which doses exceeded 100 mg/kg/day.[70; 77-83] Within the testes, DEHP appears to preferentially target Sertoli cell populations, which can impair spermatogenesis and fertility.[79; 84] Irregular seminiferous tubule structure and altered spermatogenesis were evident in male rats ingesting DEHP at 2,000 mg/kg/day via the diet for 15 days.[85] In these rats, few spermatozoa were present in the lumen of the tubules, and damaged spermatogenic cells were observed in the tubular space. Significantly increased incidences of bilateral aspermatogenesis were observed at lower exposure concentrations (29 mg/kg/day) in male rats fed DEHP-supplemented diets for 2 years.[86] Prepubertal rodents appear to be more sensitive to DEHP-mediated effects on the testes relative to sexually mature rodents.[77; 83] In contrast to studies in rodents, no changes in testes/epididymides weight or testicular histology were observed in cynomolgus monkeys following administration of 500 mg/kg/day DEHP by gavage for 14 consecutive days.[87] Decreased fertility also has been observed in female rodents exposed to DEHP, and may be related to DEHP-induced disruption of normal estrous/ovulatory cycles.[88]

DEHP is a developmental toxicant in rodents, producing embryotoxic, fetotoxic, and teratogenic effects. Decreased fetal/pup body weight, increased rates of abortion and fetal resorptions, and malformations (hydronephrosis, cardiovascular malformations, and tail malformations) have been reported in rat dams and corresponding litters after exposure to DEHP during pregnancy/gestation.[89; 90] Exposure to DEHP during the perinatal period (gestation and/or lactation) can induce abnormal development of the male reproductive tract and other androgen-sensitive tissues. Although the exact mechanism is unknown, DEHP acts as an endocrine disruptor via an antiandrogenic mode of action and decreases insulin-like hormone 3 production by Leydig cells. Normally, during the window of fetal male sexual differentiation (gestation days 15.5–21.5), androgen-dependent masculinization of the fetal reproductive tract occurs, resulting in differentiation of the internal (epididymis, vas deferens, seminal vesicles, prostate) and external (penis, scrotum, perineum) genitalia.[91; 92] Exposure to DEHP during this critical window of susceptibility decreases fetal testosterone synthesis leading to structural malformations and functional alterations of the male reproductive system.[93; 94] Reduced anogenital distance (AGD), retained nipples, penile morphological abnormalities (hypospadias), undescended testes (cryptorchidism), small/absent sex accessory glands, delays in onset of pubertal landmarks (preputial separation), and histopathological alterations in testes and epididymides have been observed in male rats following perinatal DEHP exposure.[93; 95-97] Dysmorphogenic effects in the testes include microscopic disorganization of the seminiferous tubules with detachment of the spermatogonial cells from the basal membrane and absence of spermatocytes.[98] The term “phthalate syndrome” is often used to describe the compendium of reproductive tract malformations observed in male test animals following in utero phthalate exposure.[99]

The reproductive and developmental effects of DEHP exposure were comprehensively investigated by NTP in a multigenerational reproductive assessment by continuous breeding study.[100] In this study, DEHP was administered in the diet at concentrations of 1.5 (control), 10, 30, 100, 300, 1,000, 7,500, or 10,000 ppm to Sprague Dawley rats over multiple successive generations (F_0, F_1, F_2, and F_3) throughout the breeding, gestational, lactational, and postweaning intervals. Measured feed consumption and body weights informed the calculation of approximate daily doses of 0.12, 0.78, 2.4, 7.9, 23, 77, 592, and 775 mg/kg/day in the F_0 animals; 0.09, 0.48, 1.4, 4.9, 14, 48, 391, and 543 mg/kg/day in the F_1 animals; and 0.1, 0.47, 1.4, 4.8, 14, 46, and 359 mg/kg/day in the F_2 animals. The 10,000 ppm group was removed from the study following the F_1 generation due to the inability to produce offspring (F_2 generation). Adverse reproductive and developmental effects such as decreased pregnancy index, decreased male AGD, delayed onset of pubertal landmarks (testes descent, vaginal opening, and balanopreputial separation), sperm counts, small male reproductive organs (testes, epididymis, and caudal epididymis), and seminiferous tubule atrophy were observed in all generations in the 7,500 and 10,000 ppm groups. No reproductive toxicity was evident at exposure concentrations <7,500 ppm; however, increased incidences of small testes and prostates were noted in 300 and 1,000 ppm male rats. After further review of animal studies by NTP’s Center for the Evaluation of Risks to Human Reproduction (CERHR) expert panel, a developmental no-observed-effect level of 1,000 ppm was suggested and calculated to be no more than 46 mg/kg/day based on the average dose over three generations.[14]

Humans

Given the results from animal studies, there is significant concern that DEHP can adversely affect human reproduction and male development. FDA's CDRH concluded that "children undergoing certain medical procedures may represent a population at increased risk for the effects of DEHP." A similar conclusion was reached by NTP's CERHR, which found cause for "serious concern" that certain medical treatments may result in DEHP exposure levels that could adversely affect the development of the reproductive tract in male infants.[14] Numerous epidemiological studies have found no significant association of DEHP or its metabolites with sperm abnormalities, circulating hormone concentrations, , or indications of infertility.[101-105] Other studies have reported associations between maternal urinary DEHP metabolite concentrations and effects on several markers of human male genital development. In complementary studies by Swan et al., measures of AGD and penile width in male infants were significantly associated with exposure to DEHP and three of its metabolites.[106; 107] Many parallels exist between the "phthalate syndrome" suite of effects in animal models and descriptions of human testicular dysgenesis syndrome. This syndrome is characterized by increased incidences of reproductive tract malformations in male newborns (cryptorchidism, hypospadias) and adverse effects in young adults (low sperm counts, testicular germ cell cancer) and is likely related to in utero exposure to environmental chemicals.[108; 109]

Immunotoxicity

Experimental Animals

Several studies have been conducted to assess the potential of DEHP or MEHP to modulate immune function. Studies by Larsen et al. found subcutaneous injections of MEHP (100 μg) to induce an immunosuppressive effect characterized by a reduction in immunoglobulin E (IgE) and IgG1 antibody production in BALB/cJ mice following co-administration of ovalbumin antigen.[110] However, in the same study, lower doses of MEHP (1 μg) induced an adjuvant effect characterized by increased IgE production. Administration of DEHP to male F344 rats via the diet (12 ppm) for 21 days resulted in a suppressed hepatic T-helper Type 1 (Th1) immune response initiated via intraperitoneal exposure to *Mycobacterium bovis* purified protein derivative.[111] The authors hypothesized that this effect was associated with biotransformation of DEHP to MEHP and subsequent activation of PPARα. Further studies investigating the mixed T-helper cell adjuvant properties of DEHP found that this effect occurred independent of PPARα status in mouse models.[112] Differential effects on the immune system have been noted in studies that use a developmental exposure paradigm. Increased sensitivity to DEHP exposure, characterized by altered immune parameters (T-dependent antigen response, natural killer cell activity, and tumor necrosis factor-alpha [TNF-α] production), was observed in male Wistar rats administered DEHP (0, 1, 3, 10, 30, 100, 300, or 1,000 mg/kg/day) by oral gavage during their juvenile period (postnatal day [PND] 10–50) relative to adult-only exposure (PND 50–90).[113] In contrast, no persistent effects on numerous assessed immune parameters were noted in a study by Piepenbrink et al. in which CD rats were gestationally exposed to DEHP (0, 37.5, 75, 150, or 300 mg/kg/day).[114] In the same study, no DEHP-associated immunotoxicity was noted in nulliparous exposed adults. Topical DEHP administration in adult B6C3F1 female mice did not increase serum concentrations of IgE, interleukin-4 (IL-4), or IL-13, suggesting a limited potential to induce allergic asthma.[115] Additional studies report dose-dependent increases in

some inflammatory cell numbers (macrophages, eosinophils, neutrophils, and/or lymphocytes) in bronchoalveolar lavage fluid in BALB/c or BALB/cJ mice following inhalation exposure to MEHP aerosols.[116; 117] Using median residential indoor air and worst-case exposure concentrations of 0.04 μg and 1.2 μg DEHP/m^3, respectively, Hansen et al. estimated a margin of exposure between 2,500–75,000, suggesting that immune effects from inhalation exposures are only expected at air concentrations that are well in excess of environmental inhalation exposures typically encountered by humans.[116]

Humans

Numerous case reports and epidemiological studies suggest a link between phthalate exposure from PVC products and development of allergies and/or asthma.[118] In a study of 39 PVC-processing plant workers, a higher prevalence of asthma, rhinitis, and eye and respiratory symptoms was observed in individuals exposed to PVC pyrolysis products and phthalates relative to an unexposed reference group.[119] In a population-based incident case-control study of 521 new asthma cases and 932 control cases, asthma risk was related to the presence of plastic wall materials. [120] Two epidemiological studies suggest childhood exposure to phthalates via house dust is related to the onset of allergy and/or asthma. In a nested case-control study within a 10,852 child cohort (198 persistent allergic cases, 202 control cases), higher median concentrations of DEHP (cases – 0.828 mg/g dust; control group – 0.723 mg/g dust) in house dust were significantly ($p = 0.022$) associated with asthma.[121] In a separate study in Bulgarian preschool-age children ($n = 102$), wheezing was associated with higher DEHP concentrations in dust samples collected from children's rooms (1.24 mg/g dust for children with wheezing, rhinitis, and/or eczema versus 0.86 mg/g dust for nonsymptomatic) in the preceding 12-month time interval.[122] A greater understanding of human exposure relative to animal effect levels and additional mechanistic studies are needed to support a causal inference between DEHP exposure and immunomodulatory effects in humans.

Carcinogenicity

Experimental Animals

Multiple rodent studies were identified in the literature that examined the carcinogenic activity of DEHP, all of which initiated exposure once test animals had reached adulthood. Increased incidences of hepatocellular neoplasms have been corroborated across multiple rodent studies, along with reports of increased incidences of testicular Leydig cell tumors and pancreatic acinar adenomas in male rats following chronic exposure to DEHP. In 2-year cancer bioassays conducted by NTP, F344 rats and B6C3F1 mice were administered diets containing 0, 6,000, or 12,000 ppm DEHP and 0, 3,000, or 6,000 ppm DEHP, respectively.[70; 123] DEHP was found to be carcinogenic in both F344 rats and B6C3F1 mice on the basis of increased incidences of hepatocellular adenomas/carcinomas or neoplastic nodules in both males and females. Significantly increased incidences of hepatocellular adenoma or carcinoma (combined) in F344 rats and B6C3F1 mice were observed at lower DEHP exposure concentrations (male rats: 2,500 ppm; male mice: 500 ppm; female mice: 1,500 ppm) in chronic studies by David et al.[86; 124; 125] Additionally, incidences of pancreatic acinar adenomas were increased in male F344 rats at the highest tested exposure concentration (12,500 ppm).[86] A 159-week study in male Sprague Dawley (SD-CD) rats found that the high-exposure concentration of DEHP (6,000 ppm, or 300 mg/kg/day) increased the incidence of benign Leydig cell tumors.[126]

In rats, the combination of hepatocellular, pancreatic, and testicular tumors is often referred to as the "tumor triad" and is associated with sustained peroxisome proliferator activity.[127] Although the definitive mode of action of DEHP-mediated carcinogenesis is undetermined, several key events, including activation of PPARα, perturbation of cellular proliferation and apoptosis, selective clonal expansion, and oxidative stress, are hypothesized to contribute to the onset of tumorigenesis.

Humans

The carcinogenic activity of DEHP in humans has been reviewed by numerous federal and international agencies. In the 14th Report on Carcinogens published by NTP, DEHP was listed as reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals.[128] EPA classified DEHP as a Group B2 carcinogen, probable human carcinogen, based on clear evidence of DEHP-mediated induction of liver tumors in rodent models.[36] The International Agency for Research on Cancer (IARC) previously classified DEHP as "not classifiable as to its carcinogenicity to humans" (Group 3).[74] The IARC determination was based on two assumptions: (1) that DEHP-induced hepatocellular cancer in rodents occurred as a result of induced peroxisome proliferation activity, and (2) that this mechanism is not relevant to humans due to lower PPARα expression and lack of observable peroxisome proliferation phenotypes in humans following exposure to known PPAR ligands. However, in light of new information about mechanisms of action, in 2011 IARC reclassified DEHP as a Group 2B carcinogen, indicating that there is "sufficient evidence of carcinogenicity in experimental animals" in combination with "no or limited epidemiological data."[129] The reevaluation included consideration of recent studies with novel transgenic mouse models, such as PPARα-null mice, humanized PPARα mice, and mice that express a constitutively active PPARα isoform in hepatocytes.[130-132] These studies indicate that DEHP can induce hepatocarcinogenesis through a PPARα-independent mechanism, and that other molecular signaling pathways, not just activation of PPARα, likely contribute to the development of cancer.

Epidemiological studies that investigate a link between DEHP exposure and cancer endpoints are limited. In a case-control study of female breast cancer patients, increased cancer risk was associated with elevated urinary concentrations of the DEHP metabolite MECPP, but not other identified DEHP metabolites.[133] Additional cancer epidemiology studies have been conducted in occupational groups where subjects had worked in PVC processing and plastic manufacturing facilities where increased exposure to phthalate plasticizers was probable.[134-140] However, many of these studies lacked analytical assessment of exposure to specific phthalates, limiting the ability to determine a causal relationship between DEHP exposure and human cancer.

Genetic Toxicity

The genetic toxicity of DEHP has been extensively investigated and reviewed over many years (e.g., Huber et al., IARC, and Caldwell). [141-143] Overall, DEHP shows limited evidence of genotoxic potential, and for the sporadic positive results that have been reported, associations are weak, not reproducible, obtained in a nonstandard test system, or qualified to some degree by the authors. MEHP, one of the main DEHP metabolites, does elicit positive responses, however, in some genotoxicity assays. An early study reported increases in revertant colonies in *Salmonella typhimurium* strain TA100 and *Escherichia coli* strain WP2 B/r treated with 2.5 and 5.0 mM MEHP, doses that induced marked cytotoxicity.[144] More recently, MEHP was reported to

generate reactive oxygen species and, consequently, DNA strand breaks in cultured AS52 cells[145] as well as in cultured mouse Leydig tumor cells and in human prostate adenocarcinoma cells as measured by the comet assay.[146; 147] In both cell lines in the latter two studies, the parent compound DEHP (3 mM for 24 hours) was also reported to induce DNA damage, although the concentration tested was 1,000 times higher than the concentration tested of MEHP (3 uM). Similarly, DNA damage, measured by the comet assay, was also reported for DEHP in cultured HeLa cells treated with 96.6 µM DEHP for 24 hours.[148]

NTP has conducted several in vitro and in vivo genotoxicity assays with DEHP. Unpublished NTP data are included in Appendix D of this report. Published NTP studies, results of which are consistent with most published studies, showed no induction of gene mutations in any of several strains of *S. typhimurium*[149; 150] or in cultured mouse lymphoma L5178Y $tk^{+/-}$ cells.[151] Additional bacterial mutation studies also reported negative results (e.g., Simon et al.[152]). Cytogenetic studies in cultured Chinese hamster ovary cells were negative for induction of chromosomal aberrations and were either positive or equivocal for induction of sister chromatid exchanges.[153] In vitro chromosomal aberration studies, not conducted by NTP, in human leukocytes and human fetal lung cells with DEHP also showed no significant increases in chromosomally aberrant cells,[154] as did chromosome aberration studies in Chinese hamster cells.[155-157] Studies that assessed the ability of DEHP to induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* after exposure of either adults (via injection) or larvae (via feeding) were negative.[158; 159]

Although sporadic reports of DNA damage or chromosomal effects following in vitro exposure to DEHP exist, results from in vivo studies are almost uniformly negative. In an in vivo comet assay conducted as part of the Japanese led multi-laboratory international validation effort for the assay, DEHP, administered by gavage at a top dose of 2,000 mg/kg/day for 3 days, did not induce DNA damage in cells obtained from the stomach, liver, and bone marrow of male Sprague Dawley rats.[160] In addition, bone marrow samples from those rats showed no increase in the percentage of micronucleated erythrocytes, which are biomarkers of chromosomal damage. In another study, no increases in the frequencies of *gpt* and Spi(-) mutations were seen in DNA extracted from liver cells of *gpt* transgenic rats (both F344 and Sprague Dawley backgrounds) with exposure of up to 12,000 ppm DEHP exposure in the diet for 4 weeks, a concentration that produced generalized toxicity (e.g., increased liver weights).[161] Similarly, an earlier study found that a 13-week Sprague Dawley *gpt* delta transgenic rats exposed to 12,000 ppm DEHP in the diet resulted in no increases in mutations in liver cell DNA.[162]

A study designed to investigate the potential for DEHP to induce unscheduled DNA synthesis (UDS) in liver cells of male B6C3F1 mice—a species that is sensitive to tumor induction by DEHP—found that exposures up to 500 mg/kg DEHP acutely or 6,000 ppm in the diet for up to 28 days did not induce UDS, measured using autoradiographic methods.[163] The investigators also treated primary mouse hepatocytes in culture with up to 1.0 mM DEHP and observed no UDS at time points ranging from 12–48 hours posttreatment.

The negative results from the in vivo studies described above contrast with an earlier study by Singh et al. that reported a weak positive response in an in vivo rodent dominant lethal test using ICR mice. In that study, DEHP was administered via intraperitoneal injection at 66% of the acute LD_{50} dose, determined as 38.35 mL/kg.[164] However, Jäckh et al.[165] reported that a second rodent dominant lethal test that used similar doses of DEHP but administered the chemical via oral

gavage showed no induction of dominant lethal mutations. They therefore suggested that the weak positive response in the Singh et al. study was likely related to nongenotoxic mechanisms, as covalent binding to DNA was not detected in liver cells of rats administered ^{14}C- and ^{3}H-labeled DEHP (500 mg/kg) by gavage.[165]

Study Rationale

In response to data gaps related to in utero and early life phthalate exposure and resultant adverse reproductive, developmental, and carcinogenic effects, NTP initiated a cancer hazard assessment for lifetime exposure to environmental phthalates. For DEHP, studies in rodents have established the gestational period as the time of greatest sensitivity to adverse developmental effects, specifically dysmorphogenesis of the male reproductive system. DEHP is a known rodent carcinogen; however, no previous cancer assessments have included exposure during the perinatal period (gestation and lactation). Therefore, it is unknown whether the carcinogenic response is altered when lifetime exposure encompasses these critical developmental windows.

NTP designed two 2-year studies in rats to evaluate whether DEHP lifetime exposure, including during the perinatal developmental period, would alter the dose response of the carcinogenic response relative to postweaning-only exposure. In these studies, DEHP was administered in dosed feed to mimic a common route of human exposure.

Materials and Methods

Procurement and Characterization of Di(2-ethylhexyl) Phthalate

Di(2-ethylhexyl) phthalate (DEHP) was obtained from Aldrich Chemical Company Inc. (St. Louis, MO) in a single lot (lot 01514TH) that was used in both 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at RTI International (Research Triangle Park, NC) (Appendix A). Reports on analyses performed in support of the DEHP studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 01514TH of the chemical, a clear liquid, was identified as DEHP by infrared (IR) spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, gas chromatography (GC) with mass spectrometry (MS) detection, and high-resolution MS (HRMS) (Table A-1). The IR spectrum was in good agreement with a reference spectrum and the structure was consistent with DEHP. Both ^{1}H and ^{13}C NMR spectra were consistent with reference and predicted spectra. The GC/MS spectra correlated well with the structure of DEHP and the HRMS resulted in measured mass within 0.5 ppm of the theoretical value. The elemental analysis was consistent with the composition of DEHP.

Karl Fisher titration determined the water content of lot 01514TH to be 0.145%. Ultra-performance liquid chromatography (UPLC) with photodiode array (PDA) detection and GC with flame ionization detection (FID) were used to determine a purity of 99.7% (Table A-1). The UPLC/PDA and GC/FID showed a minor peak accounting for 0.2% and 0.3%, respectively, of the total response in the chromatograms. Therefore, bulk purity was determined to be >99%.

Accelerated stability studies confirmed that lot 01514TH was stable for at least 2 weeks when stored in sealed glass vials at 5°C and 60°C. The bulk chemical of lot 01514TH was homogenized by shaking each of the 50 L plastic jugs for approximately 2 minutes and then transferred to 4 L amber glass storage bottles, which were stored at room temperature. Periodic reanalysis of the bulk chemical was performed during the studies by the study laboratory using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, and no degradation was detected (Table A-1).

Preparation and Analysis of Dose Formulations

The dose formulations were prepared approximately monthly by mixing DEHP with NIH-07 or NTP-2000 feed (Table A-2). Both the perinatal and postweaning study (Study 1) and the postweaning-only study (Study 2) used dose formulations of 300, 1,000, 3,000, and 10,000 ppm. Formulations were stored in sealed plastic bag-lined containers at room temperature (approximately 25°C) for up to 42 days. The plastic bags used by the study laboratory in the preparation and storage of blank and dosed feed were determined to have no DEHP above the limit of detection of the assay (1.27 ppm).

Homogeneity studies of the dose formulations in a 72-kg NIH-07 feed batch (300 and 10,000 ppm) and in a 92-kg NTP-2000 feed batch (300, 3,000, and 10,000 ppm) were performed prior to animal studies by the study laboratory with HPLC/UV (Table A-1). Formulations were

determined to be homogenous and stable for 42 days at room temperature and under simulated dosing conditions.

Periodic analysis of the DEHP dose formulations was conducted by the study laboratory using HPLC/UV to determine purity and concentration (Table A-3, Table A-4). All preadministration dose formulations were within 10% of the target concentrations. For the perinatal and postweaning study (Study 1), all postadministration dose formulations of DEHP were within 10% of target concentrations. In the postweaning-only study (Study 2), one sample collected from the residual feed in the feeder was below 10% of the target concentration (−12.3%). All other postadministration values were within 10% of the target concentrations.

Animal Source

Time-mated (F_0) female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN) for use in the perinatal and postweaning study (Study 1). Weanling (3 to 4 weeks old) male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were also obtained from Envigo for use in the postweaning-only study (Study 2).

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the Battelle (Columbus, OH) Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health (NIH) and National Toxicology Program (NTP) animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-year Studies

Exposure Concentration Selection Rationale

Dietary exposure concentrations of 0, 300, 1,000, 3,000, or 10,000 ppm DEHP were selected based on previous data from an NTP multigenerational reproductive assessment by continuous breeding (RACB) study, which included a perinatal exposure paradigm in the Sprague Dawley rat model. In the RACB study, the highest tested exposure concentration (10,000 ppm) was well-tolerated by pregnant dams and did not affect litter size or pup survival to weaning. However, this exposure concentration induced significant numbers of reproductive tract and testicular malformations in the F_1 male offspring and perturbed developmental androgen signaling as evidenced by decreased anogenital distance (AGD) and delayed attainment of puberty. In a previous NTP cancer bioassay using Fischer 344 (F344) rats, increased incidences of hepatocellular neoplasms occurred at exposure concentrations of 6,000 and 12,000 ppm DEHP. Together, these data suggest that the selected exposure concentrations are likely to induce a carcinogenic response and adequately challenge developmentally exposed test animals. To facilitate comparison of the results of the two 2-year studies, with and without perinatal exposure, both studies used the same exposure concentrations.

Perinatal and Postweaning Study in Rats (Study 1)

F_0 female rats were 11 to 13 weeks old upon receipt. Evidence of mating is defined as gestation day (GD) 1; F_0 females were received on GD 2 and held for 4 days. F_0 females were randomly assigned to one of five exposure groups on GD 5 (n = 45/group). Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Co., Cedar Knolls, NJ).

F_0 females were quarantined for 11 days after receipt. Ten nonmated females received with the time-mated females were designated for disease monitoring 11 days after arrival; samples were collected for serological analyses and the rats were euthanized, necropsied, and examined for the presence of disease or parasites. The health of the F_1 rats was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). Pinworms (*Syphacia* spp.) were diagnosed in sentinel animals during routine health monitoring evaluations. Infected animals did not display clinical signs and no pathological lesions were noted in relation to the presence of the pinworms. Following this finding, NTP, in coordination with the testing laboratory, developed and implemented a successful plan of pinworm containment and eradication. NTP required the testing laboratories to actively monitor animals to ensure the continued exclusion of pinworms from all studies going forward. All other test results were negative.

Beginning on GD 6, F_0 time-mated female rats were fed diets containing 0, 300, 1,000, 3,000, or 10,000 ppm DEHP throughout gestation and lactation. Groups of 50 F_1 rats/sex/exposure concentration continued on in the study after weaning and were fed diets containing the same respective DEHP concentration for 2 years.

F_0 female rats were housed individually during gestation and with their respective litters during lactation. Water and dosed feed were available ad libitum. F_0 females were weighed on GDs 5, 6, 9, 12, 15, 18, and 21 and on lactation days (LDs) 1, 4, 7, 14, and 21. During gestation, feed consumption was continuously measured over 3-day intervals from GD 6 through GD 21 (GD 6–9, 9–12, 12–15, 15–18, and 18–21). The day of parturition was considered to be LD 0. On apparent GD 26, all time-mated female rats that failed to deliver were euthanized and the uteri were examined and stained for evidence of implantation. Total litter weight and litter weights by sex were collected on postnatal day (PND) 1. Individual F_1 pups were weighed on PNDs 4, 7, 14, and 21. Clinical observations and survival were evaluated throughout lactation. During lactation, feed consumption was continuously measured over 3-day intervals from LD 1 through LD 21 (LD 1–4, 4–7, 7–10, 10–14, 14–17, 17–21).

Select dams and their litters were removed on GD 18 to quantify mono(2-ethylhexyl) phthalate plasma and tissue concentrations. On GD 18, blood was collected from the retroorbital sinus of randomly selected dams (n = 5 per exposure group). Blood samples were collected in tubes containing K_3 EDTA (tripotassium ethylene diamine tetraacetic acid), centrifuged, and the plasma harvested. Amniotic fluid was collected and pooled by dam, and each dam's fetuses were collected and pooled by litter. All samples were flash frozen in liquid nitrogen and stored frozen at approximately −20°C before shipment to RTI International (Research Triangle Park, NC). All samples were analyzed using a validated analytical method (Appendix E).

On PND 4, all litters with surviving pups were retained. Before weaning, two males and two females per litter from 25 litters in the 0, 300, 1,000, and 3,000 ppm groups and from 21 litters in

the 10,000 ppm group were randomly assigned to continue on in the 2-year postweaning phase of the study. To complete assignment in the 10,000 ppm group, two male pups and three female pups were selected from two litters, and two male pups and one female pup were selected from two additional litters. After assignments to the 2-year study were complete, 20 pups per sex from the remaining control pups were randomly selected as the sentinel animals. On the day the last litter reached PND 18, litters were randomly selected and F_1 pups from these litters were randomly selected for the 2-year study. On the day the last litter reached PND 21, dams were removed and the pups were weaned. Weaning marked the beginning of the 2-year chronic phase of the study.

After weaning, F_1 pups were housed up to two (males) or four (females) per cage. Dosed feed and water were available ad libitum. Feed consumption was measured weekly for the first 13 weeks and at 4-week intervals thereafter. Cages were changed weekly through PND 4, then changed at least twice weekly. Racks were changed and rotated at least every 2 weeks. Further details of animal maintenance are given in Table 1.

Two diets were utilized in this study: (1) NIH-07 during the perinatal phase, and (2) NTP-2000 during the postweaning phase. The NIH-07 diet is a higher protein diet that supports reproduction and lactation in rodents, whereas the NTP-2000 diet is a lower protein diet that decreases the incidence of chronic nephropathy in adult rats. Information on feed composition and contaminants for both diets is provided in Appendix B.

Postweaning-only Study in Rats (Study 2)

Male and female rats were 3 to 4 weeks old upon receipt and quarantined for 13 days prior to study start. Rats were randomly assigned to one of five exposure groups (n = 50 rats/sex/exposure group). Randomization was stratified by body weight that produced similar group mean weights using PATH/TOX SYSTEM software (Xybion Medical Systems Corporation, Cedar Knolls, NJ). Rats were 5 to 6 weeks old on the first day of the study and were provided DEHP in dosed feed for 2 years at one of five exposure concentrations (0, 300, 1,000, 3,000, or 10,000 ppm).

Five male and five female rats were designated for disease monitoring 13 days after arrival; samples were collected for serological analyses, and the rats were euthanized, necropsied, and examined for the presence of disease or parasites. The health of the rats was monitored during the study according to the protocols of the NTP Sentinel Animal Program (Appendix C). Pinworms (*Syphacia* spp.) were diagnosed in sentinel animals during routine health monitoring evaluations. All other test results were negative.

Rats were housed up to two (males) or four (females) per cage. Water and dosed feed were available ad libitum. Feed consumption was measured weekly for the first 13 weeks and at 4-week intervals thereafter. Cages were changed at least twice weekly. Racks were changed and rotated at least every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is given in Appendix B.

Clinical Examinations and Pathology

In both of the 2-year studies, animals were observed twice daily for morbidity and moribundity. Animals were weighed initially, weekly for the next 13 weeks, every 4 weeks thereafter, and at

study termination. Beginning on study day 29 (Study 1) or study week 5 (Study 2), clinical observations were recorded every 4 weeks and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all F_1 rats in Study 1 and all rats in Study 2. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin except for eyes, testes, vaginal tunics, and epididymides, which were first fixed in Davidson's solution or modified Davidson's solution. Tissues were processed and trimmed, embedded in paraffin, sectioned at a thickness of 4 to 6 μm, and stained with hematoxylin and eosin (H&E) for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. In the original evaluation of the uterus, a transverse section through each uterine horn, approximately 0.5 cm cranial to cervix, was collected for histopathology evaluation. For the residual tissue evaluation of the uterus, all remaining uterine, including the cervix, and vaginal tissue was sectioned longitudinally and examined histologically. Results from the residual uterine evaluation were combined with those from the original, transverse section of uterus. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by quality assessment (QA) pathologists at a pathology laboratory independent of the study laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For both 2-year studies, the QA pathologists evaluated slides from all neoplasms and all potential target organs, which included the liver, pancreas, kidney, heart, bone marrow, and pituitary gland of rats; testes and epididymis of male rats; and the uterus of female rats. Kidney pathology is reported only for Study 1. Additional sex-specific target tissues identified in Study 1 included the prostate glands, gubernacula, phallus, prepuce, seminal vesicles, and vagina.

The QA report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the QA/PWG coordinators to the PWG for review. The PWG consisted of the QA pathologists and other pathologists experienced in rodent toxicological pathology. The PWG examined the tissues without any knowledge of exposure groups. When the PWG consensus diagnosis differed from that of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[166] and Boorman et al.[167] For subsequent analyses of the pathology data, the decision whether or not to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al.[168]

Table 1. Experimental Design and Materials and Methods in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

Perinatal and Postweaning Study (Study 1)	Postweaning-only Study (Study 2)
Study Laboratory	
Battelle (Columbus, OH)	Same as Study 1
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®) rats	Same as Study 1
Animal Source	
Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN)	Same as Study 1
Time Held Before Studies	
F_0 female rats: 4 days	14 or 15 days
Average Age When Studies Began	
F_0 female rats: 11 to 13 weeks	5 to 6 weeks
Date of First Exposure	
F_0 female rats: May 20, 2011	February 17 (males) or 18 (females), 2011
F_1 rats: June 27 (males) or 28 (females), 2011	
Duration of Exposure	
F_0 female rats: GD 6 to LD 21	2 years
F_1: Perinatal plus 2 years	
Date of Last Exposure	
F_0 female rats: June 27, 2011	February 21 (males) or 27 (females), 2013
F_1 rats: June 27 (males) or July 3 (females), 2013	
Necropsy Dates	
F_1 rats: June 24 to 27 (males) or June 28 to July 3 (females), 2013	February 18 to 21 (males) or February 22 to 27 (females), 2013
Average Age at Necropsy	
F_1 rats: 2 years	2 years
Size of Study Groups	
F_0 female rats: 45	50/sex
F_1 rats: 50/sex	
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights	Same as Study 1
Animals per Cage	
F_0 female rats: 1 (with litter)	2 (males) or 4 (females)
F_1 rats: 2 (males) or 4 (females)	

Perinatal and Postweaning Study (Study 1)	Postweaning-only Study (Study 2)
Method of Animal Identification	
F_0 female rats: Cage card and tail marking with permanent pen	Cage card and tail tattoo
F_1 (pups): Limb tattoo	
F_1 rats (2-year study): Cage card and tail tattoo	
Diet	
Irradiated NIH-07 meal feed (perinatal phase) or irradiated NTP-2000 meal feed (2-year study) (Zeigler Brothers, Inc, Gardners, PA), available ad libitum, changed twice weekly	Irradiated NTP-2000 meal feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed twice weekly
Water	
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available ad libitum	Same as Study 1
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE); changed weekly through PND 4, then at least twice weekly; rotated every 2 weeks	Solid polycarbonate (Lab Products, Inc., Seaford, DE); changed twice weekly; rotated every 2 weeks
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as Study 1
Rack Filters	
Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH, or National Media Filter Corporation, Olive Branch, MS), changed every 2 weeks	Same as Study 1
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated at least every 2 weeks	Same as Study 1
Animal Room Environment	
Temperature: 72°F ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Same as Study 1
Exposure Concentrations	
0, 300, 1,000, 3,000, and 10,000 ppm in feed	Same as Study 1

Perinatal and Postweaning Study (Study 1)	Postweaning-only Study (Study 2)
Type and Frequency of Observation	
F_0 female rats: Observed twice daily. Weighed on GDs 5, 6, 9, 12, 15, 18, and 21 and on LDs 1, 4, 7, 14, and 21. Feed consumption was measured over 3-day intervals from GD 6 to GD 21 and LD 1 to LD 21. F_1 rats: Observed twice daily. Litter data (total litter weight, litter weights by sex, and litter observations) were recorded on PND 1. Pup survival was evaluated and recorded. Individual pups were weighed on PNDs 4, 7, 14, and 21, weekly for the first 13 weeks after weaning, every 4 weeks thereafter, and at the end of the study. Clinical findings were recorded every 4 weeks beginning on day 29 and at the end of the study. Feed consumption was recorded weekly for the first 13 weeks and at 4-week intervals thereafter.	Observed twice daily. Body weights were recorded initially, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the study. Clinical findings were recorded every 4 weeks beginning at week 5 and at the end of the study. Feed consumption was recorded weekly for the first 13 weeks and at 4-week intervals thereafter.
Method of Euthanasia	
Carbon dioxide	Same as Study 1
Necropsy	
Necropsies were performed on all animals.	Same as Study 1
Histopathology	
Complete histopathology was performed on all rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidneys, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.	Same as Study 1
Internal Dose Assessment	
Maternal plasma, amniotic fluid, and fetal (pooled by litter) mono(2-ethylhexyl) phthalate concentrations were measured at GD 18.	None

GD = gestation day; LD = lactation day; PND = postnatal day

Benchmark Dose Analysis

Benchmark doses (BMDs) were calculated using the EPA Benchmark Dose Software (BMDS), version 3.1.2.[169] and are presented in Appendix F. The dose variable for the models was the amount of DEHP consumed in mg/kg body weight/day (mg/kg/day). Numbers of animals per exposure group were poly-3-adjusted survival numbers. The response variable was the incidence of the endpoint being modeled.

All of the frequentist dichotomous models in the BMDS were used. The logistic, log-probit, and probit models were used with no parameter restrictions. Other models (dichotomous Hill, gamma, log-logistic, multistage, and Weibull) were used with default restrictions on the ranges of some of the parameters, as described in the BMDS User Guide.[170]

The benchmark response (BMR) used in the models was 0.1 (10%) extra risk, with estimated background levels. The benchmark dose lower confidence limit (BMDL) was calculated using a 95% confidence interval. The decision logic used to recommend one model from the fitted models was the default logic.[170]

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[171] and is presented graphically. Animals surviving to the end of the observation period are treated as censored observations, as are animals dying from unnatural causes within the observation period. Animals dying from natural causes are included in analyses and are treated as uncensored observations. For the postweaning-only study (Study 2), exposure concentration-related trends are identified with Tarone's life-table test,[172] and pairwise exposure concentration-related effects are assessed using Cox's method.[173] For the perinatal and postweaning study (Study 1), exposure concentration-related trends and pairwise exposure-related effects on survival are assessed using a Cox proportional hazards model[173] with a random litter effect. All reported p values for the survival analyses are two-sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented as the numbers of animals bearing such lesions at a specific anatomic site. For calculation of incidence rates, the denominator for most neoplasms and all nonneoplastic lesions is the number of animals where the site was examined microscopically. When macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, however, the denominator consists of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominator consists of the number of animals on which a necropsy was performed. Additional study data also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal euthanasia.

Analysis of Neoplasm and Nonneoplastic Lesion Incidence

Statistical analyses of neoplasm and nonneoplastic lesion incidence considered two features of the data. Some animals did not survive the entire 2 years of the study, so survival differences between groups had to be considered. Also, up to two animals per sex were randomly selected from each litter to participate in the study, except for the 10,000 ppm group in the perinatal and postweaning study (Study 1) for which additional males and females were needed to populate the

study. The statistical analysis of lesion incidence used the Poly-3 test to account for survival differences, with a Rao-Scott adjustment for litter effects, as described below.

The Poly-k test[174-176] was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to account for survival differences. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is 1 if the animal had a lesion at that site or if it survived until terminal euthanasia; if the animal died before terminal euthanasia and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only on the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time.[174] Unless otherwise specified, a value of k = 3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier[174] after an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice.[177] Bailer and Portier[174] showed that the Poly-3 test provided valid results if the true value of k is anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams.[178] Poly-3 tests used the continuity correction described by Nam.[179]

Littermates tend to be more like each other than like fetuses/pups in other litters. Failure to account for correlation within litters leads to underestimates of variance in statistical tests, resulting in higher probabilities of Type I errors ("false positives"). Because up to two pups per sex per litter were present in the perinatal and postweaning study (Study 1), the Poly-3 test was modified to accommodate litter effects using the Rao-Scott approach.[180] The Rao-Scott approach accounts for litter effects by estimating the ratio of the variance in the presence of litter effects to the variance in the absence of litter effects. This ratio is then used to adjust the sample size downward to yield the estimated variance in the presence of litter effects. The Rao-Scott approach was implemented in the Poly-3 test as recommended by Fung et al.,[181] formula $\bar{\tau}_{RS2}$.

Tests of significance included pairwise comparisons of each exposed group with control groups and a test for an overall exposure concentration-related trend. Continuity-corrected Rao-Scott-adjusted Poly-3 tests were used in the analysis of lesion incidence and reported p values are one-sided. The significance of a lower incidence or negative trend in lesions is approximated as $1 - p$ with the letter N added (e.g., p = 0.99 is presented as p = 0.01N). For neoplasms and nonneoplastic lesions observed without litter structure (e.g., at the interim evaluation), Poly-3 tests that included the continuity correction, but without adjustment for potential litter effects, were used for trend and pairwise comparisons to the control group.

To evaluate incidence rates by litter, the proportions of litters affected by each lesion type were tested among groups. The Cochran-Armitage trend test and Fisher's exact test[182] were used to test for trends and pairwise differences from the control group, respectively.

Analysis of Continuous Variables

Before statistical analysis, extreme values identified by the outlier test of Dixon and Massey,[183] for small samples ($n < 20$), and Tukey's outer fences method,[184] for large samples ($n \geq 20$), were examined by NTP personnel, and implausible values were eliminated from the analysis. Organ and body weight measurements, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[185] and Williams.[186; 187] Dam gestational and lactational feed consumption, litter sizes, pup survival, implantations, number of resorptions, and proportions of male pups per litter for all studies were analyzed using the nonparametric multiple comparison methods of Shirley[188] [as modified by Williams[189]] and Dunn[190] given that these endpoints typically have skewed distributions. For all quantitative endpoints unaffected by litter structure, the Jonckheere test[191] was used to assess the significance of exposure concentration-related trends and to determine, at the 0.01 level of significance, whether a trend-sensitive test (the Williams or Shirley test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic exposure concentration-related trend (the Dunnett or Dunn test).

Postweaning body weights were measured on two pups/sex/litter in most cases in the perinatal and postweaning study (Study 1); more than two pups/sex/litter were common in preweaning body weight measurements. The analyses of pup mean body weights and mean body weights adjusted for litter size (described below) of these animals took litter effects into account using a mixed model with litter as a random effect. To adjust for multiple comparisons, a Dunnett-Hsu adjustment was used.[192] Dam mean body weights during gestation and lactation were analyzed with the parametric multiple comparison procedures of Dunnett[185] and Williams,[186; 187] depending on whether the Jonckheere test indicated the use of a trend-sensitive test. P values for these analyses are two-sided.

Analysis of Gestational and Fertility Indices

Cochran-Armitage trend tests were used to test the significance of trends in gestational and fertility indices across exposure groups. Fisher's exact test was used to conduct pairwise comparisons of each exposed group with the control group. P values for these analyses are two-sided.

Body Weight Adjustments

To adjust preweaning pup body weights for live litter size, a linear model was fit to body weights as a function of exposure concentration and litter size. The estimated coefficient of litter size was then used to adjust each pup body weight on the basis of the difference between its litter size and the mean litter size. Preweaning pup body weights were adjusted for PND 1 live litter size. After adjustment, mean body weights were analyzed with a linear mixed model with a random litter effect.

Historical Control Data

The concurrent control group is the most valid comparison to the exposed groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential exposure-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control

data must be generally similar. Significant factors that can affect the background incidence of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period,[193-195] including the concurrent control for comparison across multiple technical reports. In general, the historical control data for a given study includes studies using the same route of administration, and the overall incidence of neoplasms in control animals for all routes of administration are included for comparison, including the current study.

Quality Assurance Methods

Both the perinatal and postweaning study (Study 1) and the postweaning-only study (Study 2) were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations.[196] In addition, both study reports were audited retrospectively by an independent QA contractor against study records submitted to the NTP Archives. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The protocols used for the conduct and evaluation of the in vivo chromosomal aberrations and micronucleus tests are described in detail in Appendix D.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage on the basis of the relationship between electrophilicity and mutagenicity[197] and the somatic mutation theory of cancer.[198; 199] It should be noted, however, that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites.[200] Information from other in vitro genotoxicity assays does not appear to increase the predictivity of the bacterial mutation assay for rodent carcinogenicity, but these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation. Positive results seen in in vivo assays that measure induction of chromosomal damage have been shown to have a high correlation with rodent carcinogenicity.[201]

Results

Data Availability

The National Toxicology Program (NTP) evaluated all study data. Data relevant for evaluating toxicological findings are presented here. All study data are available in the NTP Chemical Effects in Biological Systems (CEBS) database: https://doi.org/10.22427/NTP-DATA-TR-601.[202]

Perinatal and Postweaning Study in Rats (Study 1)

Perinatal Phase

No effects on maternal survival were observed following exposure to di(2-ethylhexyl) phthalate (DEHP), and no exposure-related maternal clinical observations were noted (Appendix H). Administration of DEHP had no effects on the percentage of pregnant females that produced pups, gestation length, or pup sex distribution (Table 2; Appendix H). The lower number of females that produced pups in the 10,000 ppm group was due to 13 mated females that were not pregnant. This was not attributed to the test article, because dam exposure to DEHP started after the period of implantation.

Table 2. Summary of the Disposition of F_0 Female Rats during Perinatal Exposure in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Reproductive Performance					
Time-mated Females (GD 6)	45	45	45	45	45
Females Pregnant (%)[a]	39 (86.7)	35 (77.8)	38 (84.4)	35 (77.8)	32 (71.1)
Females Not Pregnant (%)	6 (13.3)	10 (22.2)	7 (15.6)	10 (22.2)	13 (28.9)
Dams Not Delivering with Evidence of Pregnancy (%)	5 (12.8)	6 (17.1)	7 (18.4)	6 (17.1)	6 (18.8)
Dams with Litters on LD 0 (%)[a]	34 (87.2)	29 (82.9)	31 (81.6)	29 (82.9)	26 (81.2)
Gestation Length (Days)[b,c]	22.1 ± 0.1	22.1 ± 0.1	22.3 ± 0.1	22.0 ± 0.0	22.2 ± 0.1
Number of Litters on LD 4[d]	34	29	28	29	26
Weaned Males/Females	204/213	166/178	180/166	166/173	138/117

GD = gestation day; LD = lactation day.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]Gestation length calculated for sperm-positive females that delivered a litter. Data are presented as mean ± standard error.
[d]Litters were not standardized in this study.

Mean gestation body weights of dams receiving up to 3,000 ppm DEHP in the diet were within approximately 3.5% of control animals (Table 3). Dams that received 10,000 ppm DEHP in the diet displayed significantly decreased mean body weights (up to 10%), relative to control animals, throughout the gestational period. Lower relative body weights in 10,000 ppm dams were associated with significantly decreased body weight gain over the GD 6–9, GD 15–18, and GD 18–21 intervals (Table 3). During the gestational period (GD 6–21), the overall mean body weight gain of 10,000 ppm dams was significantly decreased 27% compared to that of the control animals. During the lactational period, there were no effects on maternal mean body weight or body weight gain among dam groups receiving up to 3,000 ppm DEHP (Table 3). Lactational body weights of 10,000 ppm dams were significantly decreased (up to approximately 25%) at all assessed lactational time points relative to control animals. This decrease was more severe in magnitude than what was observed during gestation and is likely the result of decreases in absolute body weight during the lactational period. All other exposure groups, including the control group, displayed positive weight gains during the lactation day (LD) 4–21 interval, whereas the 10,000 ppm dams lost an average of 24 g in body weight, corresponding to an approximate 10% decrease in body weight from LD 1 to LD 21.

For dams exposed at 10,000 ppm DEHP, significantly decreased feed consumption during both gestation (GD 6–21; approximately 14%) and lactation (LD 1–14; 39%), relative to control animals (Table 4), likely contributed to the observed decrements in body weight (Table 3). Decreased feed consumption (8%) was also observed in 3,000 ppm dams during the LD 17–21 interval, and attained statistical significance, relative to control animals. Gestational DEHP intake (GD 6–21) for dams in the 300, 1,000, 3,000, and 10,000 ppm groups was approximately 21, 68, 206, and 626 mg DEHP/kg body weight/day (mg/kg/day), respectively (Table 4). Lactational DEHP intake (LD 1–14) for dams in the 300, 1,000, 3,000, and 10,000 ppm groups was approximately 49, 166, 482, and 1,244 mg/kg/day, respectively (Table 4). Chemical intake for the LD 14–21 interval was not calculated due to the unknown contribution of offspring feed consumption to the overall cage-based measurements.

Table 3. Summary of Mean Body Weights and Body Weight Gains of F_0 Female Rats during Gestation and Lactation in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Parameter[a,b]	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Gestation Day					
6	237.4 ± 2.14 (39)	236.1 ± 2.63 (35)	234.6 ± 2.63 (38)	237.7 ± 1.90 (35)	236.4 ± 2.38 (32)
9	253.1 ± 2.43** (39)	251.9 ± 2.84 (35)	246.6 ± 3.67 (38)	250.1 ± 3.07 (35)	244.6 ± 1.88* (32)
12	270.4 ± 2.03** (39)	267.9 ± 2.42 (35)	264.8 ± 2.52 (38)	267.7 ± 2.05 (35)	258.2 ± 1.93** (32)
15	288.0 ± 2.06** (39)	284.5 ± 2.64 (35)	280.7 ± 3.14 (38)	286.5 ± 2.39 (35)	274.8 ± 2.30** (32)
18	326.2 ± 2.48** (39)	323.4 ± 3.57 (35)	319.1 ± 3.97 (38)	323.4 ± 4.77 (35)	304.4 ± 3.04** (32)
21[c]	374.2 ± 3.62** (34)	365.6 ± 5.14 (30)	361.0 ± 6.19 (33)	365.2 ± 6.95 (31)	335.6 ± 4.63** (28)
Gestation Weight Change					
Gestation Day Interval					
6–9	15.70 ± 1.35** (39)	15.77 ± 1.60 (35)	12.02 ± 2.04 (38)	12.34 ± 1.75 (35)	8.22 ± 1.14** (32)
9–12	17.31 ± 1.17* (39)	15.98 ± 1.19 (35)	18.24 ± 1.61 (38)	17.64 ± 1.71 (35)	13.66 ± 0.64 (32)
12–15	17.61 ± 0.68 (39)	16.68 ± 0.67 (35)	15.91 ± 1.45 (38)	18.73 ± 0.87 (35)	16.53 ± 0.72 (32)
15–18	38.18 ± 0.95** (39)	38.87 ± 1.40 (35)	38.39 ± 2.11 (38)	36.94 ± 3.14 (35)	29.60 ± 1.04** (32)
18–21	48.47 ± 1.29** (34)	44.81 ± 1.79 (30)	43.84 ± 2.22 (33)	44.50 ± 3.20 (31)	32.58 ± 1.81** (28)
6–21	137.2 ± 2.96** (34)	130.5 ± 4.49 (30)	126.2 ± 6.02 (33)	128.6 ± 6.10 (31)	100.2 ± 3.03** (28)
Lactation Day					
1	279.3 ± 2.28** (34)	278.4 ± 2.68 (29)	279.0 ± 2.21 (31)	281.3 ± 2.19 (29)	254.3 ± 2.06** (26)
4	294.4 ± 2.53** (34)	292.9 ± 2.99 (28)[d]	292.2 ± 2.36 (29)	292.7 ± 2.60 (29)	255.8 ± 2.77** (26)
7	312.7 ± 2.43** (34)	306.6 ± 3.35 (29)	309.9 ± 2.84 (29)	312.5 ± 2.35 (29)	254.2 ± 3.07** (26)
14	317.4 ± 2.29** (34)	315.2 ± 2.41 (29)	317.3 ± 3.07 (29)	315.9 ± 2.30 (29)	238.7 ± 3.84** (26)
21	293.8 ± 2.34** (34)	294.9 ± 2.52 (29)	293.6 ± 2.77 (29)	298.3 ± 2.40 (29)	230.1 ± 3.79** (26)
Lactation Weight Change					
Lactation Day Interval					
1–4	15.12 ± 1.24** (34)	14.23 ± 0.99 (28)	12.53 ± 1.44 (29)	11.34 ± 1.75 (29)	1.53 ± 1.81** (26)
4–7	18.27 ± 1.19** (34)	15.09 ± 1.46 (28)	17.73 ± 1.23 (29)	19.83 ± 1.74 (29)	−1.65 ± 1.55** (26)
7–14	4.74 ± 1.55** (34)	8.58 ± 2.22 (29)	7.42 ± 2.56 (29)	3.40 ± 1.93 (29)	−15.5 ± 2.67** (26)
14–21	−23.6 ± 1.40** (34)	−20.4 ± 1.63 (29)	−23.7 ± 2.36 (29)	−17.5 ± 1.54* (29)	−8.61 ± 3.25** (26)
4–21	14.53 ± 1.40** (34)	16.47 ± 1.44 (29)	13.93 ± 2.36 (29)	17.02 ± 2.80 (29)	−24.2 ± 3.02** (26)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Data are presented as mean ± standard error (number of dams). Body weight data are presented in grams.
[b]Each exposure group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[c]Decreased number of dams at gestation day (GD) 21 reflects animals removed at GD 18 for internal dose assessment.
[d]One dam in the 300 ppm group was removed as an outlier on lactation day 4.

Table 4. Summary of Feed and Di(2-ethylhexyl) Phthalate Consumption by F_0 Female Rats during Gestation and Lactation in the Perinatal and Postweaning Two-year Feed Study

Parameter[a]	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Gestation Day Interval[b,c]					
6–9	17.56 ± 0.41** (39)	17.64 ± 0.45 (35)	16.16 ± 0.72 (38)	16.75 ± 0.58 (35)	13.97 ± 0.22** (32)
9–12	18.64 ± 0.23** (39)	18.61 ± 0.23 (35)	18.33 ± 0.23 (38)	18.43 ± 0.24 (35)	16.11 ± 0.28** (32)
12–15	18.59 ± 0.27 (39)	18.24 ± 0.23 (35)	18.01 ± 0.42 (38)	18.53 ± 0.28 (35)	17.73 ± 0.25 (32)
15–18	20.41 ± 0.36** (39)	20.87 ± 0.32 (35)	20.74 ± 0.38 (38)	21.02 ± 0.59 (35)	18.16 ± 0.21** (32)
18–21	23.26 ± 0.29** (34)	22.59 ± 0.37 (30)	22.89 ± 0.41 (33)	23.41 ± 0.57 (31)	19.29 ± 0.29** (28)
6–21	19.69 ± 0.23** (34)	19.45 ± 0.26 (30)	19.17 ± 0.29 (33)	19.50 ± 0.30 (31)	17.03 ± 0.19** (28)
Lactation Day Interval[b,c]					
0–4	35.54 ± 0.71** (30)	34.83 ± 0.67 (28)	35.65 ± 0.70 (25)	34.97 ± 1.01 (18)	28.73 ± 0.98** (23)
4–7	47.91 ± 0.79** (33)	45.83 ± 0.78 (29)	47.26 ± 0.98 (29)	47.81 ± 1.29 (29)	30.68 ± 0.74** (26)
7–10	54.85 ± 1.02** (34)	51.92 ± 0.98 (29)	53.85 ± 1.04 (28)	53.65 ± 1.49 (29)	31.38 ± 0.75** (26)
10–14	63.36 ± 0.98** (34)	63.12 ± 0.76 (29)	61.30 ± 1.42 (29)	61.74 ± 1.70 (29)	33.36 ± 0.92** (26)
14–17	62.05 ± 1.13** (34)	63.72 ± 0.89 (29)	63.26 ± 0.98 (29)	62.52 ± 1.63 (29)	37.70 ± 1.19** (26)
17–21	86.25 ± 1.28** (33)	84.99 ± 1.64 (29)	82.42 ± 1.85 (29)	79.65 ± 2.34* (29)	45.74 ± 2.12** (26)
1–14	51.01 ± 0.80** (29)	50.13 ± 0.65 (28)	50.28 ± 0.89 (24)	49.26 ± 1.78 (18)	31.04 ± 0.74** (23)
Chemical Intake (mg/kg/day)[d,e]					
GD 6–21	0.00 ± 0.00 (34)	20.58 ± 0.16 (30)	68.11 ± 0.71 (33)	205.6 ± 2.10 (31)	625.6 ± 6.23 (28)
LD 1–14	0.00 ± 0.00 (29)	49.40 ± 0.52 (27)	165.5 ± 2.77 (24)	482.1 ± 15.93 (18)	1,244 ± 25.58 (23)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
GD = gestation day; LD = lactation day.
[a]Data are presented as mean ± standard error (number of dams).
[b]Feed consumption data are presented as grams / animal / day.
[c]Each exposure group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[d]Chemical intake calculated as: ([exposure concentration × feed consumption]/[average body weight of day range]).
[e]No statistical analysis performed on the chemical intake data.

On postnatal day (PND) 1, total litter size and total live litter size of the 10,000 ppm group were significantly decreased, relative to the control group, corresponding to a reduction of approximately two pups per litter (Table 5). This litter effect corresponded to a significantly decreased number of live female offspring in 10,000 ppm DEHP-exposed litters. A significant decrease in the survival ratio (PND 5–21) of offspring exposed to 3,000 ppm was observed; however, this effect was not considered related to exposure because the survival ratio at 10,000 ppm was not different from that of the control group. No other effect of DEHP exposure on offspring survival was observed during the preweaning intervals (PND 1–4 and PND 5–21) (Table 5).

Table 5. Summary of Mean Litter Size and Survival Ratio of F_1 Male and Female Rats during Lactation in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Parameter	0 ppm	300 ppm	1,000 ppm[a]	3,000 ppm	10,000 ppm
PND 1[b,c]					
Total	12.59 ± 0.38* (34)	12.07 ± 0.31 (29)	11.73 ± 0.66 (30)	12.24 ± 0.55 (29)	10.54 ± 0.45** (26)
Live	12.44** ± 0.36 (34)	12.07 ± 0.31 (29)	11.67 ± 0.69 (30)	12.21 ± 0.57 (29)	10.08 ± 0.46** (26)
% Male per Litter	48.73 ± 2.39 (34)	46.87 ± 2.02 (29)	51.95 ± 2.89 (28)	49.06 ± 2.80 (29)	54.83 ± 2.93 (26)
% Male[d,e]	49 (423)	47 (350)	52 (350)	48 (354)	54 (262)
Male[b,c]					
PND 1	6.12 ± 0.38 (34)	5.72 ± 0.33 (29)	6.03 ± 0.48 (30)	5.90 ± 0.42 (29)	5.42 ± 0.34 (26)
PND 4	6.09 ± 0.39 (34)	5.76 ± 0.32 (29)	6.46 ± 0.41 (28)	5.86 ± 0.41 (29)	5.42 ± 0.36 (26)
PND 21	6.00 ± 0.39 (34)	5.72 ± 0.32 (29)	6.43 ± 0.41 (28)	5.72 ± 0.41 (29)	5.31 ± 0.35 (26)
Female[b,c]					
PND 1	6.32 ± 0.33* (34)	6.34 ± 0.24 (29)	5.63 ± 0.48 (30)	6.31 ± 0.47 (29)	4.65 ± 0.35** (26)
PND 4	6.32 ± 0.33** (34)	6.24 ± 0.25 (29)	5.96 ± 0.40 (28)	6.28 ± 0.48 (29)	4.54 ± 0.36** (26)
PND 21	6.26 ± 0.33** (34)	6.14 ± 0.25 (29)	5.93 ± 0.41 (28)	5.97 ± 0.49 (29)	4.50 ± 0.36** (26)
Male and Female[b,c]					
PND 4	12.41 ± 0.36** (34)	12.00 ± 0.32 (29)	12.43 ± 0.40 (28)	12.14 ± 0.57 (29)	9.96 ± 0.44** (26)
PND 7	12.41 ± 0.36** (34)	11.93 ± 0.31 (29)	12.43 ± 0.40 (28)	11.97 ± 0.57 (29)	9.88 ± 0.44** (26)
PND 10	12.38 ± 0.36** (34)	11.90 ± 0.31 (29)	12.43 ± 0.40 (28)	11.83 ± 0.59 (29)	9.88 ± 0.44** (26)
PND 14	12.29 ± 0.35** (34)	11.86 ± 0.30 (29)	12.39 ± 0.40 (28)	11.69 ± 0.59 (29)	9.81 ± 0.43** (26)
PND 17	12.29 ± 0.35** (34)	11.86 ± 0.30 (29)	12.36 ± 0.39 (28)	11.69 ± 0.59 (29)	9.81 ± 0.43** (26)
PND 21	12.26 ± 0.35** (34)	11.86 ± 0.30 (29)	12.36 ± 0.39 (28)	11.69 ± 0.59 (29)	9.81 ± 0.43** (26)
Survival per Litter					
Total Dead: PND 1–4[f]	6 (34)	2 (29)	3 (30)	3 (29)	15 (26)
Total Dead: PND 5–21[f]	5 (34)	4 (29)	2 (28)	13 (29)	4 (26)
Dead: PND 1–4[b,c,g]	0.176 ± 0.079 (34)	0.069 ± 0.048 (29)	0.100 ± 0.056 (30)	0.103 ± 0.058 (29)	0.577 ± 0.294 (26)

Parameter	0 ppm	300 ppm	1,000 ppm[a]	3,000 ppm	10,000 ppm
Dead: PND 5–21[b,c,g]	0.147 ± 0.096 (34)	0.138 ± 0.108 (29)	0.071 ± 0.050 (28)	0.448 ± 0.183* (29)	0.154 ± 0.091 (26)
Survival Ratio: PND 1–4[b,c,h]	0.998 ± 0.002 (34)	0.994 ± 0.004 (29)	0.997 ± 0.003 (28)	0.994 ± 0.004 (29)	0.990 ± 0.005 (26)
Survival Ratio: PND 5–21[b,c,i]	0.990 ± 0.007 (34)	0.990 ± 0.008 (29)	0.995 ± 0.003 (28)	0.962 ± 0.015* (29)	0.986 ± 0.008 (26)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
PND = postnatal day.
[a]One dam in the 1,000 ppm group was not included in any endpoint calculations due to differing pup counts on PND 1 and PND 4. Two additional dams in the 1,000 ppm group produced single pups that died on PND 1; these dams were only included in the PND 1 calculations.
[b]Each exposure group was compared to the vehicle control group with the Shirley test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunn test when no trend was present.
[c]Data are presented as mean ± standard error (number of dams).
[d][100 × (number of live males in exposure group)/(number of live males and females in exposure group)] (number of pups).
[e]No statistics performed on this endpoint.
[f]Total number of dead pups in exposure group (number of dams).
[g]Number dead per litter.
[h]Survival per litter: Number of live pups on PND 4/number of live pups on PND 1.
[i]Survival per litter: Number of live pups on PND 21/number of live pups on PND 5.

Decreased PND 1 pup mean body weights were observed in male, female, and combined (male + female) offspring in 3,000 and 10,000 ppm DEHP litters (Table 6). PND 1 pup mean body weights (male, female, and combined) were significantly decreased by approximately 4% in the 3,000 ppm group, and by 15%, 12%, and 13% in males, females, and combined offspring in the 10,000 ppm group, respectively, relative to control animals. At weaning (PND 21), male and female pup mean body weights in the 1,000 and 3,000 ppm DEHP groups were significantly decreased approximately 6% compared to control animals. Severe effects on growth were evident in male and female offspring exposed to 10,000 ppm DEHP. At weaning (PND 21), mean body weights of male and female pups in the 10,000 ppm group were significantly decreased approximately 55% and 53% relative to those of control animals, respectively (Table 6). Significantly decreased mean body weights in 10,000 ppm pups were attributed to reduced body weight gain throughout the preweaning interval. Pup survival was not reduced, and there were no exposure-related clinical observations. Offspring from the 10,000 ppm group were therefore continued on study.

Table 6. Summary of Preweaning F_1 Male and Female Rat Pup Mean Body Weights Following Perinatal Exposure to Di(2-ethylhexyl) Phthalate

Parameter[a]	0 ppm	300 ppm	1,000 ppm[b]	3,000 ppm	10,000 ppm[c]
Male (g)					
PND 1[d,e,f]	7.45 ± 0.09** (34)	7.59 ± 0.08 (29)	7.43 ± 0.10 (28)	7.16 ± 0.10* (29)	6.34 ± 0.09** (26)
PND 4[g,h]	10.64 ± 0.13** (207/34)	10.51 ± 0.19 (167/29)	10.72 ± 0.16 (181/28)	10.34 ± 0.18 (170/29)	11.13 ± 0.20** (140/26)
PND 7[g,h]	15.12 ± 0.20** (207/34)	14.69 ± 0.28 (166/29)	14.71 ± 0.27 (181/28)	14.60 ± 0.23 (168/29)	11.13 ± 0.20** (140/26)
PND 14[g,h]	29.19 ± 0.30** (205/34)	28.44 ± 0.42 (166/29)	27.80 ± 0.47* (181/28)	28.49 ± 0.44 (166/29)	17.24 ± 0.36** (138/26)
PND 21[g,h]	48.65 ± 0.59** (204/34)	47.05 ± 0.72 (166/29)	45.66 ± 0.79* (180/28)	45.53 ± 0.83* (166/29)	21.82 ± 0.84** (138/26)
Female (g)					
PND 1[d,e,f]	7.01 ± 0.06** (34)	7.17 ± 0.07 (29)	7.02 ± 0.09 (28)	6.76 ± 0.09* (29)	6.19 ± 0.13** (26)
PND 4[g,h]	10.09 ± 0.11** (215/34)	10.07 ± 0.13 (181/29)	10.18 ± 0.17 (167/28)	9.67 ± 0.18 (182/29)	8.35 ± 0.21** (117/26)
PND 7[g,h]	14.25 ± 0.19** (215/34)	13.97 ± 0.23 (180/29)	13.92 ± 0.26 (167/28)	13.50 ± 0.28 (179/29)	10.97 ± 0.26** (117/26)
PND 14[g,h]	27.97 ± 0.30** (213/34)	27.41 ± 0.28 (178/29)	26.57 ± 0.45* (166/28)	27.19 ± 0.44 (173/29)	16.91 ± 0.44** (117/26)
PND 21[g,h]	45.85 ± 0.52** (213/34)	44.84 ± 0.56 (178/29)	43.27 ± 0.78* (166/28)	42.91 ± 0.84* (173/29)	21.64 ± 0.91** (117/26)
Male and Female (g)					
PND 1[d,e,f]	7.20 ± 0.07** (34)	7.35 ± 0.07 (29)	7.22 ± 0.09 (28)	6.96 ± 0.09* (29)	6.29 ± 0.10** (26)
PND 4[g,h]	10.33 ± 0.11** (422/34)	10.28 ± 0.14 (348/29)	10.47 ± 0.16 (348/28)	10.00 ± 0.17 (352/29)	8.51 ± 0.16** (258/26)
PND 7[g,h]	14.66 ± 0.19** (422/34)	14.32 ± 0.23 (346/29)	14.36 ± 0.26 (348/28)	14.05 ± 0.22 (347/29)	11.10 ± 0.20** (257/26)
PND 14[g,h]	28.52 ± 0.28** (418/34)	27.91 ± 0.32 (344/29)	27.23 ± 0.45* (347/28)	27.74 ± 0.40 (339/29)	17.14 ± 0.39** (255/26)
PND 21[g,h]	47.18 ± 0.51** (417/34)	45.90 ± 0.60 (344/29)	44.51 ± 0.77* (346/28)	44.08 ± 0.77** (339/29)	21.79 ± 0.86** (255/26)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
PND = postnatal day.
[a]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons (unless otherwise noted).
[b]All pups from one dam in the 1,000 ppm group were excluded from all body weight calculations due to that dam having differing litter size counts from PND 1 to PND 4. Additionally, one pup was removed from all body weight calculations due to litter misclassification.
[c]Two pups from two dams in the 10,000 ppm group were not included in the PND 4 body weight calculations. One pup was removed as an outlier and the other was excluded due to the pup dying on PND 4.
[d]Data are presented as mean ± standard error (number of dams).
[e]Each exposure group was compared to the vehicle control group with the Williams test when a trend was present ($p \leq 0.01$ from the Jonckheere trend test) or with the Dunnett test when no trend was present.
[f]Total pup weight at PND 1 divided by number of live pups at PND 1.
[g]Data are presented as mean of litter means ± standard error (number of pups/number of litters).
[h]Individual pup weights first adjusted for live litter size on PND 1.

Concentrations of the DEHP metabolite mono(2-ethylhexyl) phthalate (MEHP) were determined in maternal plasma, amniotic fluid, and fetal tissues collected on GD 18 (Table 7) using validated analytical methods (Appendix E). MEHP concentrations increased proportionally to exposure concentration in dam plasma in groups exposed to lower dietary DEHP concentrations (300 and 1,000 ppm). However, at higher dietary concentrations (3,000 and 10,000 ppm), the increase was greater than proportional to exposure concentration, despite proportional increases in chemical consumption, suggesting potential saturation of clearance pathways of MEHP (Figure 2). MEHP was measured in amniotic fluid and fetuses demonstrating transfer of MEHP across the placental barrier and exposure of the developing conceptus. The concentrations in fetuses were approximately 18–28% of the plasma concentrations in dams, suggesting gestational transfer was moderate. MEHP was detected in amniotic fluid and fetus samples from control animals, whereas control dam plasma concentrations were below the limit of detection.

Table 7. Summary of Internal Dose Data for Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
n	5	5	5	4[a]	4[b]
Mono(2-ethylhexyl) Phthalate Concentration[c,d]					
Gestation Day 18					
Dam plasma (ng/mL)	BD[e]	630.2 ± 84.7	2,000.0 ± 156.9	8,950.0 ± 768.4	39,800.0 ± 4,192.9
Amniotic fluid (ng/mL)[f]	45.6 ± 2.0**	73.4 ± 2.7**	123.0 ± 6.2**	456.8 ± 10.6**	1,685.0 ± 156.0**
Fetuses (ng/g)[f]	53.2 ± 7.7**	178.8 ± 22.2**	383.4 ± 18.8**	1,580.0 ± 105.4**	8,295.0 ± 813.3**

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
**Statistically significant at $p \leq 0.01$.
BD = below detection; group did not have over 20% of its values above the limit of detection.
[a]One female in the 3,000 ppm group was found not to be pregnant and was replaced with an additional dam. The replacement dam was also found not to be pregnant, and another replacement dam was not selected.
[b]Two females in the 10,000 ppm group were found not to be pregnant; one replacement dam was selected and added for analysis.
[c]Data are presented as mean ± standard error.
[d]If over 20% of the animals in a group were above the limit of detection (LOD), one-half of the LOD value was substituted for values below the LOD. LOD for dam plasma = 5 ng/mL; LOD for amniotic fluid = 12 ng/mL; LOD for fetuses = 10 ng/g.
[e]When the vehicle control group did not have over 20% of its values above the LOD, no mean or standard error were calculated and no statistical analysis was performed.
[f]Statistical analysis performed by the Jonckheere (trend) and the Shirley or Dunn (pairwise) tests.

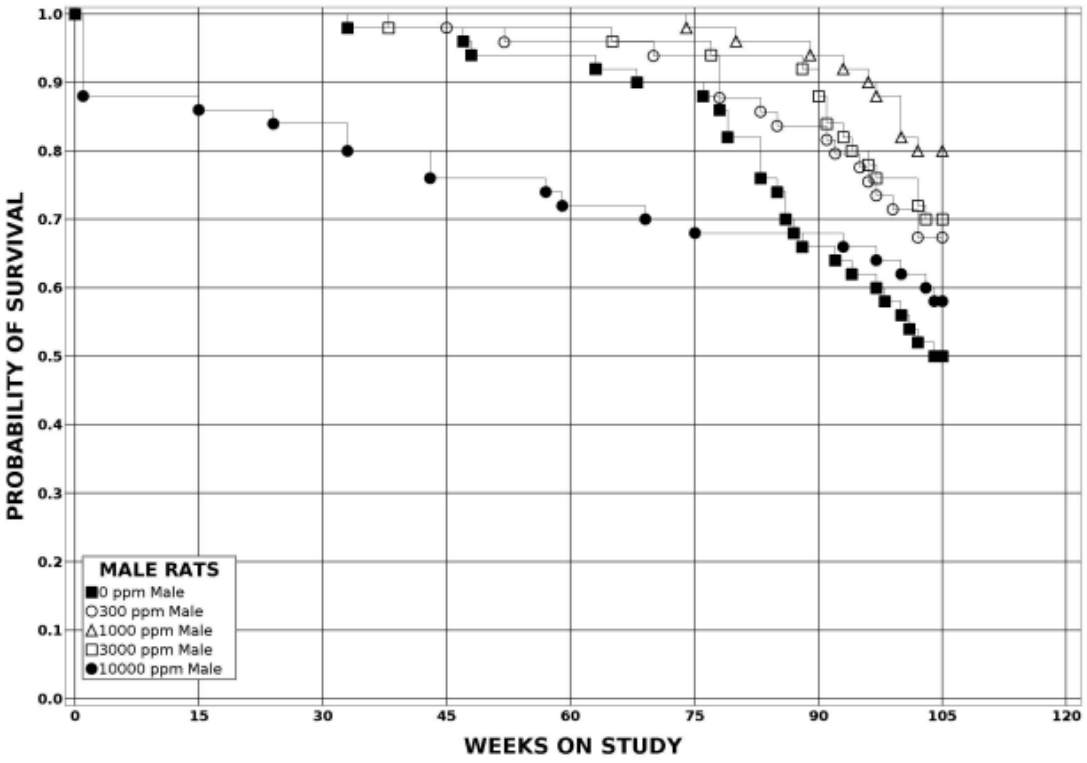
Postweaning Phase

Overall, survival at study termination of males and females in exposed groups was commensurate with control groups (Table 8; Figure 3). However, 6 to 7 rats per sex in the 10,000 ppm group died postweaning during the first two weeks of the study period. All but one of these early losses likely resulted from the severely reduced body weights of those animals.

Table 8. Summary of Survival of Male and Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
Animals Initially in Study	50	49[a]	50	50	50
Moribund	12	9	4	8	7
Natural Deaths	13	7	6	7	14
Animals Surviving to Study Termination	25[b]	33	40[b]	35	29[b]
Percent Probability of Survival at End of Study[c]	50.0	67.3	80.0	70.0	58.0
Mean Survival (Days)[d]	648	683	713	694	555
Survival Analysis[e]	p = 0.171	p = 0.118N	p = 0.004N	p = 0.059N	p = 0.938N
Female					
Animals Initially in Study	50	50	50	50	50
Moribund	13	9	9	7	12
Natural Deaths	6	9	7	9	11
Animals Surviving to Study Termination	31	32	34	34[f]	27
Percent Probability of Survival at End of Study	62.0	64.0	68.0	68.0	54.0
Mean Survival (Days)	684	671	679	681	594
Survival Analysis	p = 0.083	p = 0.995N	p = 0.693N	p = 0.667N	p = 0.185

[a]One pup was mis-sexed at the beginning of the study and was removed.
[b]Includes one animal that died naturally during the last week of the study.
[c]Kaplan-Meier determinations.
[d]Mean of litter means of all deaths (uncensored, censored, and study termination).
[e]The result of the Cox proportional hazards trend test is in the vehicle control column, and the results of the Cox proportional hazards pairwise comparisons with the vehicle control group are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by N.
[f]Includes one animal that died naturally and one animal that was euthanized moribund during the last week of the study.



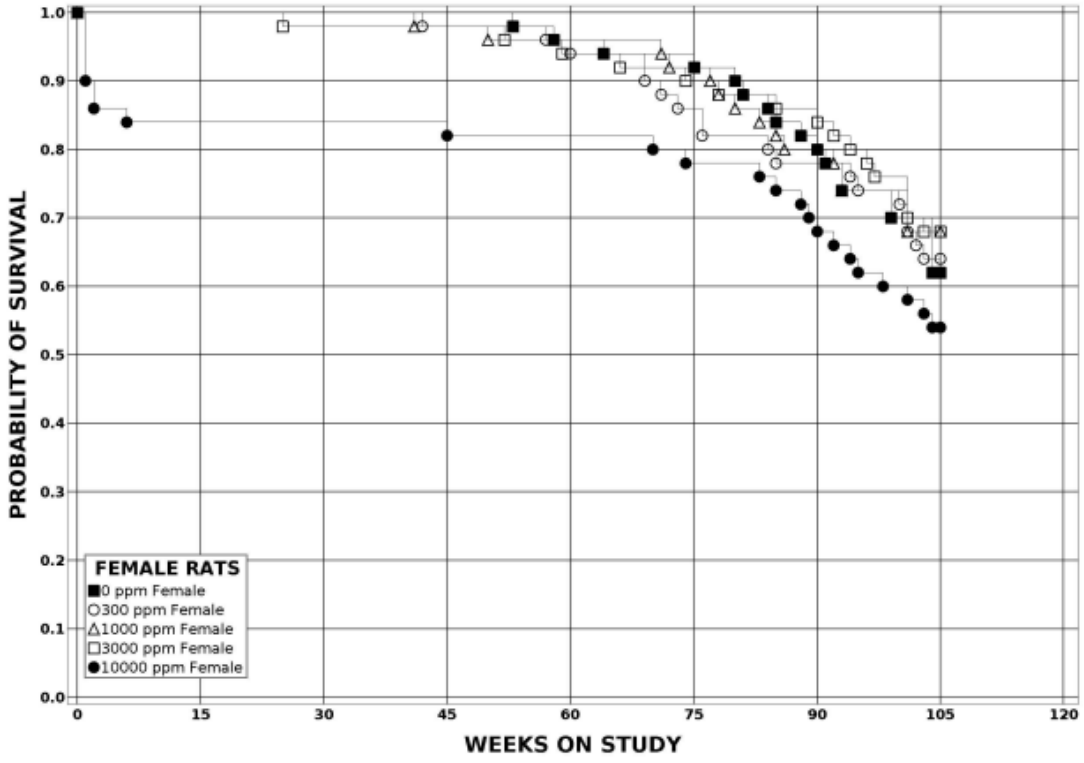


Figure 3. Kaplan-Meier Survival Curves for Rats Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study

At study termination, group mean body weights for male and female rats in the 300, 1,000, and 3,000 ppm DEHP groups were within 10% of their respective control groups (Table 9, Table 10; Figure 4). During the first 3 (females) or 6 (males) weeks on study postweaning, mean body weights of rats in the 10,000 ppm groups were approximately half that of their respective control groups. Following week 3 (females) or week 6 (males) on study, however, mean body weights of those male and female rats exhibited a moderate recovery, attaining maximum group mean body weights that were 28% and 17% lower, respectively, relative to control animals during the chronic study period. The terminal mean body weights of 10,000 ppm males and females were 30% and 32% lower than those of control animals, respectively. Lower body weights observed in the 10,000 ppm groups at study termination were attributed to reduced body weight gain observed over the duration of the study.

Feed consumption by male and female rats in the 300, 1,000, and 3,000 ppm DEHP groups was commensurate to that of the control groups throughout the study (Table 11, Table 12; Appendix H). Feed consumption was generally lower in the 10,000 ppm male and female rat groups with the largest difference directly following weaning. This finding was restricted to the early time interval and likely resulted from the significantly reduced body size of animals in the highest exposure group. Dietary concentrations of 300, 1,000, 3,000, and 10,000 ppm resulted in average daily doses of approximately 18, 58, 189, and 678 mg/kg/day for males and 18, 62, 196, and 772 mg/kg/day for females (Appendix H).

No exposure-related clinical findings were observed in any of the exposed groups (Appendix H).

Table 9. Summary of Survival and Mean Body Weights of Male Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Study Day[a]	0 ppm		300 ppm			1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)[b]	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	50.8	25	50.6	99.5	25	49.2	96.7	25	48.9	96.1	25	27.5	54.1	25
8	82.1	25	80.6	98.1	25	78.5	95.6	25	76.6	93.3	25	35.7	43.4	23
15	127.7	25	121.3	95.0	25	119.0	93.2	25	117.6	92.1	25	53.5	41.9	23
22	175.0	25	168.2	96.1	25	165.9	94.8	25	164.6	94.1	25	75.6	43.2	23
29	217.7	25	214.4	98.5	25	212.6	97.7	25	212.2	97.5	25	101.5	46.6	23
36	262.2	25	257.7	98.3	25	255.3	97.4	25	257.0	98.0	25	130.3	49.7	23
43	293.6	25	290.5	99.0	25	285.6	97.3	25	287.4	97.9	25	158.0	53.8	23
50	316.3	25	314.8	99.5	25	310.3	98.1	25	310.6	98.2	25	186.1	58.8	23
57	333.3	25	334.7	100.4	25	330.6	99.2	25	327.7	98.3	25	208.2	62.5	23
64	350.8	25	351.4	100.2	25	347.2	98.9	25	342.9	97.7	25	225.4	64.2	23
71	364.6	25	365.0	100.1	25	357.4	98.0	25	352.7	96.7	25	239.9	65.8	23
78	375.8	25	375.0	99.8	25	368.8	98.1	25	364.4	97.0	25	251.2	66.8	23
85	386.3	25	383.2	99.2	25	379.7	98.3	25	375.0	97.1	25	263.2	68.1	23
92	396.8	25	391.0	98.5	25	386.4	97.4	25	381.8	96.2	25	271.6	68.4	23
120	427.2	25	412.5	96.6	25	409.2	95.8	25	398.4	93.3	25	288.7	67.6	22
148	451.3	25	443.2	98.2	25	436.5	96.7	25	420.6	93.2	25	315.8	70.0	22
176	471.7	25	464.9	98.6	25	454.3	96.3	25	440.4	93.4	25	332.7	70.5	22
204	483.6	25	473.0	97.8	25	471.4	97.5	25	454.8	94.0	25	345.8	71.5	22
232	498.1	25	484.4	97.3	25	484.3	97.2	25	458.4	92.0	25	353.2	70.9	22
260	516.0	25	508.3	98.5	25	498.2	96.6	25	475.7	92.2	25	364.5	70.6	22
288	524.7	25	522.1	99.5	25	511.7	97.5	25	488.9	93.2	25	374.3	71.3	22
316	532.7	25	527.0	98.9	25	520.1	97.6	25	496.6	93.2	25	376.0	70.6	22
344	546.8	25	528.0	96.5	25	537.0	98.2	25	504.5	92.3	25	384.4	70.3	22
372	555.4	25	547.9	98.7	25	545.8	98.3	25	510.5	91.9	25	389.2	70.1	22
400	555.2	25	557.2	100.4	25	551.4	99.3	25	521.6	93.9	25	392.1	70.6	22
428	566.3	25	563.6	99.5	25	564.7	99.7	25	528.6	93.3	25	400.4	70.7	22
456	576.3	25	568.6	98.7	25	571.8	99.2	25	533.3	92.5	25	404.9	70.3	22
484	577.8	25	575.4	99.6	25	579.0	100.2	25	534.8	92.6	25	406.8	70.4	22
512	583.2	25	589.7	101.1	25	585.6	100.4	25	541.8	92.9	25	411.6	70.6	22
540	584.6	25	587.1	100.4	25	588.6	100.7	25	546.7	93.5	25	415.1	71.0	22
568	582.9	25	587.4	100.8	25	596.8	102.4	25	549.6	94.3	25	416.1	71.4	22
596	583.2	24	598.1	102.5	24	600.8	103.0	25	552.7	94.8	25	417.4	71.6	22
624	592.3	22	593.5	100.2	24	604.1	102.0	25	551.4	93.1	25	418.5	70.7	22
652	585.7	22	587.4	100.3	24	610.4	104.2	25	565.4	96.5	24	420.5	71.8	21
680	584.2	22	590.5	101.1	23	609.3	104.3	24	571.4	97.8	22	416.1	71.2	20
708	588.5	20	586.9	99.7	23	606.7	103.1	23	556.5	94.6	22	403.0	68.5	20
EOS	**581.2**	**18**	**585.8**	**100.8**	**23**	**594.5**	**102.3**	**23**	**569.5**	**98.0**	**21**	**408.7**	**70.3**	**20**

EOS = end of study; No. of litters = number of litters represented in weight average.

[a]Study day 1 is the day animals were placed on study after pups were weaned.

[b]Average weights shown are means of litter means.

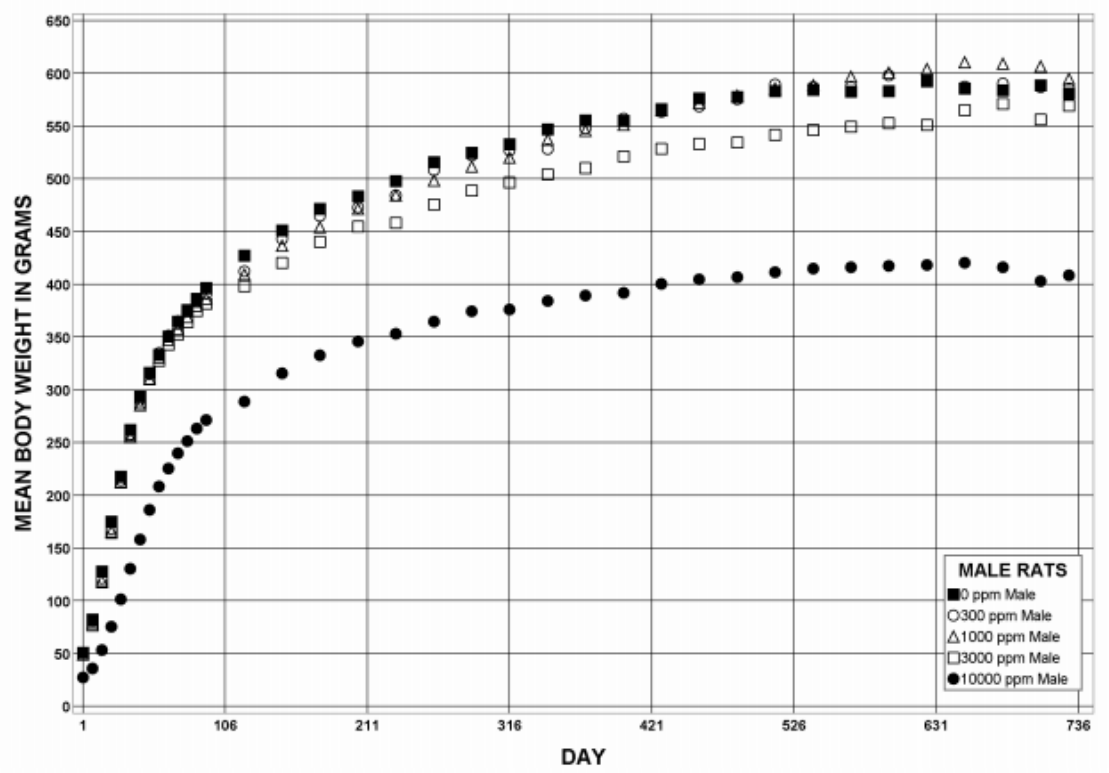
Table 10. Summary of Survival and Mean Body Weights of Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Study Day[a]	0 ppm		300 ppm			1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)[b]	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters	Av. Wt. (g)	Wt. (% of Controls)	No. of Litters
1	49.8	25	51.5	103.3	25	49.2	98.7	25	48.7	97.6	25	26.0	52.1	25
8	77.5	25	78.6	101.4	25	75.6	97.5	25	74.3	95.9	25	36.9	47.6	22
15	112.7	25	111.2	98.6	25	107.9	95.7	25	107.4	95.2	25	53.5	47.5	22
22	141.8	25	141.7	99.9	25	138.0	97.3	25	137.8	97.2	25	73.1	51.5	22
29	162.1	25	164.7	101.6	25	159.7	98.5	25	160.7	99.1	25	94.4	58.2	22
36	181.3	25	187.3	103.3	25	180.5	99.6	25	180.7	99.7	25	114.5	63.1	22
43	193.3	25	200.7	103.8	25	193.4	100.0	25	196.1	101.4	25	132.4	68.5	22
50	206.7	25	211.4	102.3	25	205.2	99.3	25	206.5	99.9	25	147.9	71.5	22
57	216.8	25	223.2	103.0	25	215.7	99.5	25	216.9	100.1	25	161.1	74.3	22
64	224.0	25	232.0	103.6	25	223.7	99.9	25	224.1	100.0	25	171.9	76.8	22
71	231.0	25	238.3	103.1	25	230.4	99.7	25	232.2	100.5	25	181.5	78.6	22
78	236.4	25	242.1	102.4	25	233.7	98.9	25	235.8	99.8	25	188.0	79.6	22
85	240.9	25	246.2	102.2	25	241.3	100.2	25	241.3	100.1	25	195.1	81.0	22
92	245.6	25	249.0	101.4	25	245.8	100.1	25	245.4	99.9	25	199.2	81.1	22
120	259.1	25	259.5	100.1	25	257.8	99.5	25	258.6	99.8	25	212.8	82.1	22
148	268.6	25	273.3	101.8	25	266.2	99.1	25	269.0	100.2	25	222.0	82.7	22
176	278.4	25	280.9	100.9	25	274.2	98.5	25	275.9	99.1	25	230.6	82.8	22
204	283.3	25	282.1	99.6	25	282.1	99.6	25	282.3	99.6	25	232.8	82.2	22
232	290.7	25	291.7	100.4	25	285.8	98.3	25	286.4	98.5	25	237.1	81.6	22
260	297.0	25	300.4	101.1	25	290.8	97.9	25	293.7	98.9	25	240.6	81.0	22
288	302.4	25	303.1	100.2	25	296.8	98.1	25	297.9	98.5	25	244.2	80.8	22
316	301.0	25	305.3	101.4	25	300.9	100.0	25	302.3	100.4	25	244.9	81.4	22
344	311.2	25	311.1	100.0	25	306.0	98.3	25	302.0	97.0	25	247.8	79.6	22
372	314.7	25	319.2	101.4	25	307.2	97.6	25	304.4	96.7	25	247.5	78.7	22
400	320.6	25	325.9	101.6	25	314.2	98.0	25	311.8	97.2	25	250.0	78.0	22
428	325.4	25	330.2	101.5	25	320.9	98.6	25	310.6	95.5	25	250.4	76.9	22
456	331.4	25	335.0	101.1	25	327.5	98.8	25	315.8	95.3	25	251.1	75.8	22
484	339.0	25	345.3	101.9	25	332.7	98.2	25	326.2	96.2	25	253.4	74.8	22
512	348.5	25	355.8	102.1	25	344.8	98.9	25	332.6	95.4	25	253.3	72.7	22
540	350.3	24	344.4	98.3	24	348.1	99.4	25	330.2	94.3	25	256.7	73.3	22
568	358.2	24	354.9	99.1	24	356.2	99.4	25	335.8	93.8	24	258.5	72.2	22
596	361.9	24	353.6	97.7	23	357.9	98.9	24	342.5	94.6	24	257.0	71.0	22
624	368.8	24	361.6	98.1	23	367.2	99.6	24	348.0	94.4	24	253.1	68.6	21
652	378.9	24	368.8	97.3	23	376.1	99.3	23	351.4	92.7	24	251.4	66.3	20
680	379.7	24	369.5	97.3	23	379.6	100.0	23	344.5	90.7	22	252.3	66.4	20
708	376.4	23	359.5	95.5	21	380.1	101.0	23	338.2	89.9	21	252.6	67.1	19
EOS	**374.3**	**22**	**371.0**	**99.1**	**21**	**391.4**	**104.6**	**23**	**337.4**	**90.1**	**20**	**255.6**	**68.3**	**18**

EOS = end of study; No. of litters = number of litters represented in weight average.

[a]Study day 1 is the day animals were placed on study after pups were weaned.

[b]Average weights shown are means of litter means.



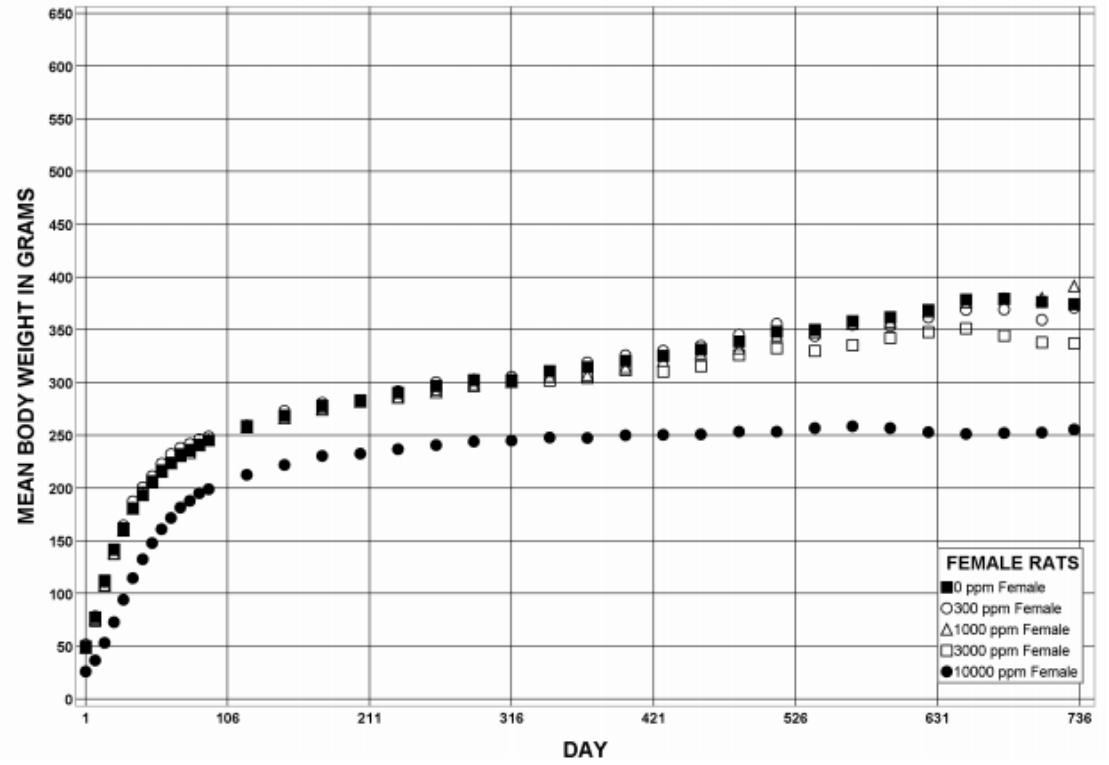


Figure 4. Growth Curves for Rats Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study

Table 11. Summary of Feed and Di(2-ethylhexyl) Phthalate Consumption of Male Rats in the Perinatal and Postweaning Two-year Feed Study

Week	0 ppm	300 ppm		1,000 ppm		3,000 ppm		10,000 ppm	
	Feed (g/day)[a]	Feed (g/day)	Dose (mg/kg/day)[b]	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)
1	9.7	9.8	58.2	9.5	193.2	8.8	540.0	2.5	908.5
13	25.0	24.0	18.8	24.4	64.3	25.3	202.4	21.0	797.7
54	27.7	28.6	15.7	26.6	48.7	28.6	168.0	22.3	569.5
102	26.6	27.6	14.1	25.0	41.3	29.0	154.4	27.4	681.4

[a]Grams of feed consumed per animal per day.
[b]Milligrams of di(2-ethylhexyl) phthalate consumed/kilogram body weight/day.

Table 12. Summary of Feed and Di(2-ethylhexyl) Phthalate Consumption of Female Rats in the Perinatal and Postweaning Two-year Feed Study

Week	0 ppm	300 ppm		1,000 ppm		3,000 ppm		10,000 ppm	
	Feed (g/day)[a]	Feed (g/day)	Dose (mg/kg/day)[b]	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)
1	9.1	9.2	53.6	9.0	182.9	8.8	542.5	2.7	1,076.8
13	15.8	16.0	19.5	16.5	68.4	16.8	208.9	15.5	796.9
54	16.9	16.6	15.6	16.5	53.6	16.9	166.6	17.6	709.8
102	20.3	20.3	17.0	21.0	56.1	23.2	204.4	22.9	899.7

[a]Grams of feed consumed per animal per day.
[b]Milligrams of di(2-ethylhexyl) phthalate consumed per kilogram body weight per day.

Pathology

This section describes statistically significant or biologically noteworthy changes in the incidences of gross lesions, neoplasms, and/or nonneoplastic lesions of the liver, pancreas, male and female reproductive organs, kidney, heart, bone marrow, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal neoplasm diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the biologically significant neoplasms mentioned in this section are presented as supplemental data in Appendix H.

Liver: There were significant increases in the incidences of hepatocellular adenoma (10,000 ppm males and 3,000 ppm females) and carcinoma (10,000 ppm females) relative to control animals and a positive trend in incidence with increasing exposure concentration in males for hepatocellular carcinomas (Table 13). The incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in the 10,000 ppm male and female groups and in the 3,000 ppm female group relative to control animals. Hepatocellular adenomas were characterized by regions that were sharply demarcated from surrounding liver parenchyma, nodular, and compressing adjacent normal hepatocytes, with loss of normal lobular architecture and an irregular growth pattern. The liver plates typically impinged obliquely on the surrounding liver parenchyma. The hepatocytes within an adenoma generally varied in size. Hepatocellular carcinomas were characterized by one or more of the following features: local infiltrating growth

and/or distinct lack of demarcation with the adjacent tissue, the presence of trabeculae composed of multiple layers of hepatocytes, cellular pleomorphism, loss of normal lobular architecture, regions of hemorrhage and/or necrosis, and increased mitotic figures.

There were significant increases in the incidences of many nonneoplastic liver lesions in DEHP-exposed groups relative to the control groups (Table 13). The incidences of hepatocellular cytoplasmic alteration were significantly increased in 3,000 and 10,000 ppm males and in all exposed groups of females relative to control animals. There were significant increases in the incidence of hepatocellular hypertrophy in the 10,000 ppm males and in the 1,000, 3,000, and 10,000 ppm females. Significant increases in the incidence of pigment were observed in the 1,000, 3,000, and 10,000 ppm males and in all exposed groups of females. There were significant increases in the incidence of hepatocellular necrosis in the 10,000 ppm males relative to control animals. The incidence of hepatocellular eosinophilic foci was significantly increased in 10,000 ppm females over that of the control group, and a positive trend was seen in males with increasing exposure concentration. The incidence of hepatocellular basophilic foci was significantly increased in the 10,000 ppm males relative to the control group, and a positive trend with increasing exposure occurred in females. There was a significant increase in the incidence of bile duct hyperplasia in 3,000 ppm females compared to control animals.

Hepatocellular cytoplasmic alteration was characterized by hepatocytes that were expanded with eosinophilic granular cytoplasm (Figure 5). A four-grade severity scale reflected the degree of tissue affected in the section of liver that was evaluated histologically: minimal (grade 1), up to 25% of hepatocyte involvement; mild (grade 2), 26% to 50% of hepatocyte involvement; moderate (grade 3), 51% to 75% of hepatocyte involvement; and marked (grade 4), at least 76% of hepatocyte involvement. Hepatocellular hypertrophy often occurred in conjunction with cytoplasmic alteration and/or pigment. Hypertrophy was characterized by enlargement of the hepatocytes. In lesser affected animals, hypertrophy was confined to centrilobular regions, but in more severely affected animals, hypertrophy extended into the midzonal and periportal areas. A four-grade severity scale was used: minimal (grade 1), up to 10% of hepatocyte involvement; mild (grade 2), 11% to 25% of hepatocyte involvement; moderate (grade 3), up to 50% of hepatocyte involvement; and severe (grade 4), >51% of hepatocyte involvement. Hypertrophy was generally centrilobular and often involved only a few cells per lobule. Although hypertrophy was only occasionally observed in males (at the 3,000 and 10,000 ppm exposure concentrations), in females it exhibited a concentration-responsive significant increase in incidence (but not severity) starting at the 1,000 ppm exposure concentration.

Pigment only occurred in exposed animals and was characterized by a pale gold-colored pigment within the hepatocellular cytoplasm (Figure 5). A four-grade severity scale was used: minimal (grade 1), up to 30% of hepatocytes contained pigment; mild (grade 2), 31% to 50% of hepatocytes contained pigment; moderate (grade 3), >51% of hepatocytes contained pigment; and marked (grade 4), >51% of hepatocytes contained pigment and the pigment was very dense. Hepatocellular necrosis was characterized by multiple adjacent hepatocytes that were swollen with increased eosinophilia, karyorrhectic nuclear debris, with or without accompanying inflammatory cells. A four-grade severity scale was used: minimal (grade 1), up to three focal areas of necrosis present; mild (grade 2), necrosis in more than three involved regions or up to 25% of the liver; moderate (grade 3), necrosis in 26% to 60% of the liver; and severe (grade 4), necrosis in >61% of the liver.

Eosinophilic and basophilic hepatocellular foci were diagnosed when there was an enlargement of the hepatocytes with increased acidophilic or basophilic staining, respectively, compared with the surrounding normal liver cells. Foci typically had a discrete lesion margin, where attenuated hepatocytes at the lesion margin (compression) involved <70% of the lesion circumference. There was preservation of lobular architecture and absence of cellular atypia. The distinction between large foci (usually eosinophilic or mixed) and hepatocellular adenomas was made on the basis of retention of normal lobular architecture in the foci, greater size of hepatocellular adenomas (usually measuring at least 3 mm), and presence of compression or bulging from the liver surface along >70% of the lesion circumference.

Bile duct hyperplasia was diagnosed when increased numbers of small bile ducts arose in portal regions. The biliary epithelium lining the ducts was well differentiated, forming normal ducts.

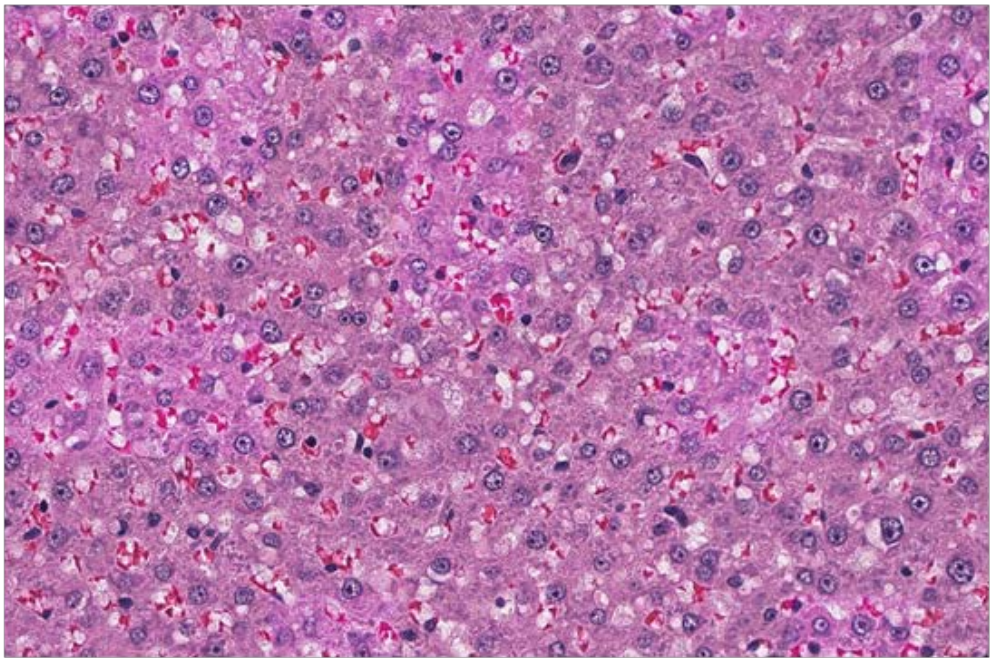
Table 13. Incidences of Neoplastic and Nonneoplastic Lesions of the Liver in Male and Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
n[a]	50	49	50	50	49
Hepatocyte, Cytoplasmic Alteration[b]	0**	0	1 (1.0)[c]	28** (1.3)	37** (2.6)
Hepatocyte, Hypertrophy	0**	0	0	3 (1.3)	17** (1.6)
Pigment	0**	1 (1.0)	5* (1.2)	40** (1.7)	38** (2.5)
Necrosis	3** (1.3)	4 (2.0)	1 (1.0)	6 (1.8)	13* (1.3)
Eosinophilic Focus	4**	1	7	2	11
Basophilic Focus	1**	1	4	4	17**
Bile Duct Hyperplasia	13 (1.2)	18 (1.1)	13 (1.2)	21 (1.0)	15 (1.1)
Hepatocellular Adenoma[d]					
Overall rate[e]	0/50 (0%)	1/49 (2%)	0/50 (0%)	3/50 (6%)	8/49 (16%)
Rate per litters[f]	0/25 (0%)	1/25 (4%)	0/25 (0%)	3/25 (12%)	7/25 (28%)
Adjusted rate[g]	0%	2.4%	0%	6.7%	22.3%
Rao-Scott-adjusted Poly-3 test[h]	p < 0.001	p = 0.604	(e)	p = 0.246	p = 0.018
Hepatocellular Carcinoma[i]					
Overall rate	1/50 (2%)	0/49 (0%)	0/50 (0%)	0/50 (0%)	3/49 (6%)
Rate per litters	1/25 (4%)	0/25 (0%)	0/25 (0%)	0/25 (0%)	3/25 (12%)
Adjusted rate	2.6%	0%	0%	0%	8.7%
Rao-Scott-adjusted Poly-3 test	p = 0.038	p = 0.589N	p = 0.587N	p = 0.587N	p = 0.341
Hepatocellular Adenoma or Carcinoma (Combined)[j]					
Overall rate	1/50 (2%)	1/49 (2%)	0/50 (0%)	3/50 (6%)	11/49 (22%)
Rate per litters	1/25 (4%)	1/25 (4%)	0/25 (0%)	3/25 (12%)	9/25 (36%)
Adjusted rate	2.6%	2.4%	0%	6.7%	30.6%
Rao-Scott-adjusted Poly-3 test	p < 0.001	p = 0.750N	p = 0.565N	p = 0.429	p = 0.009
Female					
n	49	50	50	50	48
Hepatocyte, Cytoplasmic Alteration	0**	4* (1.0)	7** (1.1)	39** (1.6)	39** (3.4)

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Hepatocyte, Hypertrophy	0**	2 (2.0)	5* (1.6)	9** (1.6)	34** (2.4)
Pigment	0**	6* (1.0)	14** (1.1)	36** (1.6)	40** (2.6)
Necrosis	3 (2.0)	9 (2.3)	3 (2.3)	7 (1.9)	8 (2.3)
Eosinophilic Focus	3**	4	4	7	12**
Basophilic Focus	4**	5	3	2	10
Bile Duct Hyperplasia	9 (1.2)	13 (1.0)	13 (1.2)	21* (1.1)	8 (1.1)
Hepatocellular Adenoma[k]					
Overall rate	1/49 (2%)	0/50 (0%)	5/50 (10%)	9/50 (18%)	5/48 (10%)
Rate per litters	1/25 (4%)	0/25 (0%)	4/25 (6%)	7/25 (28%)	5/25 (20%)
Adjusted rate	2.4%	0%	11.8%	20.9%	13.8%
Rao-Scott-adjusted Poly-3 test	p = 0.089	p = 0.587N	p = 0.170	p = 0.033	p = 0.126
Hepatocellular Carcinoma[l]					
Overall rate	0/49 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	8/48 (17%)
Rate per litters	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)	7/25 (28%)
Adjusted rate	0%	0%	0%	0%	21.8%
Rao-Scott-adjusted Poly-3 test	p < 0.001	(e)	(e)	(e)	p = 0.005
Hepatocellular Adenoma or Carcinoma (Combined)[m]					
Overall rate	1/49 (2%)	0/50 (0%)	5/50 (10%)	9/50 (18%)	13/48 (27%)
Rate per litters	1/25 (4%)	0/25 (0%)	4/25 (16%)	7/25 (28%)	11/25 (44%)
Adjusted rate	2.4%	0%	11.8%	20.9%	35.4%
Rao-Scott-adjusted Poly-3 test	p < 0.001	p = 0.568N	p = 0.158	p = 0.028	p = 0.002

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Significantly different ($p \leq 0.05$) from the control group by the Rao-Scott-adjusted Poly-3 test; **$p \leq 0.01$.
(e) = value of statistic could not be computed.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 2/489 (0.44% ± 0.88%); range: 0% to 2%.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Number of litters with neoplasm-bearing animals per number of litters examined at site.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the control group and that exposed group. The Rao-Scott test adjusts the Poly-3 test (which accounts for differential mortality in animals that do not reach study termination) for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[i]Historical control incidence: 2/489 (0.45% ± 0.89%); range: 0% to 2%.
[j]Historical control incidence: 4/489 (0.89% ± 1.06%); range: 0% to 2%.
[k]Historical control incidence: 15/489 (2.65% ± 2.59%); range: 0% to 8%.
[l]Historical control incidence: 1/489 (0.22% ± 0.67%); range: 0% to 2%.
[m]Historical control incidence: 16/489 (2.87% ± 2.8%); range: 0% to 8%.

A)



B)

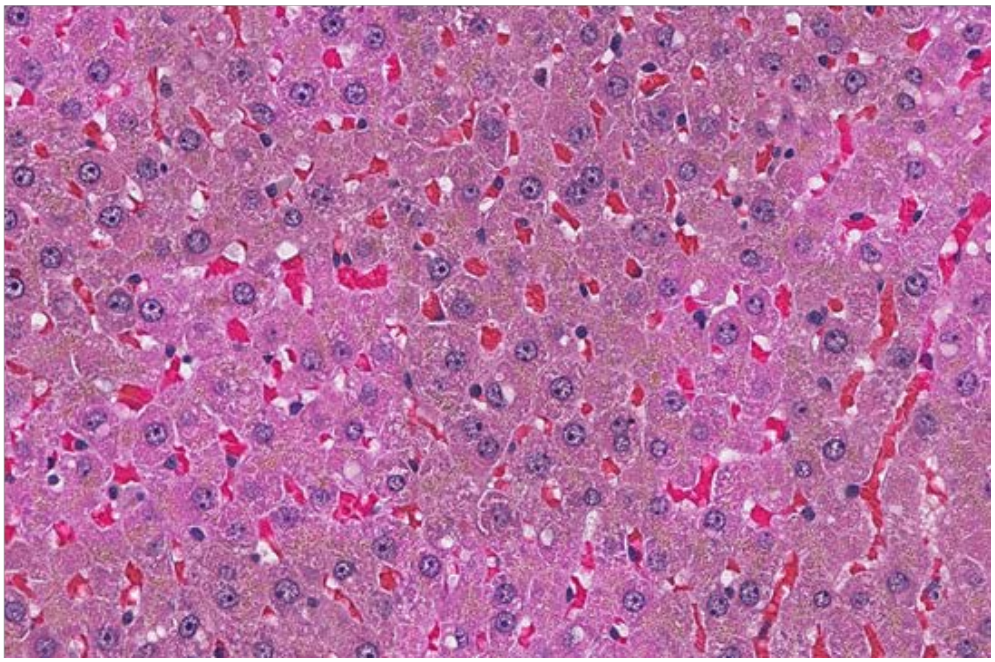


Figure 5. Hepatocellular Cytoplasmic Alteration with Pigment in Male Rats Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

Compare normal liver from a male control rat (panel A) to liver from a male rat exposed to 10,000 ppm DEHP (panel B). Hepatocytes are expanded with eosinophilic granular cytoplasm and pigment at 40x magnification.

Pancreas: In male rats, there were significant increases in the incidences of pancreatic acinar adenoma and pancreatic acinar adenoma or carcinoma (combined) in the 3,000 and 10,000 ppm groups compared to the control group (Table 14). In males, acinar carcinomas were present in the 3,000 and 10,000 ppm groups, but the incidences were not significant (Table 14). In females, acinar adenomas were observed in the 3,000 and 10,000 ppm groups, but the incidences were not significant (Table 14). Pancreatic acinar adenomas were characterized as distinct nodular masses that were not contiguous with the adjacent parenchyma, which were >3 mm in diameter, and that compressed the adjacent tissue; pleomorphism or atypia was rarely present. Pancreatic acinar carcinomas were typically larger than adenomas and frequently exhibited cellular pleomorphism and atypia; invasion or metastasis was pathognomonic. Scirrhous reactions were occasionally present, characterized by dense fibrous or connective tissue. Pancreatic acinar hyperplasias were higher in the 1,000, 3000, and 10,000 ppm male groups and in the 3,000 and 10,000 ppm female groups compared to the control group, but the differences were not significant.

There was a clear morphological continuum from focal acinar hyperplasia to adenoma and to carcinoma. Pancreatic acinar hyperplasia was characterized by circumscribed areas of enlarged acini that were <3 mm in diameter and that were contiguous with the adjacent parenchyma. Severity grades were assigned using a four-grade scale: minimal (grade 1), no more than one lobule was affected and the lesion was smaller than 1 mm; mild (grade 2), lesion was 1 to 2 mm; moderate (grade 3), lesion was 2 to 3 mm; and marked (grade 4), lesion was 3 mm but lacked features of an adenoma, such as compression. Severity grades were increased if multiple lesions were present.

Table 14. Incidences of Neoplastic and Nonneoplastic Lesions of the Pancreas in Male and Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
n[a]	50	49	50	50	49
Acinus, Hyperplasia[b]	13 (3.1)[c]	9 (2.6)	16 (3.3)	25 (3.5)	15 (3.3)
Acinar Adenoma[d]					
Overall rate[e]	10/50 (20%)	7/49 (14%)	8/50 (16%)	36/50 (72%)	22/49 (45%)
Rate per litters[f]	8/25 (32%)	5/25 (20%)	8/25 (32%)	24/25 (96%)	18/25 (72%)
Adjusted rate[g]	26%	16.6%	16.9%	77.9%	62.5%
Rao-Scott-adjusted Poly-3 test[h]	p < 0.001	p = 0.209N	p = 0.210N	p < 0.001	p < 0.001
Acinar Carcinoma[i]					
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	3/50 (6%)	1/49 (2%)
Rate per litters	0/25 (0%)	0/25 (0%)	0/25 (0%)	3/25 (12%)	1/25 (4%)
Adjusted rate	0%	0%	0%	6.6%	2.9%
Rao-Scott-adjusted Poly-3 test	p = 0.290	(e)	(e)	p = 0.250	p = 0.534
Acinar Adenoma or Carcinoma (Combined)[j]					
Overall rate	10/50 (20%)	7/49 (14%)	8/50 (16%)	38/50 (76%)	22/49 (45%)
Rate per litters	8/25 (32%)	5/25 (20%)	8/25 (32%)	25/25 (100%)	18/25 (72%)

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Adjusted rate	26%	16.6%	16.9%	81.2%	62.5%
Rao-Scott-adjusted Poly-3 test	p < 0.001	p = 0.209N	p = 0.210N	p < 0.001	p < 0.001
Female					
n	49	50	50	50	48
Acinus, Hyperplasia	0	0	0	2 (1.5)	3 (2.0)
Acinar Adenoma[k]					
Overall rate	0/49 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/48 (2%)
Rate per litters	0/25 (0%)	0/25 (0%)	0/25 (0%)	2/25 (8%)	1/25 (4%)
Adjusted rate	0%	0%	0%	4.6%	2.8%
Rao-Scott-adjusted Poly-3 test	p = 0.307	(e)	(e)	p = 0.366	p = 0.561
Acinar Carcinoma[l]					
Overall rate	0/49 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/48 (0%)
Rate per litters	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)
Adjusted rate	0%	0%	0%	0%	0%
Rao-Scott-adjusted Poly-3 test	–[m]	–	–	–	–
Acinar Adenoma or Carcinoma (Combined)[n]					
Overall rate	0/49 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/48 (2%)
Rate per litters	0/25 (0%)	0/25 (0%)	0/25 (0%)	2/25 (8%)	1/25 (4%)
Adjusted rate	0%	0%	0%	4.6%	2.8%
Rao-Scott-adjusted Poly-3 test	p = 0.307	(e)	(e)	p = 0.366	p = 0.561

(e) = value of statistic could not be computed.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 60/488 (11.58% ± 9.25%); range: 0% to 28%.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Number of litters with neoplasm-bearing animals per number of litters examined at site.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the control group and that exposed group. The Rao-Scott test adjusts the Poly-3 test (which accounts for differential mortality in animals that do not reach study termination) for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[i]Historical control incidence: 4/488 (0.8% ± 1.42%); range: 0% to 4%.
[j]Historical control incidence: 62/488 (12.03% ± 9.16%); range: 0% to 28%.
[k]Historical control incidence: 0/489.
[l]Historical control incidence: 0/489.
[m]Not applicable; no neoplasms in group.
[n]Historical control incidence: 0/489.

Gross lesions of the reproductive tract: Significantly increased incidences of several genitourinary abnormalities consistent with perturbation of developmental testosterone signaling and Wolffian duct differentiation were evident among 10,000 ppm DEHP-exposed males relative to control animals (Table 15). Small reproductive tissues were noted in 10,000 ppm males, including, but not limited to, the phallus, testes, epididymides, seminal vesicles, levator

ani/bulbocavernosus (LABC) muscle, Cowper's glands, and prostate (Table 15; Appendix H). Findings in the testes (small) were particularly prevalent, occurring in all 10,000 ppm males that survived past the initial 2 weeks of the 2-year study period relative to only two incidences in examined control rats (Table 15). Undescended testes (unilateral or bilateral) were noted in the abdomen in 19 (approximately 39%) rats and the inguinal region in 4 (approximately 8%) rats in the 10,000 ppm group relative to a single incidence of undescended testes in the abdomen (bilateral) in control males. Findings commonly associated with testicular retention in the abdomen, such as the presence of a cranial suspensory ligament (approximately 10%) and extended (>20 mm; approximately 16%) or absent gubernaculum (unilateral or bilateral; approximately 35%), were also significantly increased in the 10,000 ppm males relative to control animals (Table 15). Exposure-related increases in the left and right mean gubernaculum lengths were evident in 10,000 ppm males. Using a nonparametric (rank-based) analysis, a significant increase in the length of the right gubernaculum was observed in 10,000 ppm males. This corresponded to data in which the effect was largely associated with increased right gubernaculum lengths (≥35 mm) in seven males in the 10,000 ppm group compared to five animals with these lengths in the left gubernaculum (Table 15; Appendix H). In addition, there were 18 animals with gubernaculum not present, 12 of which had a bilateral finding. Epididymal anomalies, such as agenesis, are typically seen concomitant with other abnormalities in organs that are developed from Wolffian ducts. Agenesis of all or part of the epididymis (unilateral or bilateral) was observed only in rats in the 10,000 ppm group. Incomplete separation of the prepuce (approximately 14%), cleft phallus (approximately 6%), and/or cleft prepuce (2%) was observed in 10,000 ppm males relative to no incidences in control rats (Table 15).

Limited genitourinary abnormalities were associated with DEHP exposure in female rats. A greater incidence of vaginal nonpatency (approximately 10%) occurred in 10,000 ppm females; in contrast a similar finding was not found in any other exposure group. Additionally, observations of cleft phallus were noted in both the 3,000 ppm (approximately 4%) and 10,000 ppm (approximately 2%) females.

Table 15. Summary of Gross Lesions in the Reproductive Tract of Male and Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
Testis[a,b]	49	49	50[c]	50	49
Size, small[d]	2 (2)[e]**	2 (2)	4 (4)	2 (2)	45 (23)**
Size, enlarged (or swelling)	0	0	0	0	1 (1)
Fluid or blood filled	0	1 (1)	1 (1)	1 (1)	1 (1)
Left, abdominal, undescended	1 (1)**	0	0	0	13 (10)*
Left, inguinal, undescended	0*	1 (1)	1 (1)	0	4 (4)
Right, abdominal, undescended	1 (1)**	0	0	0	18 (14)**
Right, inguinal, undescended	0	1 (1)	1 (1)	0	3 (3)
Right or left; abdominal; undescended	1 (1)**	0	0	0	19 (14)**
Right or left; inguinal; undescended	0*	1 (1)	1 (1)	0	4 (4)
Right or left; abdominal or inguinal; undescended	1 (1)**	1 (1)	1 (1)	0	23 (16)**
Right, not present	0	0	0	0	1 (1)
Cranial suspensory ligament	0**	0	0	0	5 (4)
Epididymis[a,b]	49	49	50[c]	50	49
Size, small	0**	0	2 (2)	0	14 (12)**
Left, caput, agenesis	0	0	0	0	1 (1)
Left, corpus, agenesis	0	0	0	0	1 (1)
Left, corpus, cauda, or caput, agenesis/not present	0	0	0	0	1 (1)
Right, caput, agenesis	0**	0	0	0	4 (4)
Right, cauda, agenesis	0	0	0	0	2 (2)
Right, corpus, agenesis	0	0	0	0	2 (2)
Right, corpus, cauda, or caput, agenesis/not present	0**	0	0	0	6 (5)
Right or left, caput, agenesis	0**	0	0	0	4 (4)
Right or left, cauda, agenesis	0	0	0	0	2 (2)
Right or left, corpus, agenesis	0*	0	0	0	3 (3)
Right or left, corpus, cauda, or caput, agenesis/not present	0**	0	0	0	6 (5)
LABC Muscle	50	49	50	50	48
Size, small	0	0	0	0	2 (2)
Cowper's Glands	50	49	50	50	47

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Left, size, small	0	0	0	0	1 (1)
Right, size, small	0	0	0	0	1 (1)
Prostate Glands	50	49	50	50	47
Size, small	0	0	0	0	1 (1)
Seminal Vesicles/Coagulating Glands	50	49	50	50	47
Size, small	1 (1)**	0	1 (1)	1 (1)	8 (7)
Phallus[a,b]	50	49	49	50	49
Size, small	0*	0	0	0	3 (3)
Cleft	0*	0	0	0	3 (3)
Prepuce[a,b]	50	49	50	50	49
Cleft	0	0	0	0	1 (1)
Incomplete preputial separation	0**	0	0	0	7 (7)*
Gubernaculum[a,b]	47	49	49[c]	50	41
Left, not present	0**	0	0	0	15 (12)**
Right, not present	0**	0	0	0	15 (12)**
Right or left, not present	0**	0	0	0	18 (14)**
Gubernaculum Length[f,g]					
Left, length (mm)	15.32 ± 0.69 47 (25)[h]	15.60 ± 0.63 49 (25)	14.50 ± 0.52 49 (25)	15.64 ± 0.58 50 (25)	20.91 ± 1.91 24 (17)[i]
Right, length (mm)	15.60 ± 0.61* 47 (25)	15.46 ± 0.57 49 (25)	14.34 ± 0.52 48 (25)	15.62 ± 0.44 50 (25)	24.22 ± 2.61** 24 (16)
Female					
Vagina[b]	50	50	50	50	48
Not patent	0*	0	0	0	5 (3)
Phallus[b]	50	50	50	50	48
Cleft	0	0	0	2 (1)	1 (1)

LABC = levator ani/bulbocavernosus.
Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
[a]Number of animals examined for each tissue.
[b]Statistical analysis performed by the Cochran-Armitage test with a Rao-Scott modification for the random effect due to litter. All trend and pairwise p values are reported as one-sided.
[c]One animal in the 1,000 ppm group was not examined for right-sided gross lesion in this tissue.
[d]Number of animals affected given for each observation.
[e]Number of litters with observations shown in parentheses for F_1 animals. F_1 litter incidence based on the number of F_0 dams.
[f]Statistical analysis performed using a bootstrapped Jonckheere test for trend, and a Datta-Satten modified Wilcoxon test with Hommel adjustment for pairwise comparisons.
[g]Data are presented as mean ± standard error.
[h]n = number of animals examined (number of litters represented).
[i]15 animals from the 10,000 ppm group were excluded from statistical analysis due to at least one (left or right) of the gubernaculum observations listed as "not present." An additional two animals from the 10,000 ppm group were excluded from statistical analysis with values listed as "within normal limits," and eight animals had no data collected.

Testis: There were significant increases in germinal epithelium degeneration (includes bilateral), interstitial cell hyperplasia (includes bilateral), and seminiferous tubule dysgenesis (includes bilateral) in the 10,000 ppm group compared to the control group (Table 16). Two of the occurrences of seminiferous tubule dysgenesis were bilateral (Table 16, Appendix H). Germinal epithelium degeneration was recorded when one or more of the following features was present in tubules not adjacent to the rete testis: tubular vacuolation, partial depletion of germ cells, degenerating (multinucleated or apoptotic) germ cells, disordered arrangement of the germ cell layers, or seminiferous tubules completely devoid of germ cells (atrophy) and lined only by Sertoli cells. Germinal epithelium degeneration was scored for severity on a four-grade scale: minimal (grade 1), up to 25% of at least one testis involved; mild (grade 2), 26% to 50% of at least one testis involved; moderate (grade 3), 51% to 75% of at least one testis involved; and marked (grade 4), rare to no normal seminiferous tubules in either testis were present (i.e., remaining seminiferous tubules solely lined by Sertoli cells).

Interstitial cell hyperplasia, defined as focal aggregates of Leydig cells, was scored using a four-grade scale: minimal (grade 1), when only a thin rim of interstitial cells or a cluster of cells one-fourth the size of a normal seminiferous tubule was present; mild (grade 2), when several such areas were present or one cluster was present that was one-half the size of a normal seminiferous tubule; moderate (grade 3), when a cluster three-fourths the size of a normal seminiferous tubule was present; and marked (grade 4), when a cluster of interstitial cells approached the diameter of a normal seminiferous tubule. The interstitial cells involved were frequently elongated and flattened in profile. Interstitial cell adenomas were characterized by regions of increased interstitial cells, described as mostly uniform polyhedral cells with abundant eosinophilic, finely granular, or vacuolated cytoplasm, which exceeded the diameter of three seminiferous tubules. Circumferential compression of adjacent seminiferous tubules was observed occasionally. Seminiferous tubule dysgenesis only occurred in the highest exposure group (10,000 ppm) and was characterized by seminiferous tubules that were misshapen and convoluted, lined by only Sertoli cells, and surrounded by a thickened basement membrane (Figure 6). The lumen was often not patent and was commonly associated with an undescended testis but was also identified in scrotal testes. Three of the 10 animals with seminiferous tubule dysgenesis died early, each on day 3 of the study (two were 24 days old and one was 25 days old), and all seminiferous tubule dysgenesis lesions were focal. Focal lesion locations were variable, sometimes being present in sections that did not include the rete testis.

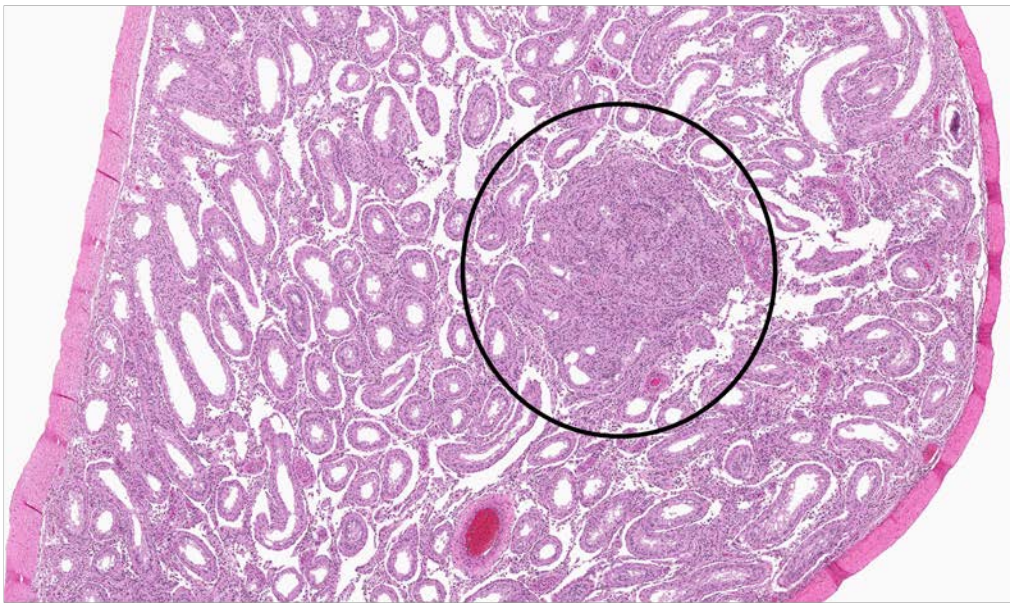
Epididymis: There were significant increases in the incidences of epididymis hypospermia (includes bilateral) in the 10,000 ppm group compared to the control group (Table 16). Epididymis hypospermia was characterized by a reduced density of sperm in the lumen of the epididymal duct, often accompanied by luminal cell debris. It was scored using a four-grade scale: minimal (grade 1), 25% to 50% reduction of spermatozoa; mild (grade 2), 51% to 66% reduction; moderate (grade 3), 67% to 80% reduction; and marked (grade 4), 81% to 100% reduction.

Table 16. Incidences of Neoplastic and Nonneoplastic Lesions of the Testis and Epididymis in Male Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
n[a]	49	49	50	50	49
Testis					
Germinal epithelium, degeneration (includes bilateral)[b,c]	16** (1.6)[d]	25 (1.6)	21 (2.0)	21 (1.5)	44** (4.0)
Interstitial cell, hyperplasia, focal (includes bilateral)	4** (2.0)	3 (2.0)	6 (2.0)	5 (1.4)	30** (2.3)
Seminiferous tubule, dysgenesis (includes bilateral)	0**	0	0	0	10* (1.6)
Epididymis					
Hypospermia (includes bilateral)	4** (2.3)	5 (3.4)	12 (2.8)	8 (2.4)	43** (4.0)
Testis					
Interstitial cell, adenoma[e]					
Overall rate[f]	3/49 (6%)	1/49 (2%)	3/50 (6%)	5/50 (10%)	5/49 (10%)
Rate per litters[g]	3/25 (12%)	1/25 (4%)	3/25 (12%)	5/25 (20%)	4/25 (16%)
Adjusted rate[h]	7.9%	2.4%	6.4%	11.2%	14.1%
Rao-Scott-adjusted Poly-3 test[i]	p = 0.097	p = 0.295N	p = 0.526N	p = 0.461	p = 0.330

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Significantly different ($p \leq 0.05$) from the control group by the Rao-Scott-adjusted Poly-3 test; **$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Incidence reported is the combination of unilateral and bilateral lesions.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[e]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 19/487 (4.06% ± 4.36%); range: 0% to 14%.
[f]Number of animals with neoplasm per number of animals necropsied.
[g]Number of litters with neoplasm-bearing animals per number of litters examined at site.
[h]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[i]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the control group and that exposed group. The Rao-Scott test adjusts the Poly-3 test (which accounts for differential mortality in animals that do not reach study termination) for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.

A)



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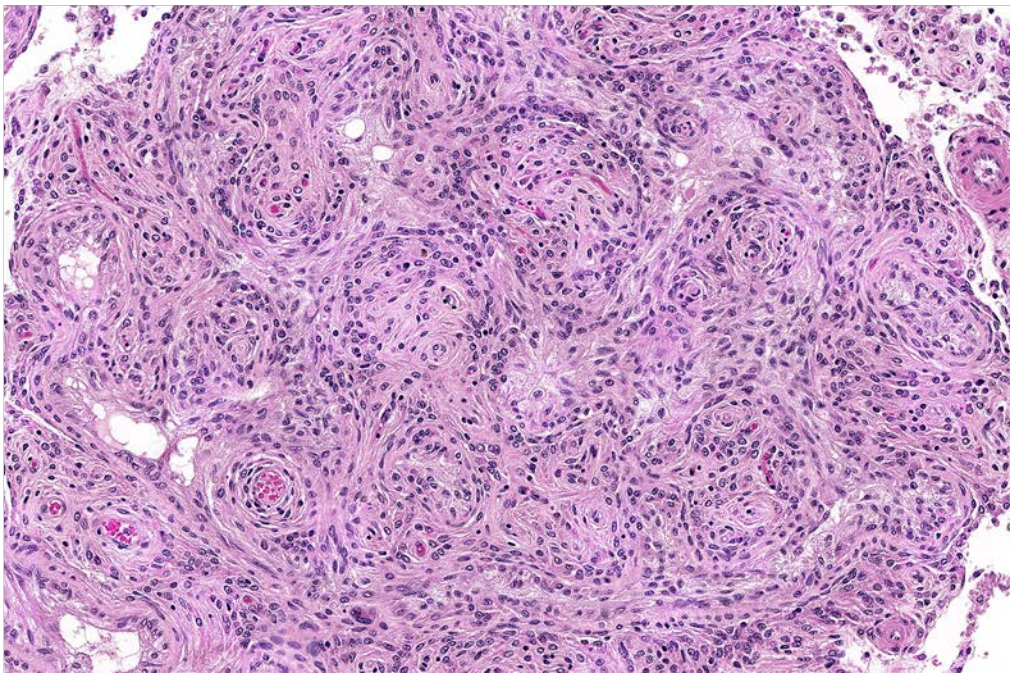


Figure 6. Seminiferous Tubule Dysgenesis in a Male Rat Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

This image highlights the misshapen, convoluted, and anastomosing tubules in a male rat exposed to 10,000 ppm DEHP. Panel B is a higher magnification (20x) of the region within the circle of panel A (4x magnification).

Uterus: There was a significant increase in the incidence of uterus acute inflammation in the 1,000 ppm group and a positive trend with increasing exposure concentration in uterus endometrium adenocarcinoma and uterus adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) (Table 17). Uterus endometrium adenocarcinoma typically is poorly circumscribed and invades the myometrium. The neoplastic epithelial cells form solid nests, cords, papillary, or acinar structures that are within, or supported by, stroma.

Table 17. Incidences of Neoplastic and Nonneoplastic Lesions of the Uterus (Including Cervix) in Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
n[a]	50	50	50	50	50
Inflammation, Acute[b]	0	0	6* (1.8)[c]	2 (2.5)	0[d]
Adenoma[e]	0	1	0	0	0
Adenocarcinoma[f]					
Overall rate[g]	3/50 (6%)	0/50 (0%)	1/50 (2%)	3/50 (6%)	6/50 (12%)
Rate per litters[h]	3/25 (12%)	0/25 (0%)	1/25 (4%)	3/25 (12%)	6/25 (24%)
Adjusted rate[i]	7%	0%	2.4%	7%	16.4%
Rao-Scott-adjusted Poly-3 test[j]	p = 0.008	p = 0.147N	p = 0.325N	p = 0.653N	p = 0.182
Squamous Cell Carcinoma (Includes Multiple)[k]	0	1	0	0	1
Squamous Cell Papilloma[l]	0	0	0	1	0
Adenoma, Adenocarcinoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined)[m]					
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	3/50 (6%)	7/50 (14%)
Rate per litters	3/25 (12%)	1/25 (4%)	1/25 (4%)	3/25 (12%)	7/25 (28%)
Adjusted rate	7%	2.4%	2.4%	7%	19%
Rao-Scott-adjusted Poly-3 test	p = 0.005	p = 0.325N	p = 0.317N	p = 0.651N	p = 0.112

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
*Significantly different ($p \leq 0.05$) from the control group by the Rao-Scott-adjusted Poly-3 test.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Only 48 animals were examined microscopically for this nonneoplastic lesion.
[e]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 1/350 (0.29% ± 0.76%); range: 0% to 2%.
[f]Historical control incidence: 20/350 (5.71% ± 3.35%); range: 2% to 10%.
[g]Number of animals with neoplasm per number of animals necropsied.
[h]Number of litters with neoplasm-bearing animals per number of litters examined at site.
[i]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[j]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the control group and that exposed group. The Rao-Scott test adjusts the Poly-3 test (which accounts for differential mortality in animals that do not reach study termination) for within-litter correlation. A negative trend or a lower incidence in an exposure group is indicated by N.
[k]Historical control incidence: 2/350 (0.57% ± 1.51%); range: 0% to 4%.
[l]Historical control incidence: 0/350.
[m]Historical control incidence: 23/350 (6.57% ± 3.41%); range: 2% to 10%.

Kidney: There were significant increases in the incidences of nonneoplastic kidney lesions in male and female DEHP-exposed groups relative to the control groups (Table 18). The incidences of papilla edema and papilla epithelium hyperplasia were significantly increased in the 10,000 ppm males and females relative to control animals; there was a significant increase in the incidence of papilla hemorrhage in the 10,000 ppm males. There were significant increases in the incidence of kidney infarct in the 300 and 10,000 ppm males and the 1,000 and 10,000 ppm females. A significant increase in the incidence of renal tubule cyst was observed in the 10,000 ppm female group compared to that of the control group. A positive trend in the incidence of renal tubule dilation occurred with increasing exposure concentration in the females.

Papilla edema (Figure 7) occurred only in exposed animals, was present in most males and females exposed to 10,000 ppm, and was observed in two females exposed to 1,000 ppm DEHP. Papillary edema affected the kidneys bilaterally and was characterized by expansion of the papillary interstitium by fibrillary amphophilic to pale eosinophilic material. Collecting ducts were often dilated and distorted and lined by a continuous layer of thin attenuated epithelium. The dilatation of collecting ducts sometimes extended to include renal tubules within the medulla and/or cortex. Representative sections were stained with Alcian blue, which identifies glycosaminoglycans (GAGs), and with periodic acid-Schiff (PAS) to evaluate basement membrane integrity. The interstitial material stained positively for Alcian blue, confirming that the material contained GAGs. The basement membranes of vascular structures stained intensely positive for PAS throughout the kidney sections and were not compromised in any areas, indicating they were intact. The renal tubules in the cortex and outer medulla stained uniformly and intensely positive for PAS, but there was an abrupt loss of staining at the junction of the outer and inner medulla. Tubules and collecting ducts within the inner medulla and papilla lacked staining for PAS, indicating disruption of the basement membrane of tubules/ducts within this region.

Papillary hemorrhage occurred in regions of papillary edema (Figure 8). Papilla epithelium hyperplasia was diagnosed when there was thickening and/or variably sized outgrowths (with clear spaces) of the epithelium overlying the renal papilla (Figure 9). Occasionally, these spaces contained eosinophilic material or cells. Although papillary epithelial hyperplasia is commonly associated with advanced chronic progressive nephropathy (CPN), there was no direct correlation with CPN severity in this study.

Kidney infarct consisted of well-demarcated, wedge-shaped regions characterized by renal interstitial fibrosis and depression of the overlying capsule; lesions extended from the capsular surface into the medulla. Infarcts were scored using a four-grade severity scale: minimal (grade 1), <25% of renal involvement; mild (grade 2), 25% to 50% of renal involvement; moderate (grade 3), 51% to 75% of renal involvement; and marked (grade 4), >75% renal involvement. Renal tubule cysts were characterized by dilated renal tubules lined by cuboidal to thin attenuated epithelial cells. There was a positive trend in renal tubule dilation in females.

Table 18. Incidences of Nonneoplastic Lesions of the Kidney in Male and Female Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
n[a]	50	49	50	50	49
Papilla, Edema[b]	0**	0	0	0	39** (2.2)[c]
Papilla, Hemorrhage	0**	1 (2.0)	0	2 (1.0)	12** (1.7)
Epithelium, Papilla, Hyperplasia	9** (1.3)	4 (1.3)	4 (1.8)	3 (1.0)	17* (1.2)
Infarct	2** (1.0)	10* (1.0)	9 (1.0)	7 (1.0)	17** (1.1)
Renal Tubule, Cyst	9	2	4	7	5
Renal Tubule, Dilation	0	0	0	2 (2.5)	0
Female					
n	50	50	50	50	49
Papilla, Edema	0**	0	2 (1.5)	0	38** (1.6)
Papilla, Hemorrhage	0	0	0	0	2 (1.0)
Epithelium, Papilla, Hyperplasia	2** (1.0)	1 (3.0)	2 (1.0)	4 (1.3)	15** (1.3)
Infarct	0**	3 (2.0)	7* (1.1)	5 (1.0)	12** (1.5)
Renal Tubule, Cyst	0**	0	2	0	7*
Renal Tubule, Dilation	0*	0	0	0	3 (2.7)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant ($p \leq 0.05$) from the vehicle control group by the Rao-Scott-adjusted Poly-3 test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

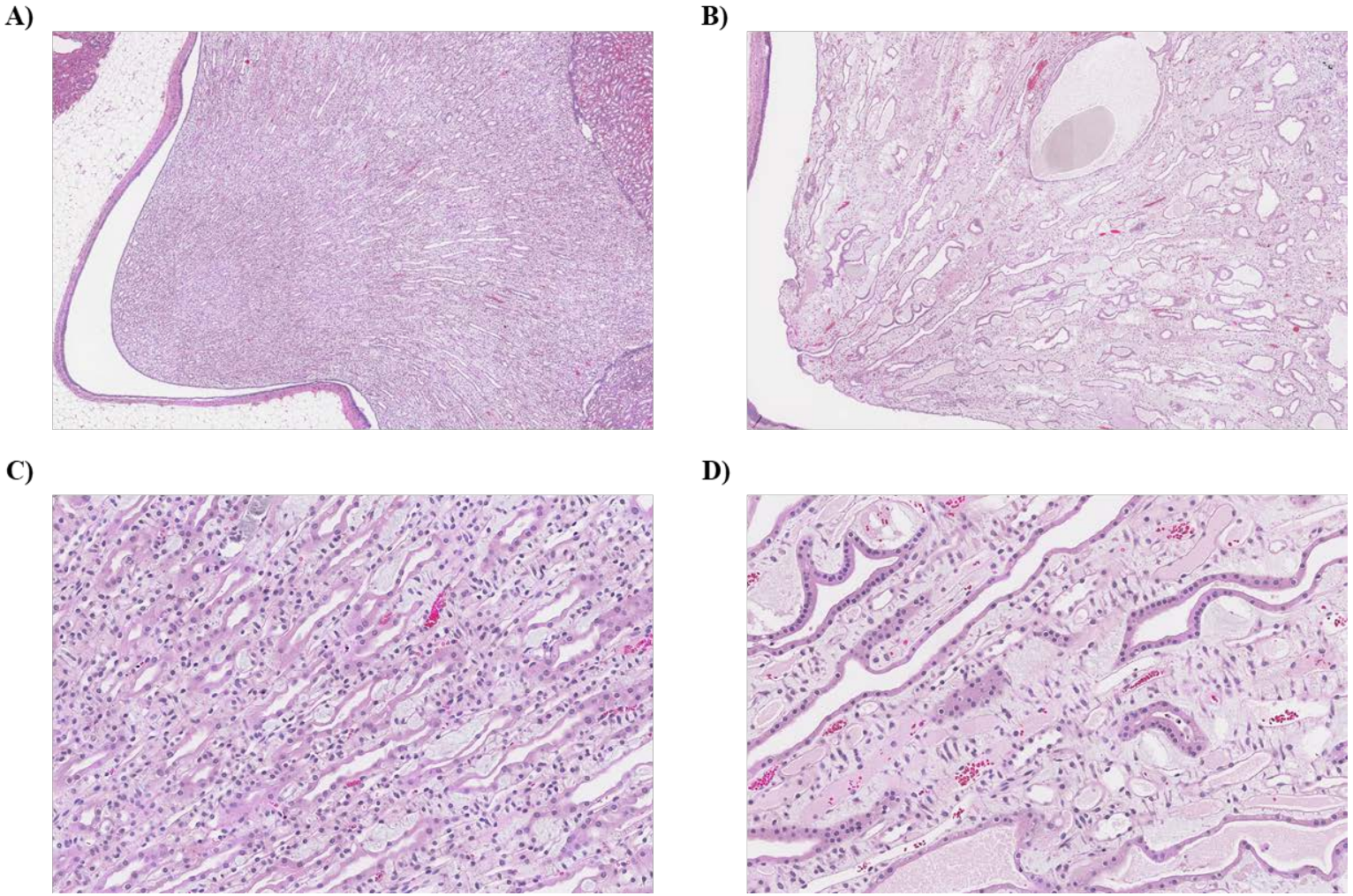


Figure 7. Kidney Papilla Edema in Male and Female Rats Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

Kidney from a control female rat with normal papilla structure (panel A at 4x magnification, panel C at 20x magnification) compared to kidney from a male 10,000 ppm rat with papillary edema (panel B at 4x magnification, panel D at 20x magnification). This lesion was characterized by expansion of the papillary interstitium by fibrillary amphophilic to pale eosinophilic material. Collecting ducts were often dilated, distorted, and lined by a continuous layer of thin attenuated epithelium.

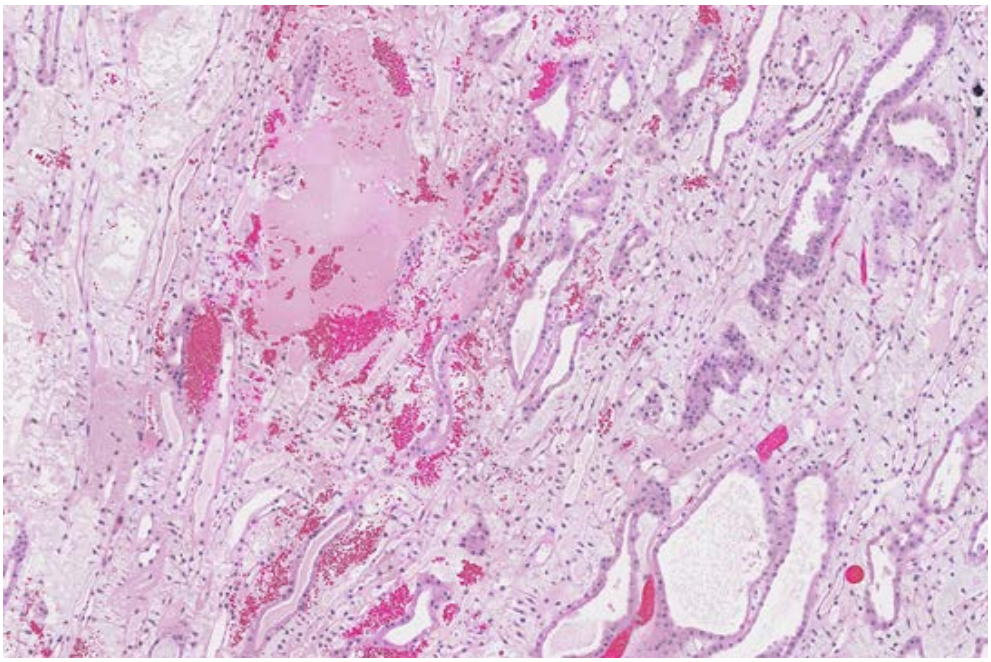


Figure 8. Renal Papillary Hemorrhage in a Male Rat Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

This image is an example of mild hemorrhage in a kidney from a 1,000 ppm male rat with moderate papillary edema at 10x magnification.

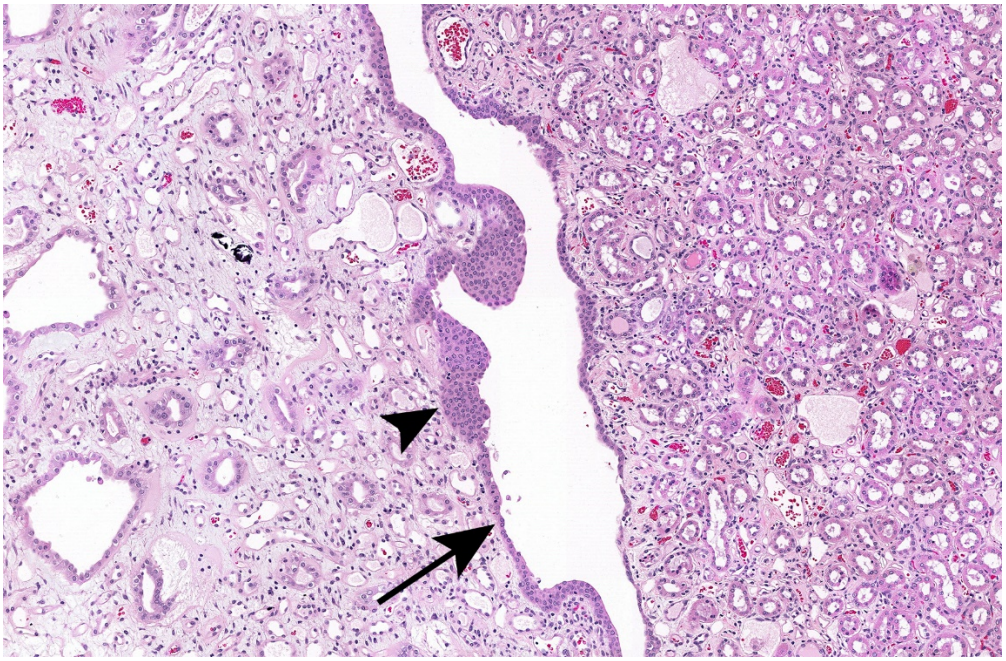


Figure 9. Renal Papillary Epithelium Hyperplasia in a Male Rat Exposed to Di(2-Ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

This image is an example of mild epithelial hyperplasia in a 10,000 ppm male rat with normal low cuboidal lining cells (long arrow) are increased in thickness (arrowhead) at 10x magnification.

Heart: A significant increase in the incidences of heart valve fibrosis and heart valve thrombus occurred in the 10,000 ppm males, relative to the control animals (Table 19). Heart valve fibrosis was diagnosed when valves were expanded by fibrous connective tissue that was more densely eosinophilic than the loose lightly basophilic to amphophilic tissue of a normal heart valve (Figure 10). Severity was scored using a four-grade scale: minimal (grade 1), change was barely detectable to thickening of the valve up to double the normal thickness; mild (grade 2), thickening up to three times the normal thickness, involvement of several portions of the valve, or more than one valve up to double the normal thickness; moderate (grade 3), thickening of several regions of the valve or valves, at least one of which was greater than double the normal thickness of a valve at that location; marked (grade 4) valve fibrosis was not diagnosed. Heart valve thrombus was characterized by fibrin, admixed with variable numbers of blood cells, that covered the cardiac valves. Severity grades were assigned according to the following grading scheme: minimal (grade 1), amount of fibrin and cells was less than the thickness of the widest part of the valve; mild (grade 2), layering upon the valves that could occlude approximately 50% of the valve lumen; moderate (grade 3), lesions that occluded 51% to 80% of the valve lumen; marked (grade 4), lesions that occluded >81% of the valve lumen.

Bone Marrow: There was a significant increase in the incidence of bone marrow hypercellularity in the 3,000 and 10,000 ppm male groups, relative to the control group (Table 19). Bone marrow hypercellularity was characterized by an increase in one or more hematopoietic cell lines, generally with a decrease in adipocytes.

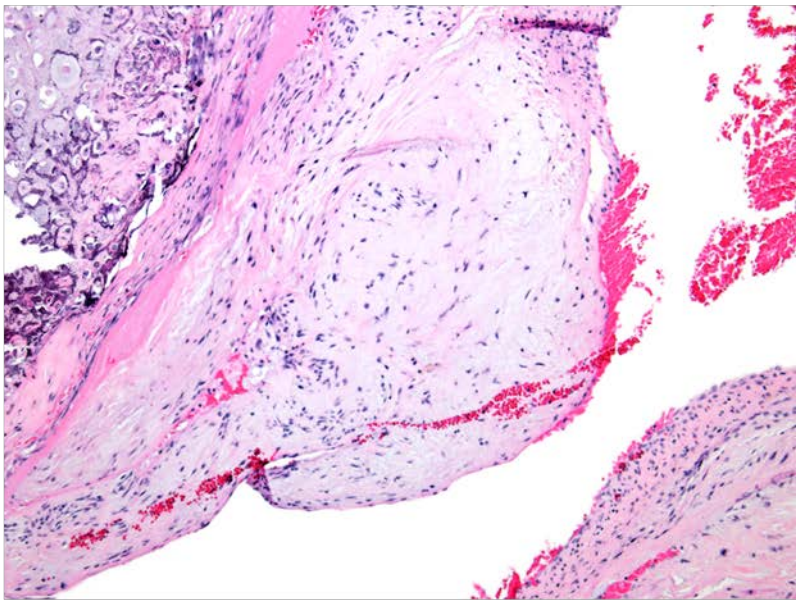
Pituitary Gland: There was a significant increase in the incidence of pars distalis hypertrophy in the 3,000 and 10,000 ppm males compared to control animals (Table 19). Pars distalis hypertrophy was characterized by clusters of cells that were round, with abundant vacuolated amorphous amphophilic or pale eosinophilic cytoplasm and peripherally compressed nuclei, scattered throughout the pars distalis (Figure 11). A severity grade was assigned based on the numbers of affected cells.

Table 19. Incidences of Select Nonneoplastic Lesions of the Heart, Bone Marrow, and Pituitary Gland in Male Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Heart[a]	50	49	50	50	49
Valve, fibrosis[b]	0**	2 (1.0)[c]	1 (2.0)	3 (1.3)	11** (1.7)
Valve, thrombus	0**	0	0	0	6* (1.8)
Bone Marrow	50	49	50	50	50
Hypercellularity	21** (2.2)	17 (1.9)	29 (1.9)	34* (1.9)	36** (2.1)
Pituitary Gland	50	49	50	50	49
Pars distalis, hypertrophy	3** (1.0)	7 (1.1)	5 (1.0)	15** (1.3)	37** (2.4)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant ($p \leq 0.05$) from the vehicle control group by the Rao-Scott-adjusted Poly-3 test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

A)



B)

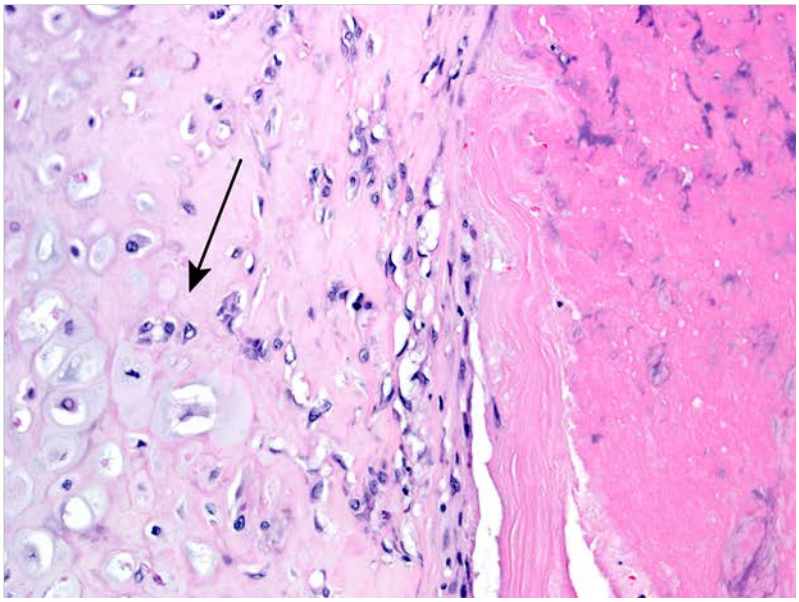
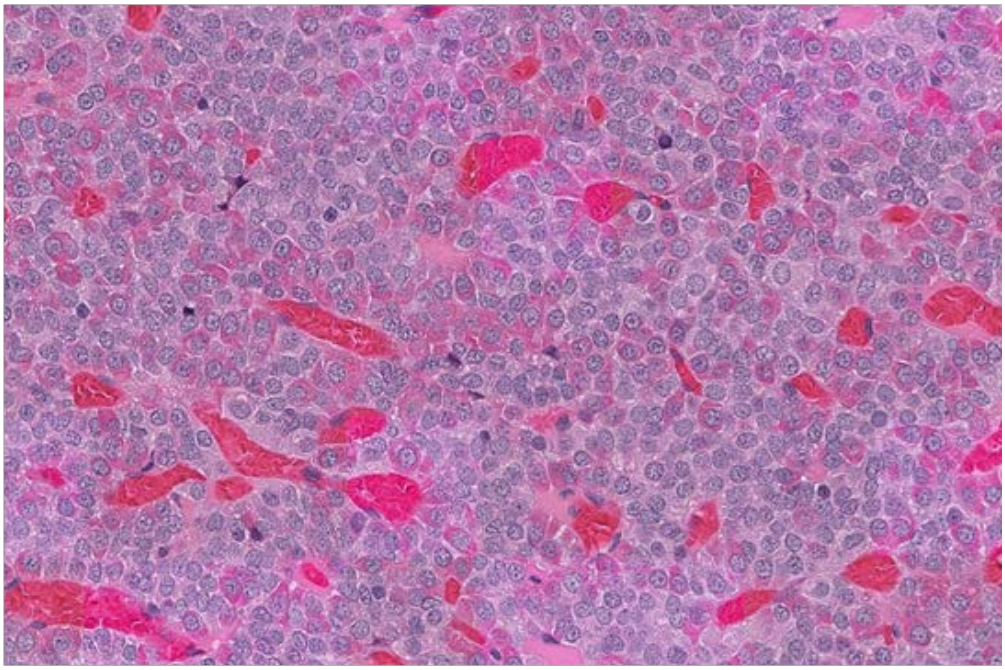


Figure 10. Heart Valve Fibrosis in a Male Rat Exposed to Di(2-Ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

The images show examples of heart valve fibrosis in a 1,000 ppm male at 10x magnification (A) and 20x magnification (B). The lesion was characterized by valves that were expanded by fibrous connective tissue that was more densely eosinophilic than the loose lightly basophilic to amphophilic tissue of a normal heart valve. Cartilaginous metaplasia (arrow) was sometimes associated with the valvular fibrosis.

A)



B)

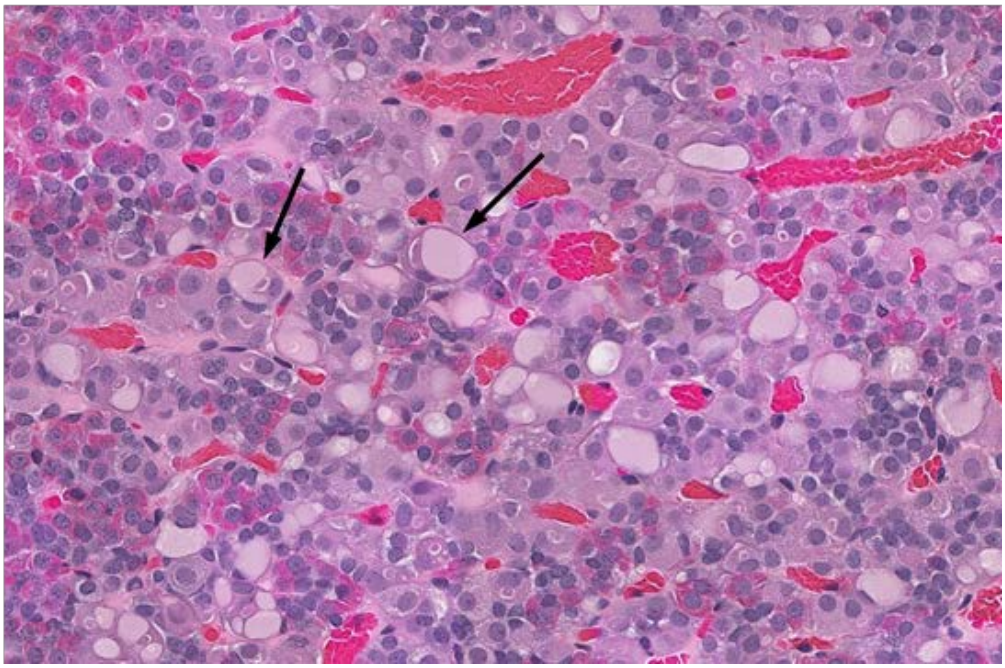


Figure 11. Pituitary Pars Distalis Hypertrophy in Male Rats Exposed to Di(2-ethylhexyl) Phthalate in the Perinatal and Postweaning Two-year Feed Study (H&E)

The images compare normal pars distalis in a control male (panel A) with one from a 10,000 ppm male that is hypertrophied (panel B) and characterized by clusters of cells that are round, with abundant amorphous amphophilic or pale eosinophilic cytoplasm and peripherally compressed nuclei (arrows) at 40x magnification.

The biological significance of other lesions is unknown (Appendix H). In males, these lesions included: adrenal cortex focal hyperplasia, adrenal medulla focal hyperplasia, testis polyarteritis nodosa, bilateral testis polyarteritis nodosa, thyroid gland C-cell adenoma, and thyroid gland C-cell adenoma or carcinoma (combined). In females, these lesions included: nose respiratory epithelium hyaline droplet accumulation, ovary atrophy, uterus endometrium squamous metaplasia, mammary gland fibroadenoma, pituitary gland pars distalis, or unspecified site adenoma in females.

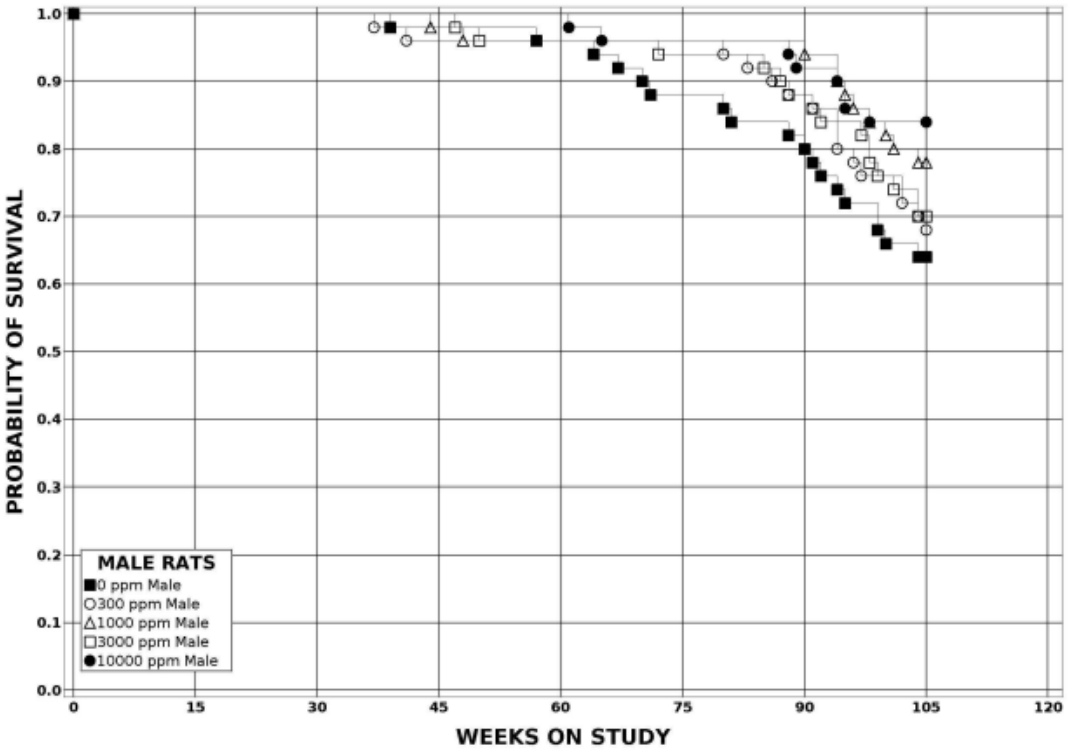
Postweaning-only Study in Rats (Study 2)

Overall, survival at study termination of male and female rats exposed to DEHP was commensurate with or greater than that of control animals (Table 20; Figure 12). Survival to study termination was significantly increased in 10,000 ppm males (approximately 84%) relative to control males (approximately 64%).

Table 20. Summary of Survival of Male and Female Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
Animals Initially in Study	50	50	50	50	50
Moribund	4	8	2	8	4
Natural Deaths	14	8	9	7	4
Animals Surviving to Study Termination	32	34[a]	39	35	42
Percent Probability of Survival at End of Study[b]	64.0	68.0	78.0	70.0	84.0
Mean Survival (Days)[c]	675	692	706	696	711
Survival Analysis[d]	p = 0.061N	p = 0.692N	p = 0.139N	p = 0.553N	p = 0.037N
Female					
Animals Initially in Study	50	50	50	50	50
Moribund	9	10	13	9	6
Natural Deaths	8	6	4	7	12
Animals Surviving to Study Termination	33	34	33[e]	34	32[f]
Percent Probability of Survival at End of Study	66.0	68.0	66.0	68.0	64.0
Mean Survival (Days)	691	668	678	700	684
Survival Analysis	p = 0.834	p = 1.000N	p = 1.000	p = 0.932N	p = 0.904

[a]Includes one animal that died naturally during the last week of the study.
[b]Kaplan-Meier determinations.
[c]Mean of all deaths (uncensored, censored, and study termination).
[d]The result of the life-table trend test is in the vehicle control column, and the results of the life-table pairwise comparisons with the vehicle control group are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by N.
[e]Includes one animal that died naturally and one animal that was euthanized moribund during the last week of the study.
[f]Includes one animal that died naturally during the last week of the study.



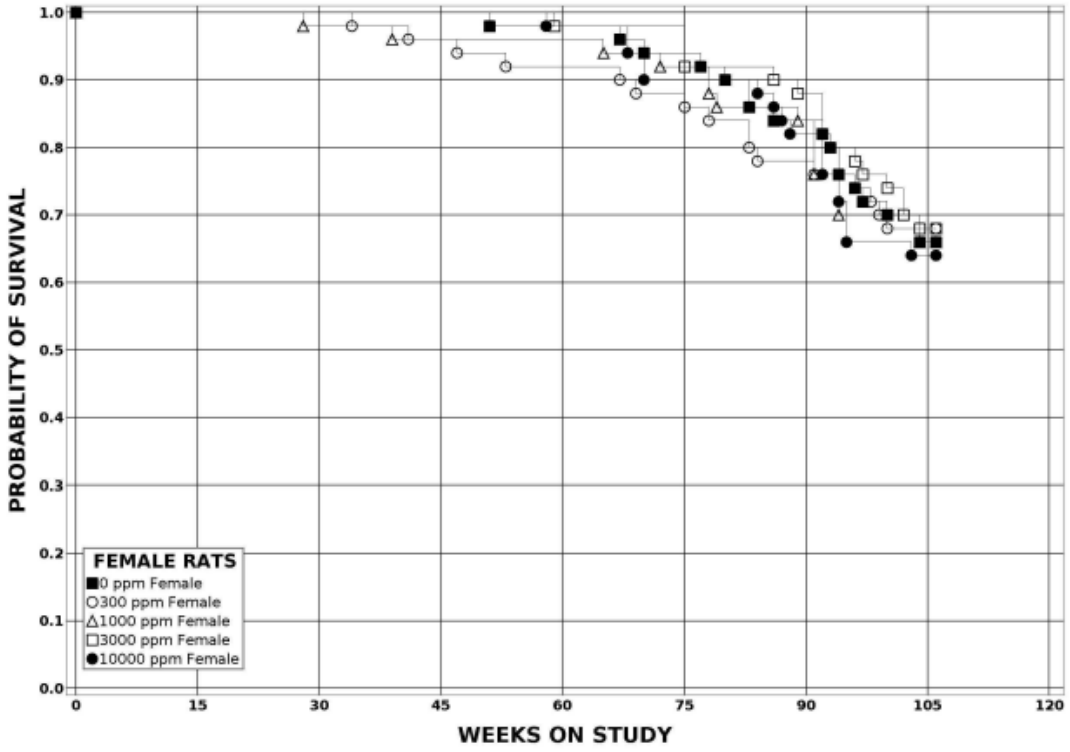


Figure 12. Kaplan-Meier Survival Curves for Rats Exposed to Di(2-ethylhexyl) Phthalate in the Postweaning-only Two-year Feed Study

At study termination, group mean body weights for the 300, 1,000, and 3,000 ppm DEHP groups were within 6% of control animals in both male and female rats (Table 21, Table 22; Figure 13). Lower body weights were noted in males (approximately 16%) and females (approximately 22%) in the 10,000 ppm groups at the end of study relative to control animals. These effects were attributed to reduced body weight gain relative to control animals, which occurred throughout the study.

Feed consumption by male and female rats in the 300, 1,000, 3,000, and 10,000 ppm DEHP groups was commensurate with that of the control group throughout the study with the exception of study week 1, when feed consumption was approximately 21% lower in the 10,000 ppm male and female groups (Table 23, Table 24). This finding might reflect an initial adjustment related to the palatability of feed containing high concentrations (1%) of DEHP. Dietary concentrations of 300, 1,000, 3,000, and 10,000 ppm resulted in average daily doses of approximately 17, 54, 170, and 602 mg/kg/day for males and 17, 60, 177, and 646 mg/kg/day for females (Appendix H).

No exposure-related clinical findings were observed in any of the exposed groups (Appendix H).

Table 21. Summary of Survival and Mean Body Weights of Male Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Study Day[a]	0 ppm		300 ppm			1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	138.2	50	138.5	100.2	50	139.0	100.5	50	138.7	100.3	50	139.3	100.8	50
8	182.5	50	182.4	100.0	50	184.1	100.9	50	182.3	99.9	50	174.1	95.4	50
15	222.3	50	225.4	101.4	50	224.7	101.1	50	223.2	100.4	50	215.1	96.8	50
22	253.2	50	256.9	101.5	50	254.6	100.6	50	253.4	100.1	50	243.0	96.0	50
29	273.8	50	279.4	102.1	50	275.8	100.8	50	273.1	99.8	50	259.4	94.8	50
36	290.0	50	296.6	102.3	50	293.0	101.0	50	288.7	99.6	50	271.2	93.5	50
43	303.0	50	310.6	102.5	50	306.6	101.2	50	301.3	99.4	50	283.8	93.7	50
50	314.9	50	318.7	101.2	50	316.7	100.6	50	311.8	99.0	50	294.0	93.4	50
57	326.4	50	326.5	100.0	50	325.9	99.9	50	319.1	97.8	50	301.2	92.3	50
64	337.6	50	337.4	99.9	50	336.8	99.8	50	328.6	97.3	50	309.5	91.7	50
71	346.3	50	347.1	100.2	50	345.2	99.7	50	335.4	96.9	50	316.4	91.4	50
78	357.7	50	354.7	99.1	50	354.3	99.0	50	343.8	96.1	50	323.0	90.3	50
85	365.2	50	360.9	98.8	50	361.7	99.0	50	346.3	94.8	50	324.2	88.8	50
92	372.1	50	365.2	98.2	50	366.3	98.5	50	351.0	94.3	50	329.4	88.5	50
120	386.2	50	388.4	100.6	50	380.2	98.4	50	365.1	94.5	50	341.9	88.5	50
148	404.6	50	409.5	101.2	50	398.5	98.5	50	385.6	95.3	50	361.1	89.3	50
176	420.8	50	426.9	101.4	50	415.8	98.8	50	400.2	95.1	50	373.2	88.7	50
204	435.4	50	435.6	100.0	50	428.4	98.4	50	409.0	93.9	50	375.8	86.3	50
232	443.7	50	447.1	100.8	50	436.8	98.4	50	412.8	93.0	50	380.5	85.8	50
260	455.5	50	463.2	101.7	49	451.1	99.0	50	430.5	94.5	50	393.2	86.3	50
288	463.2	49	468.7	101.2	48	460.0	99.3	50	437.8	94.5	50	396.5	85.6	50
316	475.3	49	477.8	100.5	48	467.8	98.4	49	444.2	93.5	50	401.4	84.4	50
344	478.3	49	489.5	102.3	48	477.9	99.9	48	448.0	93.7	49	406.9	85.1	50
372	481.2	49	490.2	101.9	48	481.6	100.1	48	457.6	95.1	48	412.7	85.8	50
400	499.7	48	508.9	101.8	48	492.3	98.5	48	468.2	93.7	48	420.6	84.2	50
428	508.5	48	510.8	100.5	48	501.2	98.6	48	472.1	92.8	48	422.7	83.1	49
456	507.2	47	513.9	101.3	48	505.4	99.6	48	476.4	93.9	48	424.2	83.6	48
484	515.8	46	521.1	101.0	48	514.3	99.7	48	481.2	93.3	48	430.8	83.5	48
512	515.5	44	522.5	101.4	48	511.6	99.2	48	481.9	93.5	47	432.7	83.9	48
540	522.6	44	519.9	99.5	48	516.3	98.8	48	489.0	93.6	47	435.8	83.4	48
568	525.8	42	528.0	100.4	47	524.2	99.7	48	490.8	93.4	47	436.0	82.9	48
596	523.6	42	527.8	100.8	46	524.2	100.1	48	493.7	94.3	46	436.3	83.3	48
624	517.5	41	536.6	103.7	44	520.8	100.6	47	496.8	96.0	44	435.0	84.1	46
652	525.7	38	530.6	100.9	43	520.5	99.0	47	501.6	95.4	42	428.5	81.5	46
680	515.6	36	530.7	102.9	38	522.5	101.3	43	501.8	97.3	40	427.4	82.9	43
708	512.4	33	537.6	104.9	38	524.9	102.4	40	501.0	97.8	37	430.2	83.9	42
EOS	**505.9**	**32**	**524.8**	**103.7**	**33**	**520.6**	**102.9**	**39**	**500.9**	**99.0**	**35**	**426.9**	**84.4**	**42**

EOS = end of study.

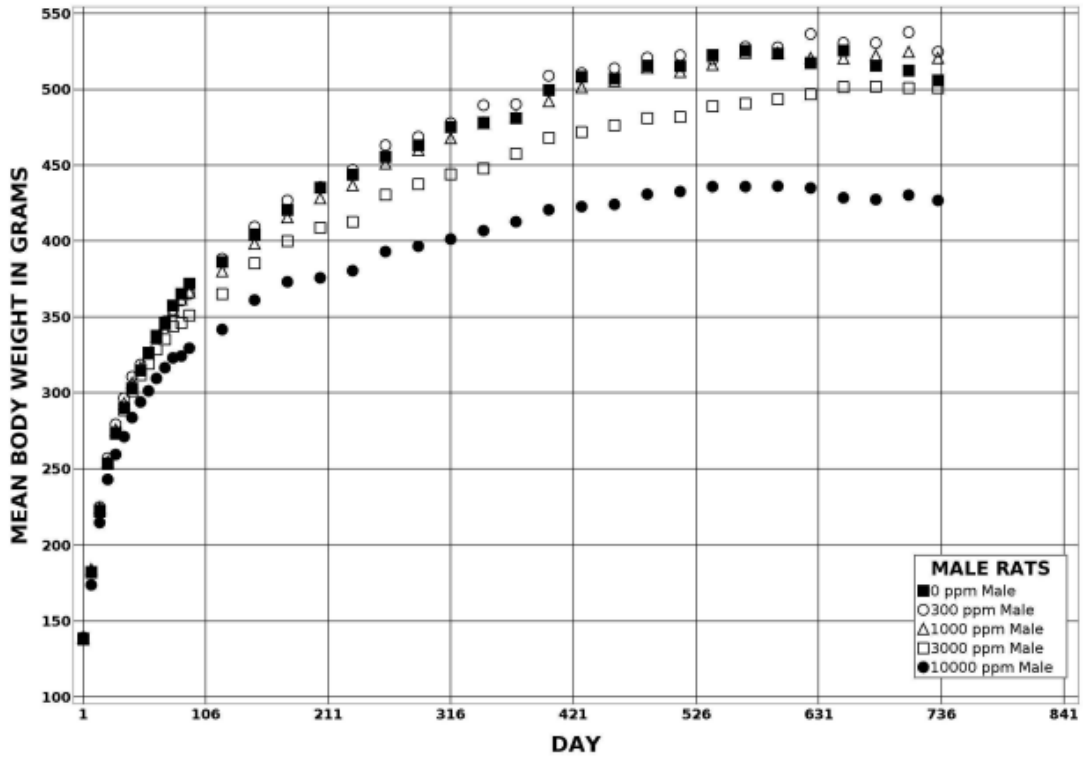
[a]Study day 1 is the day animals were placed on study.

Table 22. Summary of Survival and Mean Body Weights of Female Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Study Day[a]	0 ppm		300 ppm			1,000 ppm			3,000 ppm			10,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	118.4	50	121.2	102.4	50	120.2	101.6	50	121.2	102.4	50	119.7	101.1	50
8	141.7	50	141.0	99.5	50	141.2	99.6	50	144.2	101.8	50	139.3	98.3	50
15	158.6	50	161.4	101.8	50	158.4	99.9	50	162.4	102.4	50	156.8	98.9	50
22	172.5	50	177.4	102.9	50	174.7	101.3	50	179.9	104.3	50	170.6	98.9	50
29	185.0	50	193.2	104.4	50	185.5	100.2	50	189.4	102.3	50	178.3	96.3	50
36	193.7	50	200.9	103.7	50	194.1	100.2	50	200.0	103.2	50	185.8	95.9	50
43	200.1	50	207.7	103.8	50	203.0	101.5	50	206.8	103.4	50	191.9	95.9	50
50	205.8	50	212.4	103.2	50	210.6	102.3	50	210.9	102.5	50	196.0	95.3	50
57	211.4	50	219.0	103.6	50	217.2	102.7	50	216.0	102.2	50	201.7	95.4	50
64	216.4	50	221.1	102.2	50	223.0	103.0	50	220.8	102.1	50	205.0	94.7	50
71	220.5	50	226.4	102.7	50	225.4	102.2	50	225.1	102.1	50	208.3	94.5	50
78	224.9	50	228.6	101.7	50	230.8	102.7	50	229.7	102.1	50	213.0	94.7	50
85	228.8	50	231.0	100.9	50	231.8	101.3	50	233.0	101.8	50	215.0	94.0	50
92	233.4	50	232.7	99.7	50	234.4	100.4	50	233.4	100.0	50	216.8	92.9	50
120	241.1	50	248.2	102.9	50	240.8	99.9	50	242.7	100.6	50	221.6	91.9	50
148	248.1	50	255.7	103.1	50	250.7	101.1	50	251.2	101.3	50	229.3	92.4	50
176	253.9	50	267.2	105.2	50	259.7	102.3	50	258.6	101.8	50	234.5	92.4	50
204	258.3	50	266.6	103.2	50	261.3	101.1	49	260.8	100.9	50	235.4	91.1	50
232	264.1	50	276.6	104.8	50	262.1	99.3	49	266.2	100.8	50	236.3	89.5	50
260	265.8	50	280.1	105.4	49	270.9	101.9	49	269.5	101.4	50	240.7	90.5	50
288	272.7	50	285.2	104.6	48	271.4	99.5	48	273.2	100.2	50	241.5	88.6	50
316	274.2	50	289.6	105.6	48	274.0	99.9	48	273.8	99.8	50	241.9	88.2	50
344	279.5	50	291.4	104.3	47	277.8	99.4	48	278.6	99.7	50	242.5	86.8	50
372	283.3	49	295.7	104.4	46	282.1	99.6	48	281.3	99.3	50	242.8	85.7	50
400	290.1	49	301.8	104.0	46	286.3	98.7	48	284.9	98.2	50	244.4	84.2	50
428	295.3	49	303.2	102.7	46	293.0	99.2	48	291.1	98.6	49	247.7	83.9	49
456	300.7	49	309.8	103.0	46	296.3	98.6	47	293.5	97.6	49	249.0	82.8	49
484	304.6	48	305.8	100.4	44	302.5	99.3	47	297.4	97.6	49	252.0	82.7	47
512	308.3	47	313.1	101.6	44	307.0	99.6	46	302.8	98.2	49	251.7	81.6	45
540	308.6	46	314.6	101.9	43	316.8	102.6	46	300.2	97.3	46	251.9	81.6	45
568	311.3	45	324.0	104.1	42	313.8	100.8	43	299.2	96.1	46	253.2	81.3	45
596	315.8	43	319.2	101.1	39	320.9	101.6	43	303.2	96.0	46	251.5	79.6	43
624	321.7	42	322.6	100.3	39	327.3	101.7	42	304.4	94.6	44	251.4	78.2	41
652	325.1	40	325.6	100.1	38	327.3	100.7	36	307.6	94.6	40	247.4	76.1	37
680	324.4	36	328.8	101.4	36	322.6	99.5	35	311.3	96.0	38	247.3	76.2	33
708	329.5	35	328.9	99.8	34	334.3	101.4	35	312.0	94.7	36	247.7	75.2	33
EOS	**322.5**	**33**	**336.3**	**104.3**	**34**	**341.5**	**105.9**	**31**	**312.5**	**96.9**	**34**	**252.0**	**78.1**	**33**

EOS = end of study.

[a]Study day 1 is the day animals were placed on study.



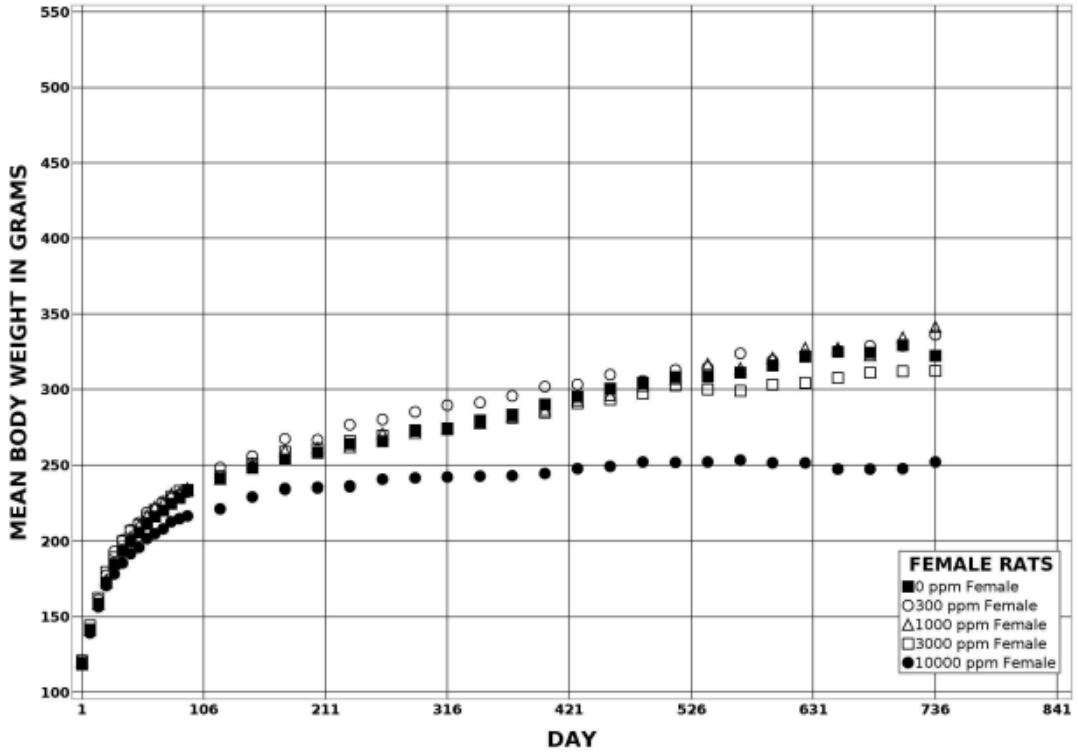


Figure 13. Growth Curves for Rats Exposed to Di(2-ethylhexyl) Phthalate in the Postweaning-only Two-year Feed Study

Table 23. Summary of Feed and Di(2-ethylhexyl) Phthalate Consumption of Male Rats in the Postweaning-only Two-year Feed Study

Week	0 ppm	300 ppm		1,000 ppm		3,000 ppm		10,000 ppm	
	Feed (g/day)[a]	Feed (g/day)	Dose (mg/kg/day)[b]	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)
1	20.2	20.4	44.2	20.4	146.8	19.8	428.2	15.9	1,141.5
13	21.6	20.2	16.8	20.2	55.9	20.5	177.6	19.3	595.3
54	27.4	26.3	16.1	26.7	55.4	26.3	172.4	24.0	581.5
102	25.1	26.4	14.7	25.1	47.8	23.6	141.3	24.5	569.5

[a]Grams of feed consumed per animal per day.
[b]Milligrams of di(2-ethylhexyl) phthalate consumed/kilogram body weight/day.

Table 24. Summary of Feed and Di(2-ethylhexyl) Phthalate Consumption of Female Rats in the Postweaning-only Two-year Feed Study

Week	0 ppm	300 ppm		1,000 ppm		3,000 ppm		10,000 ppm	
	Feed (g/day)[a]	Feed (g/day)	Dose (mg/kg/day)[b]	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)	Feed (g/day)	Dose (mg/kg/day)
1	14.5	14.5	35.9	14.2	118.1	14.6	361.5	11.5	960.6
13	14.0	13.2	17.1	14.6	63.0	14.2	182.8	13.6	632.7
54	15.0	17.2	17.4	16.5	58.5	16.3	173.9	15.1	621.9
102	18.1	18.3	16.7	18.8	56.2	18.6	175.5	16.9	682.2

[a]Grams of feed consumed per animal per day.
[b]Milligrams of di(2-ethylhexyl) phthalate consumed/kilogram body weight/day.

Pathology

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, pancreas, male and female reproductive organs, thyroid, heart, and bone marrow. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal neoplasm diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the biologically significant neoplasms mentioned in this section are presented as supplemental data in Appendix H.

Liver: There were significant increases in the incidences of hepatocellular adenomas (10,000 ppm males and females) and carcinomas (10,000 ppm males) relative to that of control animals and a positive trend in females for hepatocellular carcinomas with increasing exposure concentration (Table 25). The incidence of adenoma or carcinoma (combined) was significantly increased in the 10,000 ppm male and female groups relative to the respective control groups. Hepatocellular adenomas were characterized by regions that were sharply demarcated from surrounding liver parenchyma, nodular, and compressing adjacent normal hepatocytes, with loss of normal lobular architecture and an irregular growth pattern. The liver plates typically impinged obliquely on the surrounding liver parenchyma. The hepatocytes within an adenoma generally varied in size. Hepatocellular carcinomas were characterized by one or more of the following features: local infiltrating growth and/or distinct lack of demarcation with the adjacent

tissue, the presence of trabeculae composed of multiple layers of hepatocytes, cellular pleomorphism, loss of normal lobular architecture, regions of hemorrhage and/or necrosis, and increased mitotic figures.

There were significant increases in the incidences of many nonneoplastic liver lesions in DEHP-exposed groups relative to the control groups (Table 25). The incidence of hepatocellular cytoplasmic alteration was significantly increased in 3,000 and 10,000 ppm males and in 1,000, 3,000, and 10,000 ppm females relative to that of the respective control animals. There were significant increases in the incidence of hepatocellular hypertrophy in the 10,000 ppm males and in the 1,000, 3,000, and 10,000 ppm females. Significant increases in the incidence of liver pigment were observed in the 1,000, 3,000, and 10,000 ppm males and females. There were significant increases in the incidence of liver necrosis in the 3,000 and 10,000 ppm males. There was a significant increase in the incidence of hepatocellular eosinophilic foci in the 10,000 ppm males, and a positive trend in the incidence of hepatocellular clear cell foci in exposed males with increasing exposure concentration.

Hepatocellular cytoplasmic alteration was characterized by hepatocytes that were expanded with eosinophilic granular cytoplasm (see Figure 5 as an example). A four-grade severity scale was used based on degree of tissue affected in the section of liver that was evaluated histologically: minimal (grade 1), up to 25% of hepatocyte involvement; mild (grade 2), 26% to 50% of hepatocyte involvement; moderate (grade 3), 51% to 75% of hepatocyte involvement; and marked (grade 4) at least 76% of hepatocyte involvement. Hepatocellular hypertrophy often occurred in conjunction with cytoplasmic alteration and/or pigment. Hypertrophy was characterized by enlargement of the hepatocytes. In lesser affected animals, hypertrophy was confined to centrilobular regions, but in more severely affected animals, hypertrophy extended into the midzonal and periportal areas. A four-grade severity scale was used: minimal (grade 1), up to 10% of hepatocyte involvement; mild (grade 2), 11% to 25% of hepatocyte involvement; moderate (grade 3), 26% to 50% of hepatocyte involvement; and severe (grade 4), >51% of hepatic involvement. Hypertrophy was generally centrilobular and often involved only a few cells per lobule. Although hypertrophy was only occasionally observed in males (at the 3,000 and 10,000 ppm concentrations), in females, its incidence (but not severity) increased significantly with exposure concentrations starting at 1,000 ppm.

Pigment was characterized by a pale gold-colored pigment within the hepatocellular cytoplasm (see Figure 5 as an example). A four-grade severity scale was used: minimal (grade 1), up to 30% of hepatocytes contained pigment; mild (grade 2), 31% to 50% of hepatocytes contained pigment; moderate (grade 3), >51% of hepatocytes contained pigment; and marked (grade 4), >51% of hepatocytes contained pigment, and the pigment was very dense. Hepatocellular necrosis was characterized by multiple adjacent hepatocytes that were swollen with increased eosinophilia, karyorrhectic nuclear debris, with or without accompanying inflammatory cells. Necrosis was scored using a four-grade severity scale: minimal (grade 1), up to three focal areas of necrosis present; mild (grade 2), necrosis in up to 25% of the liver; moderate (grade 3), necrosis in 26% to 60% of the liver; and severe (grade 4), necrosis in >61% of the liver.

Hepatocellular foci were diagnosed when there was an alteration in the arrangement of hepatocytes involving at least six cells, with a discrete lesion margin, where attenuated hepatocytes at the lesion margin (compression) involved <70% of the lesion circumference. Lobular architecture was preserved in the absence of cellular atypia, and the hepatocytes within

the eosinophilic foci were more eosinophilic than those within the surrounding parenchyma. Clear cell foci were characterized by circular or ovoid regions composed of normal-sized or enlarged cells with distinct cytoplasmic clear spaces compared with the surrounding parenchyma. The nuclei were often small and dense, prominent, and centrally located, occasionally exhibiting increased volume. The distinction between large foci (usually eosinophilic) and hepatocellular adenomas was based on retention of normal lobular architecture in the foci, greater size of hepatocellular adenomas (usually measuring at least 3 mm), and presence of compression or bulging of the adenoma from the liver surface along >70% of the lesion circumference.

Table 25. Incidences of Neoplastic and Nonneoplastic Lesions of the Liver in Male and Female Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
n[a]	50	50	50	50	50
Hepatocyte, Cytoplasmic Alteration[b]	0**	1 (2.0)[c]	0	38** (1.3)	49** (3.6)
Hepatocyte, Hypertrophy	0**	0	0	2 (1.0)	6* (1.2)
Pigment	0**	0	7* (1.0)	45** (1.8)	50** (2.5)
Necrosis	0**	2 (1.5)	4 (1.0)	7* (1.3)	8** (1.3)
Eosinophilic Focus	1**	0	4	2	24**
Clear Cell Focus	29*	31	33	35	39
Hepatocellular Adenoma[d]					
Overall rate[e]	0/50 (0%)	2/50 (4%)	0/50 (0%)	1/50 (2%)	6/50 (12%)
Adjusted rate[f]	0%	4.5%	0%	2.2%	12.9%
Poly-3 test[g]	p < 0.001	p = 0.251	(e)	p = 0.514	p = 0.022
Hepatocellular Carcinoma[h]					
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted rate	0%	0%	0%	0%	12.8%
Poly-3 test	p < 0.001	(e)	(e)	(e)	p = 0.022
Hepatocellular Adenoma or Carcinoma (Combined)[i]					
Overall rate	0/50 (0%)	2/50 (4%)	0/50 (0%)	1/50 (2%)	12/50 (24%)
Adjusted rate	0%	4.5%	0%	2.2%	25.6%
Poly-3 test	p < 0.001	p = 0.251	(e)	p = 0.514	p < 0.001
Female					
n	50	50	50	50	49
Hepatocyte, Cytoplasmic Alteration	0**	2 (1.0)	15** (1.1)	38** (1.3)	45** (2.8)
Hepatocyte, Hypertrophy	0**	0	6* (1.2)	14** (1.0)	28** (1.3)
Pigment	3** (1.0)	0	18** (1.1)	30** (1.3)	48** (2.5)

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Necrosis	2 (1.0)	5 (1.8)	4 (1.5)	2 (2.5)	4 (2.0)
Eosinophilic Focus	7	6	6	3	7
Clear Cell Focus	8	10	14	7	5
Hepatocellular Adenoma[j]					
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)	13/49 (27%)
Adjusted rate	0%	0%	2.4%	2.3%	31.3%
Poly-3 test	p < 0.001	(e)	p = 0.495	p = 0.505	p < 0.001
Hepatocellular Carcinoma[k]					
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/49 (4%)
Adjusted rate	0%	0%	0%	0%	4.9%
Poly-3 test	p = 0.018	(e)	(e)	(e)	p = 0.226
Hepatocellular Adenoma or Carcinoma (Combined)[l]					
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)	14/49 (29%)
Adjusted rate	0%	0%	2.4%	2.3%	33.7%
Poly-3 test	p < 0.001	(e)	p = 0.495	p = 0.505	p < 0.001

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test; **$p \leq 0.01$.
(e) = value of statistic could not be computed.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 2/489 (0.44% ± 0.88%); range: 0% to 2%.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[g]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach study termination. A negative trend or a lower incidence in an exposure group is indicated by N.
[h]Historical control incidence: 2/489 (0.45% ± 0.89%); range: 0% to 2%.
[i]Historical control incidence: 4/489 (0.89% ± 1.06%); range: 0% to 2%.
[j]Historical control incidence: 15/489 (2.65% ± 2.59%); range: 0% to 8%.
[k]Historical control incidence: 1/489 (0.22% ± 0.67%); range: 0% to 2%.
[l]Historical control incidence: 16/489 (2.87% ± 2.8%); range: 0% to 8%.

Pancreas: In male rats, there were significant increases in the incidences of acinar adenoma and acinar adenoma or carcinoma (combined) in the 3,000 and 10,000 ppm groups relative to the control group. There was a significant increase in the incidence of acinar carcinoma in the 10,000 ppm male group (Table 26). In females, there was a positive trend for pancreas acinar adenoma or carcinoma (combined). Pancreatic acinar adenomas were distinct nodular masses that were not contiguous with the adjacent parenchyma, which were >3 mm in diameter, and that compressed the adjacent tissue; pleomorphism or atypia was rarely present. Pancreatic acinar carcinomas were typically larger than adenomas and frequently exhibited cellular pleomorphism and atypia; invasion or metastasis was pathognomonic. Scirrhous reactions were occasionally present, characterized by dense fibrous or connective tissue.

There were significant increases in the incidences of pancreatic acinus hyperplasia in male rats in the 3,000 and 10,000 ppm groups relative to the control group. In females, there was a significant increase in the incidence of pancreatic acinus hyperplasia in the 10,000 ppm group. Pancreatic acinus hyperplasia was characterized by circumscribed areas of enlarged acini that were <3 mm in diameter and that were contiguous with the adjacent parenchyma. A four-grade severity scale was used: minimal (grade 1), no more than one lobule was affected, and the lesion was smaller than 1 mm; mild (grade 2), lesion was 1 to 2 mm; moderate (grade 3), lesion was 2 to 3 mm; and marked (grade 4), lesion was 3 mm but lacked features of an adenoma, such as compression. Severity grades were increased if multiple hyperplastic lesions were present within the pancreas.

Table 26. Incidences of Neoplastic and Nonneoplastic Lesions of the Pancreas in Male and Female Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Male					
n[a]	49	50	50	50	50
Acinus, Hyperplasia[b]	7** (2.6)[c]	8 (2.3)	9 (1.8)	24** (3.3)	26** (3.0)
Acinar Adenoma[d]					
Overall rate[e]	1/49 (2%)	4/50 (8%)	5/50 (10%)	23/50 (46%)	30/50 (60%)
Adjusted rate[f]	2.4%	9%	10.7%	49.9%	64%
Poly-3 test[g]	p < 0.001	p = 0.202	p = 0.131	p < 0.001	p < 0.001
Acinar Carcinoma[h]					
Overall rate	0/49 (0%)	1/50 (2%)	0/50 (0%)	1/50 (2%)	5/50 (10%)
Adjusted rate	0%	2.3%	0%	2.2%	10.6%
Poly-3 test	p < 0.001	p = 0.513	(e)	p = 0.515	p = 0.043
Acinar Adenoma or Carcinoma (Combined)[i]					
Overall rate	1/49 (2%)	5/50 (10%)	5/50 (10%)	23/50 (46%)	33/50 (66%)
Adjusted rate	2.4%	11.2%	10.7%	49.9%	69.8%
Poly-3 test	p < 0.001	p = 0.119	p = 0.131	p < 0.001	p < 0.001
Female					
n	50	50	50	50	47
Acinus, Hyperplasia	0**	1 (2.0)	1 (1.0)	1 (1.0)	5* (3.0)
Acinar Adenoma[j]					
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/47 (2%)
Adjusted rate	0%	0%	0%	2.3%	2.5%
Poly-3 test	(n)	(n)	(n)	(n)	(n)
Acinar Carcinoma[j]					
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/47 (2%)
Adjusted rate	0%	0%	0%	0%	2.5%
Poly-3 test	(n)	(n)	(n)	(n)	(n)

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Acinar Adenoma or Carcinoma (Combined)[j]					
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/47 (4%)
Adjusted rate	0%	0%	0%	2.3%	5%
Poly-3 test	p = 0.038	(e)	(e)	p = 0.505	p = 0.219

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test; **$p \leq 0.01$.
(e) = value of statistic could not be computed; (n) = no statistics were calculated.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 60/488 (11.58% ± 9.25%); range: 0% to 28%.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[g]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach study termination. A negative trend or a lower incidence in an exposure group is indicated by N.
[h]Historical control incidence: 4/488 (0.8% ± 1.42%); range: 0% to 4%.
[i]Historical control incidence: 62/488 (12.03% ± 9.16%); range: 0% to 28%.
[j]Historical control incidence: 0/489.

Testis: There was a positive trend in the incidence of testicular interstitial cell adenoma in male rats (Table 27). Interstitial cell adenomas were characterized by regions of increased Leydig cells, described as mostly uniform polyhedral cells with abundant eosinophilic, finely granular, or vacuolated cytoplasm, which exceeded the diameter of three seminiferous tubules. Circumferential compression of adjacent seminiferous tubules was observed occasionally.

There were significant increases in the incidences of germinal epithelium degeneration, bilateral germinal epithelium degeneration, testis edema, and bilateral testis edema in the 10,000 ppm group compared to the control males; there was a positive trend in the incidence of focal interstitial cell hyperplasia (Table 27). Germinal epithelium degeneration was recorded when one or more of the following features was present in tubules not adjacent to the rete testis: tubular vacuolation, partial depletion of germ cells, degenerating (multinucleated or apoptotic) germ cells, and disordered arrangement of the germ cell layers and/or seminiferous tubules completely devoid of germ cells and lined only by Sertoli cells. Germinal epithelium degeneration was scored using a four-grade severity scale: minimal (grade 1), up to 25% of at least one testis involved; mild (grade 2), 26% to 50% of at least one testis involved; moderate (grade 3), 51% to 75% of at least one testis involved; and marked (grade 4), rare to no normal seminiferous tubules in either testis were present (i.e., remaining seminiferous tubules solely lined by Sertoli cells).

Testis edema was characterized by the presence of acellular, finely granular or fibrillar pale eosinophilic material in the interstitium. In most animals, this finding was bilateral. A severity grade was determined by the amount of interstitial fluid. Generally, the severity of edema was higher in testes with reduced numbers of seminiferous tubules, because the interstitial fluid filled the intervening space. In some animals with reduced numbers of seminiferous tubules, however, the testis was collapsed and shrunken, leaving no interstitial space.

Interstitial cell hyperplasia was scored using a four-grade severity scale: minimal (grade 1), when only a thin rim of interstitial cells or a cluster of cells one-fourth the size of a normal seminiferous tubule was present; mild (grade 2), when several such areas were present or one cluster was present that was one-half the size of a normal seminiferous tubule; moderate (grade 3), when a cluster three-fourths the size of a normal seminiferous tubule was present; and marked (grade 4), when a cluster of interstitial cells approached the diameter of a normal seminiferous tubule. The interstitial cells involved were frequently very elongated and flattened in profile.

Epididymis: There were significant increases in the incidences of bilateral hypospermia and bilateral epididymis duct exfoliated germ cell in the 10,000 ppm group relative to the control males (Table 27). Epididymis hypospermia was characterized by a reduced density of sperm in the lumen of the epididymal duct, often accompanied by luminal cell debris. Its severity was scored using a four-grade scale: minimal (grade 1), 25–50% reduction of spermatozoa; mild (grade 2), 51–66% reduction; moderate (grade 3), 67–80% reduction; and marked (grade 4), 81–100% reduction. The lesion of epididymis duct exfoliated germ cell was characterized by the presence of nondegenerate germ cells and debris in the epididymal lumen. This was often accompanied by depletion of germ cells from the seminiferous epithelium in testes diagnosed with germinal epithelium degeneration.

Table 27. Incidences of Neoplastic and Nonneoplastic Lesions of the Testis and Epididymis in Male Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
n[a]	50	50	50	50	50
Testis					
Germinal epithelium, degeneration (includes bilateral)[b,c]	31** (1.6)[d]	25 (1.7)	21* (1.5)	22* (1.6)	50** (3.6)
Edema (includes bilateral)	27** (1.3)	23 (1.1)	29 (1.1)	24 (1.2)	45** (2.7)
Interstitial cell, hyperplasia, focal (includes bilateral)	1* (3.0)	1 (3.0)	0	4 (2.0)	4 (2.3)
Epididymis					
Hypospermia (includes bilateral)	4** (3.8)	4 (3.5)	4 (3.3)	3 (3.7)	43** (4.0)
Duct, exfoliated germ cell (includes bilateral)	2** (2.0)	3 (2.0)	4 (2.0)	4 (2.0)	36** (1.4)
Testis					
Interstitial cell, adenoma[e]					
Overall rate[f]	7/50 (14%)	3/50 (6%)	3/50 (6%)	6/50 (12%)	15/50 (30%)
Adjusted rate[g]	16.7%	6.8%	6.5%	13.4%	32.3%
Poly-3 test[h]	p < 0.001	p = 0.135N	p = 0.119N	p = 0.451N	p = 0.073

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test; **$p \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Incidence reported is the combination of unilateral and bilateral lesions.
[d]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[e]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 19/487 (4.06% ± 4.36%); range: 0% to 14%.
[f]Number of animals with neoplasm per number of animals necropsied.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach study termination. A negative trend or a lower incidence in an exposure group is indicated by N.

Uterus: There was a significant increase in the incidence of endometrium adenocarcinoma in the 10,000 ppm group and a positive trend in the incidence of uterine squamous cell papilloma with increasing exposure concentration (Table 28). Uterus adenocarcinomas were typically poorly circumscribed and invaded the myometrium. The neoplastic epithelial cells formed solid nests, cords, papillary, or acinar structures. Uterine squamous cell papillomas were characterized by a neoplasm that arose from the surface epithelium with either a broad base or a delicate stalk. The epithelium was well differentiated and arranged in papillary, glandular, or tubular structures that were lined by cuboidal to columnar cells, one to two cell layers thick. The combined incidence of these was significantly increased in the 10,000 ppm group (Table 28).

There were significant increases in the incidences of uterine inflammation in the 300, 1,000, and 10,000 ppm groups, compared to the control group (Table 28). Uterine inflammation was characterized by a spectrum of changes from mostly mononuclear cells (recorded as chronic

inflammation) to a mixture of mononuclear cells and neutrophils (recorded as chronic active inflammation); both diagnoses were considered a part of the same process.

Table 28. Incidences of Neoplastic and Nonneoplastic Lesions of the Uterus (Including Cervix) in Female Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
n[a]	50	50	50	50	49
Inflammation, Chronic[b]	2 (2.5)[c]	9* (2.0)	6* (2.5)	8 (2.0)	8* (3.0)
Adenoma[d]	0	1	0	0	0
Adenocarcinoma[e]					
Overall rate[f]	2/50 (4%)	2/50 (4%)	1/50 (2%)	4/50 (8%)	10/50 (20%)
Adjusted rate[g]	4.7%	4.9%	2.4%	9%	23.8%
Poly-3 test[h]	p < 0.001	p = 0.678	p = 0.508N	p = 0.352	p = 0.011
Squamous Cell Carcinoma[i]	0	1	0	2	1
Squamous Cell Papilloma (Includes Multiple)[j]	0*	0	0	0	2
Adenoma, Adenocarcinoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined)[k]					
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	6/50 (12%)	13/50 (26%)
Adjusted rate	4.7%	9.7%	2.4%	13.4%	30.7%
Poly-3 test	p < 0.001	p = 0.315	p = 0.508N	p = 0.145	p < 0.001

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 1/350 (0.29% ± 0.76%); range: 0% to 2%.
[e]Historical control incidence: 20/350 (5.71% ± 3.35%); range: 2% to 10%.
[f]Number of animals with neoplasm per number of animals necropsied.
[g]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[h]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach study termination. A negative trend or a lower incidence in an exposure group is indicated by N.
[i]Historical control incidence: 2/350 (0.57% ± 1.51%); range: 0% to 4%.
[j]Historical control incidence: 0/350.
[k]Historical control incidence: 23/350 (6.57% ± 3.41%); range: 2% to 10%.

Heart: There were significant increases in the incidences of heart valve fibrosis and heart valve thrombus in the 10,000 ppm male group relative to the control group (Table 29). Valve fibrosis was diagnosed when valves were expanded by fibrous connective tissue that was more densely eosinophilic than the loose lightly basophilic to amphophilic tissue of a normal heart valve. Heart valve thrombus was characterized by fibrin, admixed with variable numbers of blood cells, which covered the cardiac valves.

Bone Marrow: There was a significant increase in the incidence of bone marrow hypercellularity in the 1,000 and 10,000 ppm male groups relative to the control group, and a positive trend in

incidence with increasing exposure concentration in females (Table 29). Bone marrow hypercellularity was characterized by an increase in one or more hematopoietic cell lines, generally with a decrease in adipocytes.

Pituitary Gland: There was a significant increase in the incidence of pars distalis hypertrophy in the 10,000 ppm males compared to the control group (Table 29). Pars distalis hypertrophy was characterized by clusters of cells that were enlarged, with abundant amorphous amphophilic or pale eosinophilic cytoplasm and peripherally compressed nuclei ("signet ring" cells). A severity grade was assigned based on the numbers of affected cells.

Table 29. Incidences of Nonneoplastic Lesions of the Heart, Bone Marrow, and Pituitary Gland in Male and Female Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
n[a]	50	50	50	50	50
Male					
Heart					
Valve, fibrosis[b]	2** (1.5)[c]	0	0	1 (1.0)	9* (1.9)
Valve, thrombus	0**	0	0	2 (2.5)	6* (1.8)
Bone Marrow					
Hypercellularity	18** (2.1)	22 (2.1)	30* (1.8)	25 (1.8)	34** (1.9)
Pituitary Gland					
Pars distalis, hypertrophy	8** (1.0)	10 (1.2)	11 (1.2)	14 (1.1)	37** (1.9)
Female					
Bone Marrow					
Hypercellularity	43* (2.7)	39 (2.8)	43 (2.7)	43 (2.7)	47 (2.9)

Statistical significance for an exposure group indicates a significant pairwise test compared to the vehicle control group.
Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$ by the Poly-3 test; **$p \leq 0.01$.
[a]Number of animals examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

There were lower incidences of lesions in other tissues relative to control animals in the postweaning-only study; the biological significance of these differences is unknown (Appendix H). In males, these lesions included heart cardiomyopathy and parathyroid gland diffuse hyperplasia. In females, these lesions included: heart cardiomyopathy; uterus endometrium metaplasia; mammary gland fibroadenoma; mammary gland fibroma, fibroadenoma, or adenoma; mammary gland fibroma, fibroadenoma, carcinoma, or adenoma; nose, olfactory epithelium, hyaline droplet accumulation; pituitary gland pars distalis adenoma; thyroid gland C-cell hyperplasia; and thyroid gland C-cell adenoma or carcinoma.

Comparative Carcinogenic Benchmark Dose Analysis

Exposure-related neoplastic lesions were further assessed via benchmark dose (BMD) analyses. Daily doses for each exposure group were calculated using time-weighted averages of postweaning feed consumption and corresponding chemical intake during the 2-year exposure

period for each study. All available dichotomous models in U.S. EPA's BMD Software (BMDS version 3.1.2)[169] were fit to the adjusted incidence data for assessed neoplastic lesions. Model fit was assessed by a chi-square goodness-of-fit test, visual inspection of the respective plots of observed versus predicted values from the various models, and Akaike information criterion (AIC) values (Appendix F). A benchmark response (BMR) of 0.1, corresponding to a 10% extra risk of a DEHP carcinogenic response, was used to determine benchmark doses. Benchmark doses (i.e., BMD_{10} [BMD corresponding to a 10% extra risk] and $BMDL_{10}$ [95% lower bound on the BMD corresponding to a 10% extra risk]) were determined for incidences of hepatocellular adenoma or carcinoma (combined), pancreatic acinar adenoma or carcinoma (combined), testicular interstitial cell adenoma, and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined). The BMD_{10} and $BMDL_{10}$ were calculated separately for the perinatal and postweaning study (Study 1) and for the postweaning-only study (Study 2).

Higher adjusted incidences of hepatocellular adenoma or carcinoma (combined) occurred in the 3,000 and 10,000 ppm male rats exposed during the perinatal and postweaning periods (6.7% and 30.6%), relative to postweaning-only exposure (2.2% and 25.6%) (Table 30). A probit model provided the best relative model fit for the adjusted rates of hepatocellular adenoma or carcinoma (combined) in the perinatal and postweaning study (Figure 14A). Using this model, a BMD_{10} of 382.90 mg/kg/day was estimated for hepatocellular adenoma or carcinoma (combined) in male rats. A multistage degree 4 model provided the best relative model fit for the adjusted rates of hepatocellular adenoma or carcinoma (combined) in the postweaning-only study (Figure 14B). Using this model, a BMD_{10} of 434.41 mg/kg/day was estimated for hepatocellular adenoma or carcinoma (combined) observed in male rats.

Table 30**. Adjusted Incidence Data and Benchmark Dose Modeling for Select Neoplasms in Male Rats in the Two-year Feed Studies of Di(2-Ethylhexyl) Phthalate**

Neoplasm	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm	BMD10 (mg/kg/day)	BMDL10 (mg/kg/day)	Model
Perinatal and Postweaning Study (Study 1)								
DEHP Intake (mg/kg/day)	0	17.6	57.5	188.5	678.3	–	–	–
Hepatocellular Adenoma or Carcinoma (Combined)[a]	2.6%[b]	2.4%	0%	6.7%	30.6%	382.90[c]	306.05[c]	Probit
Pancreatic Acinar Adenoma or Carcinoma (Combined)[d]	26%	16.6%	16.9%	81.2%	62.5%	85.92[c]	56.78[c]	Dichotomous Hill
Postweaning-only Study (Study 2)								
DEHP Intake (mg/kg/day)	0	16.8	53.5	169.9	602.3	–	–	–
Hepatocellular Adenoma or Carcinoma (Combined)[a]	0%	4.5%	0%	2.2%	25.6%	434.41	263.52	Multistage degree 4
Pancreatic Acinar Adenoma or Carcinoma (Combined)[d]	2.4%	11.2%	10.7%	49.9%	69.8%	30.99	20.20	Log-logistic
Testis Interstitial Cell Adenoma[e]	16.7%	6.8%	6.5%	13.4%	32.3%	366.69	164.41	Multistage degree 4

BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

[a]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 4/489 (0.89% ± 1.06%); range: 0% to 2%.

[b]Percentages represent adjusted incidence rate based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]BMD models excluded incidences in the 10,000 ppm group due to the nonmonotonicity of the dose response.
[d]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 62/488 (12.03% ± 9.16%); range: 0% to 28%.
[e]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 19/487 (4.06% ± 4.36%); range: 0% to 14%.

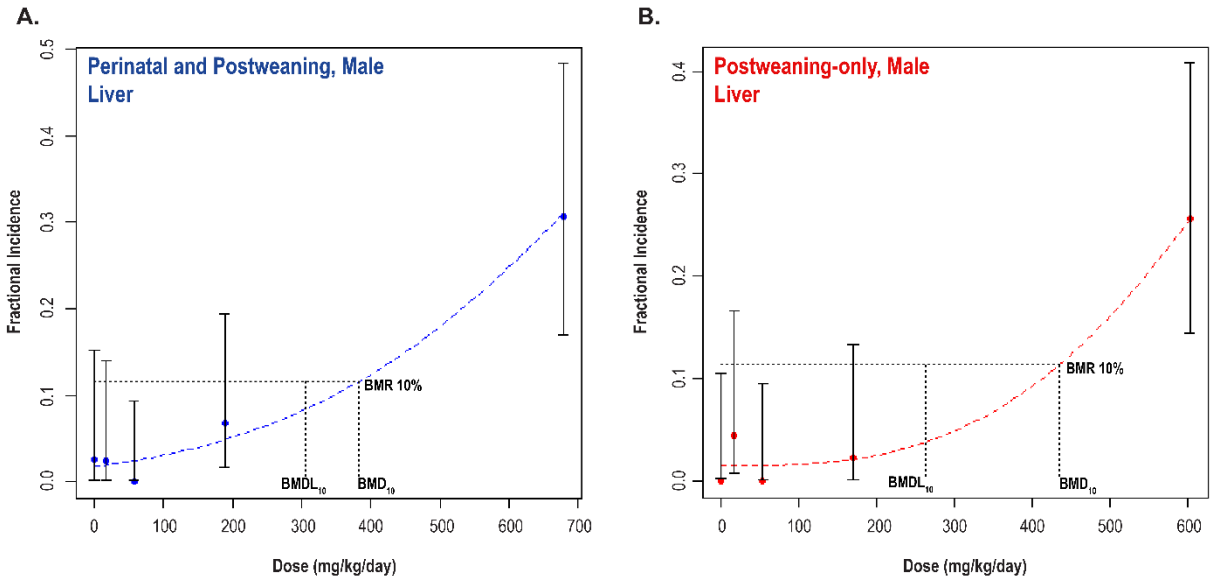


Figure 14. Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma (Combined) in Male Rats

Frequentist (A) probit model (perinatal and postweaning; Study 1) and (B) multistage degree 4 model (postweaning-only; Study 2) with BMR of 10% extra risk for the BMD_{10} and 0.95 lower confidence limit for the $BMDL_{10}$ for the incidence of hepatocellular adenoma or carcinoma (combined) in male rats.

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

A higher adjusted incidence of hepatocellular adenoma or carcinoma (combined) occurred in 3,000 ppm female rats exposed during the perinatal and postweaning periods (20.9%) relative to postweaning-only exposure (2.3%) (Table 31). A log-logistic model provided the best relative model fit for the adjusted rates of hepatocellular adenoma or carcinoma (combined) in the perinatal and postweaning study (Figure 15A). Using this model, a BMD_{10} of 122.95 mg/kg/day was estimated for hepatocellular adenoma or carcinoma (combined) in female rats. A multistage degree 4 model provided the best relative model fit for the adjusted rates of hepatocellular adenoma or carcinoma (combined) in the postweaning-only study (Figure 15B). Using this model, a BMD_{10} of 383.63 mg/kg/day was estimated for hepatocellular adenoma or carcinoma (combined) in female rats.

Table 31**. Adjusted Incidence Data and Benchmark Dose Modeling for Select Neoplasms in Female Rats in the Two-year Feed Studies of Di(2-Ethylhexyl) Phthalate**

Neoplasm	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	Model
Perinatal and Postweaning Study (Study 1)								
DEHP Intake (mg/kg/day)	0	17.9	61.7	195.6	772.3	–	–	–
Hepatocellular Adenoma or Carcinoma (Combined)[a]	2.4%[b]	0%	11.8%	20.9%	35.4%	122.95	79.74	Log-logistic
Uterus (Including Cervix) Adenocarcinoma, Adenoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined)[c]	7.0%	2.4%	2.4%	7.0%	19.0%	594.19	432.23	Logistic
Postweaning-only Study (Study 2)								
DEHP Intake (mg/kg/day)	0	17.2	59.5	177.1	646.3	–	–	–
Hepatocellular Adenoma or Carcinoma (Combined)[a]	0%	0%	2.4%	2.3%	33.7%	383.63	207.99	Multistage degree 4
Uterus (Including Cervix) Adenocarcinoma, Adenoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined)[c]	4.7%	9.7%	2.4%	13.4%	30.7%	324.15	249.01	Probit

BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

[a]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 16/489 (2.87% ± 2.8%); range: 0% to 8%.

[b]Percentages represent adjusted incidence rate based on Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

[c]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 23/350 (6.57% ± 3.41%); range: 2% to 10%.

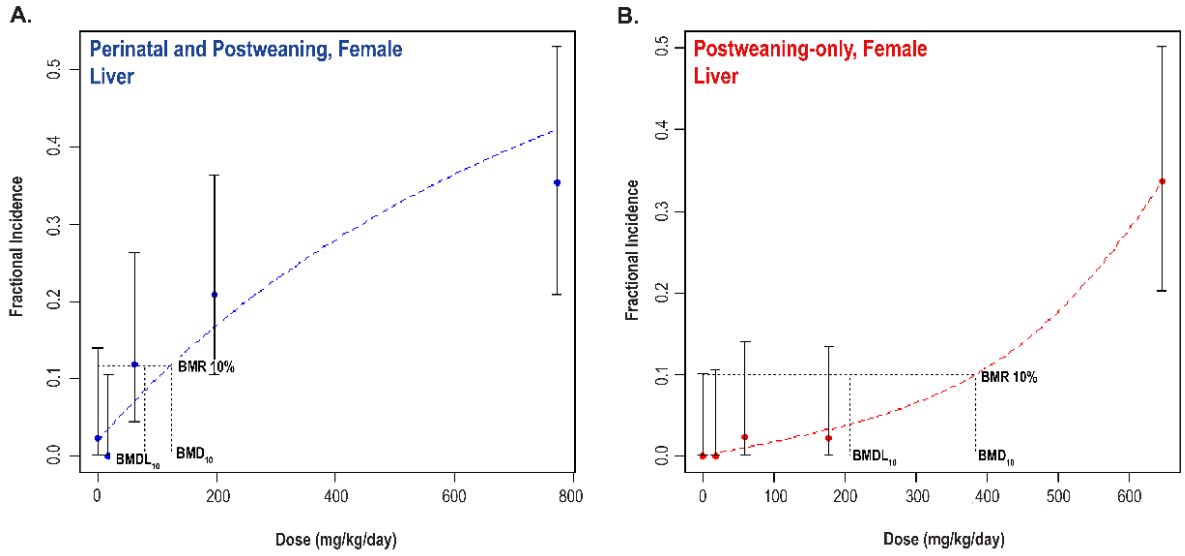


Figure 15. Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma (Combined) in Female Rats

Frequentist (A) log-logistic model (perinatal and postweaning; Study 1) and (B) multistage degree 4 model (postweaning-only; Study 2) with BMR of 10% extra risk for the BMD_{10} and 0.95 lower confidence limit for the $BMDL_{10}$ for the incidence of hepatocellular adenoma or carcinoma (combined) in female rats.

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

A higher adjusted incidence of pancreatic acinar adenoma or carcinoma (combined) occurred in 3,000 ppm male rats exposed during the perinatal and postweaning periods (81.2%), relative to postweaning-only exposure (49.9%); however, incidences in the 10,000 ppm group were similar between the two studies (62.5% versus 69.8%, respectively) (Table 30). A dichotomous Hill model provided the best relative model fit for the adjusted rates of pancreatic acinar adenoma or carcinoma (combined) in the perinatal and postweaning study (Figure 16A). Using this model, a BMD_{10} of 85.92 mg/kg/day was estimated for pancreatic acinar adenoma or carcinoma (combined) in male rats (Table 30). A log-logistic model provided the best relative model fit for the adjusted rates of pancreatic acinar adenoma or carcinoma (combined) in the postweaning-only study (Figure 16B). Using this model, a BMD_{10} of 30.99 mg/kg/day was estimated for pancreatic acinar adenoma or carcinoma (combined) in male rats (Table 30).

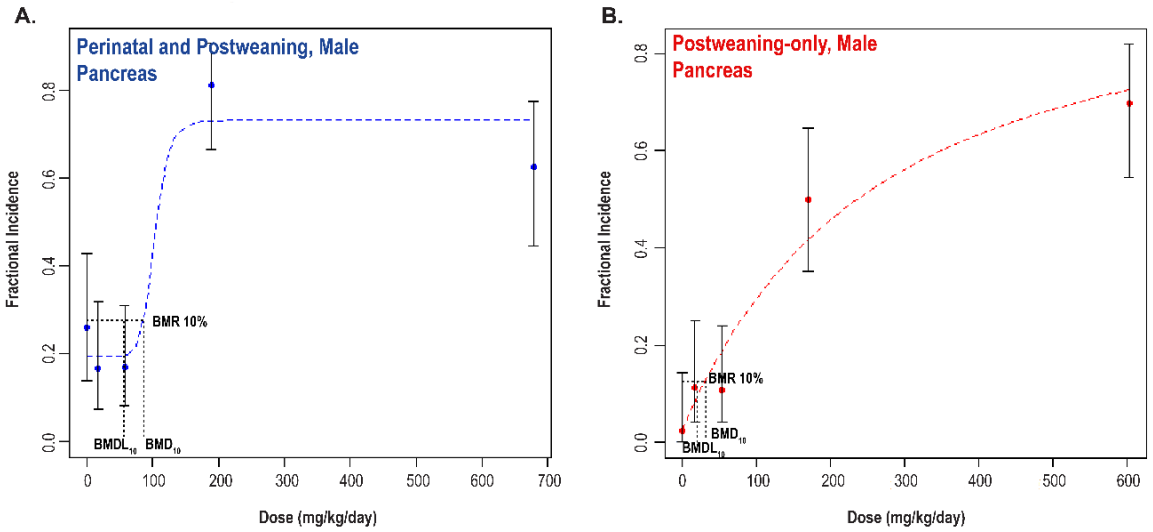


Figure 16. Benchmark Dose Modeling Results for Pancreatic Acinar Adenoma or Carcinoma (Combined) in Male Rats

Frequentist (A) dichotomous Hill model (perinatal and postweaning; Study 1) and (B) log-logistic model (postweaning-only; Study 2) with BMR of 10% extra risk for the BMD_{10} and 0.95 lower confidence limit for the $BMDL_{10}$ for the incidence of pancreatic acinar adenoma or carcinoma (combined) in male rats.

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

The incidences of pancreatic acinar adenoma or carcinoma (combined) in female rats were not amenable to BMD modeling. These lesions are considered rare in female rats and were only observed at adjusted rates up to 5% in any single exposed group across both studies. Therefore, an estimated BMR corresponding to 10% extra risk would be greater than the maximum exposure concentration used in the study.

A higher adjusted incidence of testicular interstitial cell adenoma occurred only in 10,000 ppm male rats exposed during the postweaning period (32.3%), relative to perinatal and postweaning exposure (14.1%) (Table 27, Table 16, Table 32). Although there was no exposure-related response in the testis from the perinatal and postweaning study (Study 1), there was a marginal response in the testis with postweaning-only exposure (Study 2). A multistage degree 4 model provided the best relative model fit for the adjusted rates of testicular interstitial cell adenoma in the postweaning-only study (Table 32; Figure 16; Appendix H). Using this model, a BMD_{10} of 366.69 mg/kg/day was estimated for testicular interstitial cell adenoma in male rats.

Table 32. Incidence Data and Benchmark Dose Modeling Results for Testicular Interstitial Cell Adenoma in Male Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Neoplasm	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	Model
Postweaning-only Study (Study 2)								
n[a]	50	50	50	50	50			
Testicular Interstitial Cell Adenoma[b]								
Overall rate[c]	7/50 (14%)	3/50 (6%)	3/50 (6%)	6/50 (12%)	15/50 (30%)	366.69	164.41	Multistage degree 4
Adjusted rate[d]	16.7%	6.8%	6.5%	13.4%	32.3%			
Poly-3 test[e]	p < 0.001	p = 0.135N	p = 0.119N	p = 0.451N	p = 0.073			

BMD_{10} = benchmark dose corresponding to a 10% extra risk; BMD_{L10} = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.
[a]Number of animals with tissue examined microscopically.
[b]Historical control incidence for all routes of 2-year studies (mean ± standard deviation): 19/487 (4.06% ± 4.36%); range: 0% to 14%.
[c]Number of animals with neoplasm per number of animals necropsied.
[d]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[e]Beneath the control incidence is the p value associated with the trend test. Beneath the exposed group incidence are the p values corresponding to pairwise comparisons between the vehicle control group and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A negative trend or a lower incidence in an exposure group is indicated by N.

Higher adjusted incidences of uterine (including cervix) adenocarcinoma, adenoma, squamous cell carcinoma, or squamous cell papilloma (combined) occurred in 3,000 and 10,000 ppm female rats exposed postweaning-only (13.4% and 30.7%, respectively), relative to exposure during the perinatal and postweaning periods (7% and 19%, respectively) (Table 31). A logistic model provided the best relative model fit for the adjusted rates of uterine neoplasms in the perinatal and postweaning study (Figure 17A). Using this model, a BMD_{10} of 594.19 mg/kg/day was estimated for uterine (including cervix) adenocarcinoma, adenoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats (Table 31). A probit model provided the best relative model fit for the adjusted rates of uterine (including cervix) adenocarcinoma, adenoma, squamous cell carcinoma, or squamous cell papilloma (combined) in the postweaning-only study (Figure 17B). Using this model, a BMD_{10} of 324.15 mg/kg/day was estimated for uterine (including cervix) adenocarcinoma, adenoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats (Table 31).

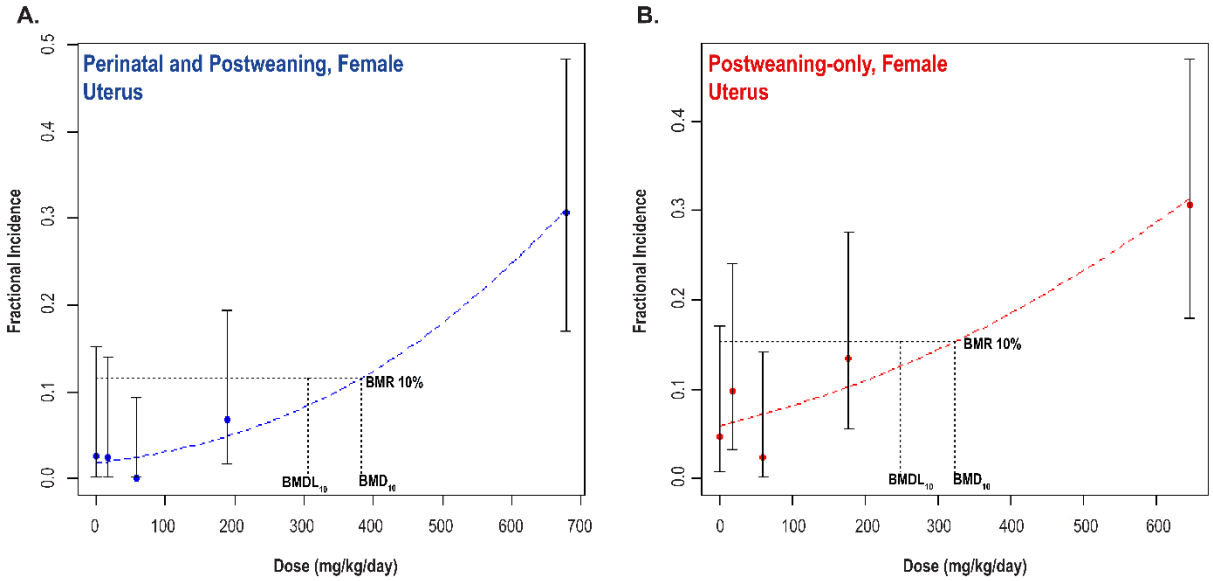


Figure 17. Benchmark Dose Modeling Results for Uterine Adenocarcinoma, Adenoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined) in Female Rats

Frequentist (A) log-logistic model (perinatal and postweaning; Study 1) and (B) probit model (postweaning-only; Study 2) with BMR of 10% extra risk for the BMD_{10} and 0.95 lower confidence limit for the $BMDL_{10}$ for the incidence of uterine adenocarcinoma, adenoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats.

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

Genetic Toxicology

DEHP was tested in a variety of genotoxicity assays in vitro and in vivo; most results were negative. DEHP (100–10,000 µg/plate) was tested in six independent bacterial mutation assays using a variety of strains of *Salmonella typhimurium* (TA100, TA1535, TA1537, TA97, and TA98) and exogenous metabolic activation systems (induced hamster, rat, or mouse liver S9 plus cofactors); results from all bacterial assays were negative (Appendix H).[149] A single mouse lymphoma gene mutation assay was conducted with DEHP (0.125–3.0 µL/mL) and was negative

overall, with and without induced rat liver S9 mix (Appendix H). In three independent studies, no increases in chromosomal aberrations were observed in Chinese hamster ovary (CHO) cells exposed to DEHP (concentrations up to 5,000 µg/mL) with or without induced rat liver S9 (Appendix H).[153] In a series of nine in vitro sister chromatid exchange (SCE) tests conducted in CHO cells with and without S9, DEHP produced positive responses in four tests, equivocal results in three, and negative results in two (Appendix H).[153] All of the increases in SCEs judged to be positive or equivocal were observed only in the absence of S9 and at concentrations of DEHP that induced severe cell cycle delay, necessitating longer incubation prior to cell harvesting. The level of cytotoxicity and the extended incubation times might have contributed to the increased SCE levels observed in these seven studies, rather than the SCE reflecting a direct interaction of DEHP with chromosomal DNA. DEHP was tested for induction of sex-linked recessive lethal mutations in male *Drosophila melanogaster* in two independent studies, one using adult injection and one using larval feeding as the route of exposure; both studies yielded negative results (Appendix H).[158; 159]

In vivo, no significant increases in chromosomal aberrations were observed in bone marrow cells of female B6C3F1 mice following exposure to DEHP (3,000–12,000 ppm) in dosed feed for 14 days (Table D-1). DEHP was tested in three independent erythrocyte micronucleus tests and produced varying results. In one test, B6C3F1 female mice were exposed to DEHP (3,000–12,000 ppm) in dosed feed for 14 days; results were judged to be equivocal overall—the response was negative in the immature erythrocyte population (polychromatic erythrocytes) and positive in the mature erythrocyte population (normochromatic erythrocytes) (Table D-2). In a second test, DEHP (1,500–6,000 ppm) induced an equivocal response in male TgAC (FVB/N) mice and a positive response in female TgAC (FVB/N) mice following exposure via dosed feed for 26 weeks (Table D-3). Another 26-week exposure test in TgAC (FVB/N) mice used dermal application of DEHP (100–400 mg/kg/day) and produced negative results in both male and female mice (Table D-2).

Discussion

Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer to which humans are exposed, as evidenced by detection of DEHP metabolites in serum and urine samples. The presence of DEHP metabolites in human amniotic fluid samples shows exposure occurs in utero. Rodent studies report that DEHP produces adverse effects on the developing male rat reproductive tract and induces hepatic, pancreatic, and testicular neoplasms. Prior to the current studies, data were insufficient to assess whether developmental exposure would alter lifetime DEHP carcinogenic risk. To address this knowledge gap, the National Toxicology Program (NTP) conducted two 2-year bioassays with DEHP administered in feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) rats to evaluate whether neoplasm incidence during lifetime exposure that included the perinatal period (gestation and lactation) would increase the incidence of neoplasms or lead to the appearance of different neoplasm types relative to chronic exposure initiated in early adulthood.

In the perinatal and postweaning study (Study 1), exposure was associated with significantly decreased maternal mean body weights during gestation and lactation in the 10,000 ppm group compared to the control group, with the magnitude of the effect increasing throughout the perinatal period. This effect was attributed to significantly decreased body weight gain during gestation, likely in part due to cumulative effects of reduced maternal feed consumption (g/animal/day) throughout the gestation and lactation period in the 10,000 ppm group.

Estimated DEHP intake increased in proportion to exposure concentration, with the exception of 10,000 ppm dams during lactation (lactation days [LDs] 1–14), for which significantly decreased feed consumption resulted in a less-than-proportional higher intake. In gestation day (GD) 18 dams, the mean concentration of the DEHP metabolite, mono(2-ethylhexyl) phthalate (MEHP), increased with exposure concentration although the increase was more than proportional (63-fold increase in plasma concentration versus 30-fold increase in DEHP intake [mg DEHP/kg body weight/day or mg/kg/day] during gestation from lowest [300 ppm] to highest [10,000 ppm] exposure groups). Amniotic fluid and fetus concentrations of MEHP increased 23- and 46-fold, respectively, from the lowest to highest exposure groups (300 to 10,000 ppm). Gestational transfer of MEHP from the dam to the fetus was moderate.

MEHP concentrations in dam plasma on GD 18 at the lowest exposure concentration were 630 ng/mL, approximately 60-fold higher than the median blood MEHP concentration of 10.4 ng/mL observed in pregnant women in the Hokkaido Study Sapporo Cohort.[203] Moreover, the GD 18 MEHP concentration measured in amniotic fluid at the lowest exposure concentration was 73.4 ng/mL, which is 28-fold higher than the upper 95th percentile of MEHP levels measured in human amniotic fluid samples.[27] Additional studies have detected MEHP in human amniotic fluid and cord blood plasma samples, indicating that DEHP or its metabolites cross the placental barrier and result in exposure to the developing conceptus.[204-206] DEHP levels in control feed were below the limit of detection (1.27 ppm) of the analytical method; however, detectable levels of MEHP were measured in control amniotic fluid and fetuses, but not in control dam plasma (GD 18). Detection of MEHP in control animal samples might have resulted from sample contamination during collection or analysis, due to the presence of phthalates in manufactured laboratory plasticware. Although not assessed in the present study, DEHP and MEHP can be transferred from dam to offspring via lactation.[55] DEHP exposure during the

perinatal period was associated with significantly decreased total and live litter size, due to a significantly decreased number of female pups per litter in the 10,000 ppm group (626 mg/kg/day). In previous studies, increased resorptions, postimplantation loss, and whole-litter loss have been observed following DEHP exposure at doses >500 mg/kg/day in pregnant rats.[90; 207-209] In a multigenerational reproductive assessment of DEHP, previously conducted by NTP, significant effects on litter size and sex ratio were observed following perinatal exposure of Sprague Dawley rat F_0 dams at concentrations of 7,500 and 10,000 ppm.[210] In this perinatal and postweaning study (Study 1), exposure-related decreases in birth and weaning mean body weights were observed in both male and female groups. Gestational DEHP exposure was associated with 15% and 12% decreases in postnatal day (PND) 1 mean body weights of 10,000 ppm male and female pups, respectively. Further growth retardation during lactation was observed with male and female pup weights. Postweaning, mean body weights of the 10,000 ppm offspring remained significantly decreased relative to control groups throughout the 2-year exposure period. The magnitude of effect on body weight observed in 10,000 ppm offspring was higher than the 30% decrease in postweaning body weights observed at the same dose level in the NTP multigeneration assessment of DEHP.[210]

No significant differences in overall survival were observed in either the perinatal and postweaning study (Study 1) or the postweaning-only study (Study 2) relative to concurrent control groups, although there was some early postweaning mortality (Study 1). Lower mean body weights (postweaning to study termination) were observed in 10,000 ppm male and female rats in both studies relative to control rats. In both studies, lower mean body weights were associated with lower body weight gain; however, the magnitude of effect was higher following perinatal and postweaning exposure compared to postweaning-only exposure, due to early life exposure that included gestation, lactation, and a brief period after weaning. In the 10,000 ppm male and female rat groups, the largest difference in feed consumption relative to the control groups occurred directly following weaning. In rats, increased rates of feed and water consumption, relative to body weight, are commonly observed in younger animals and decrease with subsequent growth and development. Furthermore, in the perinatal and postweaning study (Study 1), the 2-year direct exposure period began at weaning, 3 weeks earlier than in the postweaning-only study (PND 42) (Study 2). Although this 3-week interval represents a small fraction of the total exposure timeframe, perinatal and postweaning exposure groups were exposed to DEHP at earlier ages and therefore at higher doses than the corresponding groups in the postweaning-only study (Study 2), likely contributing to the 3–20% higher mean chemical consumption (mg/kg/day) postweaning in Study 1 versus Study 2.

The following conclusions on the carcinogenicity of DEHP were determined based on the weight-of-evidence approach described in the Explanation of Levels of Evidence of Carcinogenic Activity. Conclusions on DEHP carcinogenic activity are described separately for the perinatal and postweaning and postweaning-only studies, based on the independent results observed in each study. Although some variability in carcinogenic and noncarcinogenic outcomes was observed between rats exposed to DEHP during the perinatal and postweaning periods and those only exposed postweaning, it is unclear whether any differences correspond to specific developmental mechanisms during the perinatal period.

At the conclusion of both studies, numerous neoplastic and nonneoplastic lesions in the liver were identified. In male rats, increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were observed in both 2-year studies. In the perinatal and

postweaning study (Study 1), there was an increase in rare hepatocellular carcinomas (historical control 2/489; range 0% to 2%) in the 10,000 ppm group (8.7%), whereas a positive trend was observed in these neoplasms in the postweaning-only study (Study 2). In both perinatal and postweaning and postweaning-only studies, higher incidences of hepatocellular cytoplasmic alteration, liver pigmentation, and liver necrosis were observed in male rats. Although considered minimal in severity, a higher incidence of hepatocellular hypertrophy was observed in 10,000 ppm male rats exposed during the perinatal and postweaning periods (35%) compared to male rats in the postweaning-only study (12%). Additionally, a significantly increased incidence of basophilic focus was observed in the livers of 10,000 ppm male rats in the perinatal and postweaning study, but not in their counterparts in the postweaning-only study (Study 2). Taken together, the significantly increased incidence of hepatocellular adenoma or carcinoma (combined) supported clear evidence of carcinogenicity in male rats in both 2-year studies.

In female rats, increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were observed in both 2-year studies. In female rats in the perinatal and postweaning study, the incidence of hepatocellular adenomas in the 3,000 ppm group that was higher (18%) than in the historical control range (15/489; range 0% to 8%); in females in the postweaning-only study (Study 2), the incidence of hepatocellular adenomas was above the historical control range in the 10,000 ppm group. Furthermore, an increased incidence of hepatocellular carcinoma (4.9%), a rare neoplasm type (historical control 1/489; range 0% to 2%), was also observed in 10,000 ppm female rats in the perinatal and postweaning study, whereas there was occurrence of hepatocellular carcinomas (0% versus 4% compared to the control group) in the 10,000 ppm group of the postweaning-only study (Study 2). Taken together, the increased incidence of hepatocellular adenoma or carcinoma (combined) supported clear evidence of carcinogenicity in the liver of female rats in both studies.

Significantly increased incidences of hepatocellular cytoplasmic alteration, hepatocellular hypertrophy, and liver pigmentation were observed in female rats in both 2-year studies. Higher incidences of eosinophilic foci were observed in 10,000 ppm female rats in the perinatal and postweaning study (Study 1), but not in the postweaning-only study (Study 2). A significantly increased incidence of hepatocellular carcinomas was observed only in 10,000 ppm females of the perinatal and postweaning study (17%) compared to the postweaning-only study (4%). This observation could be due to a higher rate of progression from hepatocellular adenoma to carcinoma because of early life or prolonged exposure, observations that are similar to those made in the perfluorooctanoic acid (PFOA) 2-year study[211] in which perinatal and postweaning exposure led to a marginally higher carcinoma rate (4%) of this rare neoplasm in male rats relative to the rats with postweaning-only exposure (0%). Males with perinatal and postweaning exposure to DEHP also had a marginally higher hepatocellular carcinoma incidence compared to males with postweaning-only exposures.

Numerous chronic exposure studies have found that DEHP induces hepatic neoplasms in rats and mice. In the current study, estimated DEHP daily exposure concentrations (mg/kg/day) associated with higher incidences of hepatocellular adenomas or carcinomas were comparable to concentrations reported in previous studies. In a previous NTP study, chronic DEHP exposure via dosed feed resulted in increased incidences of hepatocellular carcinomas in male and female Fischer 344 (F344) rats at estimated daily exposures of 322 and 674 mg/kg/day in males and 394 and 774 mg/kg/day in females.[70] In another study, increased incidences of hepatocellular adenomas and carcinomas were observed in male Sprague Dawley rats following lifetime

exposure to 300 mg/kg/day.[126] The precise mechanism by which DEHP induces hepatic neoplasms is not fully characterized. However, activation of peroxisome proliferator-activated receptor alpha (PPARα) by the DEHP proximal metabolite MEHP has been defined as a key molecular event by which DEHP causes hepatic neoplasms in rodents. The human relevance of mechanisms of carcinogenesis of the peroxisome proliferator class of chemicals is frequently debated.[212] Additional research suggests that multiple signaling pathways and downstream mediators likely contribute to DEHP-induced hepatic carcinogenesis, rather than a single hallmark event such as activation of PPAR.[74; 212]

Increased incidences of pancreatic acinar adenomas, carcinomas, and adenoma or carcinoma (combined) were observed in male rats in both studies. In both perinatal and postweaning and postweaning-only exposed male rats, increased incidences of pancreatic acinar adenomas occurred in the 3,000 and 10,000 ppm groups at higher rates than the historical control range (60/488; range 0% to 28%). Notably, a higher incidence of pancreatic cell adenomas occurred in 3,000 ppm perinatal and postweaning-exposed males (72%) when compared to males exposed to the same concentration postweaning-only (46%). Due to the potentially high background incidence of pancreatic acinar adenoma in the test rat strain (up to 28% in historical control groups), observed differences between perinatal and postweaning and postweaning-only exposure groups might have resulted from background variability of this lesion and not increased sensitivity related to perinatal exposure. A higher incidence of pancreatic acinar carcinoma, a rare neoplasm type (historical control 4/488; range 0% to 4%), was observed in 3,000 ppm male rats (6%) exposed during the perinatal and postweaning periods, and this neoplasm was increased in the 10,000 ppm male rats exposed postweaning-only. Furthermore, increased incidences of pancreatic acinar hyperplasia were noted in 3,000 and 10,000 ppm males exposed postweaning-only. The increased incidence of pancreatic acinar adenoma or carcinoma (combined) was considered clear evidence of carcinogenicity in male rats in both 2-year studies.

In female rats, occurrences of pancreatic acinar adenoma and carcinomas were observed in the postweaning-only study (Study 2), whereas occurrences of pancreatic acinar adenomas were observed in the perinatal and postweaning study (Study 1). In contrast to males, pancreatic acinar neoplasms are very rare in female rats (historic control 0/489). Occurrences of pancreatic acinus hyperplasia were also observed in exposed groups in both studies. After considering the rarity of this lesion type in female rats, the corroborating effect in male rats, and findings supportive of neoplastic progression, the incidence of pancreatic acinar adenoma or carcinoma (combined) was considered related to DEHP exposure in female rats.

Pancreatic acinar adenomas and carcinomas have been observed previously in male F344 rats following chronic DEHP exposure.[86] Pancreatic adenomas have been reported in rodent models following exposure to various chemicals known to activate PPARα, such as PFOA, butyl benzyl phthalate (BBP), and Wyeth-14,643 (WY).[211; 213; 214] Although direct activity of PPARα agonists on acinar cells has yet to be established, some data suggest that induction of pancreatic neoplasms by PPARα agonists is secondary to functional alterations in the liver. One proposed mode of action suggests that hepatic PPARα activation and subsequent alteration of transcriptional activity leads to alteration in bile acid composition and flow, resulting in cholestasis and increased expression of cholecystokinin (CCK).[127] CCK is a growth factor reported to induce normal, adaptive, and neoplastic growth of pancreatic acinar cells in rats.[215-217]

Numerous gross lesions in the male reproductive tract were observed in male rats in the 10,000 ppm group exposed during both the perinatal and postweaning period, consistent with the "phthalate syndrome" suite of effects.[99; 218; 219] These findings included decreased size of the phallus, testes, epididymides, prostate, seminal vesicles, and levator ani/bulbocavernosus (LABC) muscle; gubernacular length exceeding 20 mm; no gubernaculum present; nonregression of the cranial suspensory ligament (CSL); cleft phallus or prepuce; undescended testes (cryptorchid); epididymal agenesis (caput, corpus, or cauda); and incomplete preputial separation. All examined males exposed to 10,000 ppm DEHP in the perinatal and postweaning study presented with at least one of the aforementioned reproductive tract malformations; small or undescended testes were the most frequently observed reproductive tract malformations at 10,000 ppm. Male reproductive tract malformations have been observed in rodents following perinatal exposure to various phthalates, such as DEHP, di-*n*-butyl phthalate (DBP), di-isobutyl phthalate (DiBP), BBP, and diisononyl phthalate (DINP), and are indicative of hormone disruption of developmental androgen and insulin-like peptide 3 (Insl3) dependent signaling pathways.[95; 220; 221] Differentiation of Wolffian structures (e.g., the epididymis, vas deferens, seminal vesicles) depends on fetal testosterone signaling, and masculinization of the prostate and external genitalia depends on the biosynthesis and signaling of the more potent androgen, dihydrotestosterone.[222] Targeted disruption of Insl3 signaling alters gubernaculum development and CSL regression, leading to cryptorchidism.[223; 224] Developmental exposure to phthalates, such as DEHP, DBP, and BBP, disrupts Insl3 signaling leading to complete agenesis or hypoplasia of the gubernacular ligaments and retention of the testes in the inguinal or abdominal position.[95; 225] In the study presented here, undescended testes were consistently reduced in size and were more often retained in the abdominal region compared to the inguinal region.

Additional microscopic nonneoplastic lesions diagnosed in the testis and epididymis of male rats exposed during both the perinatal and postweaning periods were considered related to DEHP exposure. Observations of testicular germinal epithelium degeneration were noted in 10,000 ppm male rats with or without perinatal exposure and occurred concomitantly with epididymal hypospermia. Findings of testicular edema and exfoliated germ cells in the epididymal duct were observed in 10,000 ppm males of both studies; however, these effects were not significant in the perinatal and postweaning study and therefore considered related to exposure only in the postweaning-only study (Study 2). Seminiferous tubule dysgenesis was only present in 10,000 ppm males with perinatal and postweaning exposure (10/49). Seminiferous tubule dysgenesis is characterized as a developmental malformation seen microscopically as aberrant or misshapen seminiferous tubules, either with no or dilated lumens, which are often surrounded by focal Leydig cell aggregates. The Leydig cell aggregates within foci of dysgenesis differ morphologically from the Leydig cells in adenomas. The Leydig cells in these foci of dysgenesis appear to be poorly differentiated, are spindle-shaped and resemble embryonic Leydig cells, and do not have the abundant eosinophilic or vacuolated cytoplasm often apparent in hyperplasia or adenoma.[226] Dysgenetic lesions might occur as one or more small foci per testis, and tend to be located near the center of the testis or might occupy the entire testis.[226] The malformed tubules can appear to form anastomotic networks. The dysgenetic tubules contain poorly differentiated Sertoli cells, with small, elongated, and sometimes cleaved nuclei and less prominent nucleoli than the typical, prominent, tripartite nucleoli seen in mature Sertoli cells. Spermatogenesis is absent in these foci of dysgenesis but can be present elsewhere in the testis. Dysgenetic foci might be present in one or both testes and can be more severe in undescended than in scrotal testes.[227; 228] In men, similar microscopic dysgenetic foci have been reported in cryptorchid

(undescended) testes[229], in testes also containing testicular cancer (both scrotal and cryptorchid testes[230]), and from testicular biopsies from the contralateral testis in men undergoing orchiectomy for testicular cancer.[231]

Increased pituitary pars distalis hypertrophy occurred in 10,000 ppm male rats in both studies. This lesion is commonly associated with disruption of the hypothalamus-pituitary-gonad signaling axis. Loss of negative feedback signaling by testicular-derived androgens, due to the antiandrogenic activity of phthalates, leads to increased hypothalamic release of gonadotropin-releasing hormone and subsequent increased releases of luteinizing hormone and follicle-stimulating hormone by gonadotrophs, or "castration cells," in the pars distalis of the pituitary gland.[232]

There was a significant positive trend with testicular interstitial cell adenoma neoplasms in the postweaning-only study (Study 2), and the incidence of testicular interstitial cell adenoma (15/50; 30%) observed in 10,000 ppm male rats was above NTP's historical control range (19/487; range 0% to 14%). However, there were no significant pairwise differences among the exposed groups compared to the control groups in the incidences of neoplasms or hyperplasias. Taken together, the data suggest that testicular interstitial cell adenomas may have been related to DEHP exposure in postweaning-only exposed male rats.

In contrast, perinatal and postweaning exposure did not increase the incidence of Leydig cell (interstitial) neoplasms relative to control animals, although the incidence of interstitial cell hyperplasia was considerably higher. Currently, it is unclear whether developmental malformations in the male reproductive tract, such as altered seminiferous tubule morphology or structural and functional alterations in Sertoli and Leydig cell populations, affect the carcinogenic potential of DEHP in testes in perinatally exposed rats relative to functional effects observed following adult exposure only. Increased incidences of focal interstitial cell hyperplasia were observed in both 2-year studies. Focal hyperplasia is considered a preneoplastic lesion that commonly forms as a part of a continuum leading to interstitial cell adenoma; it is distinct from diffuse hyperplasia, generally considered a physiological response to hormonal imbalance.[233-235]

Several PPARα agonists, including DEHP, PFOA, and WY, have been shown to induce Leydig cell neoplasms in rats.[126; 214] Multiple mechanisms by which PPARα agonists might induce testicular neoplasms through disruption of the hypothalamus-pituitary-thyroid axis have been postulated; however, the weight of evidence is currently inadequate to establish a mode of action.[127] The marginal to no response in Leydig cell neoplasms to DEHP in this study is inconsistent with published studies and could be due to differential diagnoses. Varying morphological criteria distinguishing Leydig cell adenomas from seminiferous tubule dysgenesis may account for differential diagnoses;[226] the rodent strain studies may also be a factor as there were no Leydig cell neoplasms observed in the NTP PFOA studies, [211] which used the Hsd:Sprague Dawley® SD® rat.

In female rats, increased incidences of adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) (mostly adenocarcinoma) were observed in the uterus (including cervix). Higher incidences of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) (26%) were observed in 10,000 ppm postweaning-only exposed females, which was above the NTP historical control incidence of this combination of lesions of 2% to 10%. Uterine inflammation was increased in all

DEHP-exposed groups in the postweaning-only study (Study 2). This supported clear evidence of carcinogenic activity from DEHP postweaning-only exposure in female rats.

In the perinatal and postweaning study (Study 1), however, there was a marginally higher incidence of uterine neoplasms in DEHP-exposed groups compared to the control group and none of the pairwise comparisons to the control group were significant. The magnitude of the difference between the 10,000 ppm group and the control group in each study was lower in the perinatal and postweaning study (8% difference) compared to the postweaning-only study (22% difference). There was reduced certainty in this marginal response such that the incidence of uterine neoplasms may have been related to perinatal and postweaning exposure. The reason for this is not clear, but it is noteworthy that the testis and uterus, sites of endocrine action, had a lower response in general with the perinatal and postweaning exposure compared to the postweaning-only exposure.

The present study is the first to identify an association between DEHP exposure and induction of uterine neoplasms in female rats. The mechanism for this response is unclear. For previous chronic studies, no alterations in female reproductive organ histology were reported.[70; 86; 123] Induction of the "tumor triad," including liver, Leydig cell, and pancreatic acinar cell tumors, is a finding characteristic of sustained exposure to PPARα agonists in rats;[214] however, the relevance of this finding to humans is uncertain. The uterine neoplasm response in the NTP PFOA carcinogenicity study[211] was considered equivocal evidence of carcinogenic activity. The magnitude of the response and level of evidence for PFOA is similar to that of the DEHP perinatal and postweaning study, whereas the response in the DEHP postweaning-only study was considerably higher. Further work will be required to assess the mode of action for these outcomes.

Evidence of DEHP-associated renal toxicity was specific to male and female rats with perinatal and postweaning exposure. Numerous nonneoplastic kidney lesions were increased in DEHP-exposed groups relative to control groups, such as papilla edema, papilla epithelium hyperplasia, papilla hemorrhage, infarct, and renal tubule cysts. Papillary edema was the most prevalent kidney lesion in 10,000 ppm male (39/49) and female (38/49) rats in the perinatal and postweaning study and was not present in the postweaning-only study (Study 2). This highly unusual bilateral lesion was characterized by marked dilation and/or distortion of the collecting ducts and moderate to marked expansion of the papilla interstitium by pale eosinophilic to fibrillary amphophilic material, consistent with edema. Periodic acid-Schiff (PAS) staining demonstrated that the basement of vascular structures, in addition to the renal tubule basement membranes in the cortex and medulla, were intact. An abrupt loss of PAS staining of the basement membranes of collecting tubules was observed, however, at the junction of the outer and inner medulla. Therefore, perinatal exposure to DEHP is presumed to interfere directly or indirectly with the proper development of the collecting tubules. The normal function of the collecting duct system is urine transport as well as electrolyte and fluid balance through reabsorption and excretion, processes regulated by aldosterone and vasopressin. Additional studies have reported DEHP-associated renal toxicity. Chronic dietary exposure to DEHP (≥789 mg/kg/day) was associated with increased severity of routinely occurring renal tubule pigmentation and chronic progressive nephropathy in male and female rats.[86] Altered kidney function and kidney lesions have been reported in rats following developmental DEHP exposure. Impaired kidney development and function were observed in adult Wistar rats following

maternal exposure to DEHP at doses of 0.25 and 6.25 mg/kg/day from GD 0 through offspring PND 21.[236] Maternal exposure resulted in a decreased number of nephrons, higher glomerular volume, and smaller Bowman's capsule in offspring at weaning, as well as glomerulosclerosis, interstitial fibrosis, and effacement of podocyte foot processes in 33-week-old F_1 rats. Taken together, these data suggest the developing kidney may be a sensitive target of DEHP toxicity.

Cardiovascular findings of increased heart valve fibrosis and thrombus were present in 10,000 ppm male rats in both of the present studies. Thrombosis in male rats has been associated with pancreatic cancer related to onset of an intrinsic hypercoagulable state caused by elevated activation of platelets and increased expression of procoagulant factors.[237] However, a low concurrence of pancreatic cancer and cardiovascular thrombosis was observed in the present studies. Additionally, increased systolic blood pressure has been observed in rats and mice exposed to DEHP; however, this effect is thought to be secondary to renal dysfunction or alterations in renin and angiotensin II signaling.[236; 238]

NTP has tested DEHP in a range of in vitro and in vivo genotoxicity assays, and the results were generally negative. The positive results seen in some of the in vitro assays for induction of sister chromatid exchanges (SCE) were seen in the presence of excessive cytotoxicity. The Organization for Economic Co-operation and Development test guideline[239] for the in vitro SCE test was withdrawn in 2014, and the test is no longer requested by regulatory agencies. In vivo, the nonnegative responses that were observed in some of the NTP micronucleus assays were generally weak. The consensus from published data is that DEHP shows limited evidence of genotoxic potential, and for the sporadic positive results that have been reported, the response is either weak, not reproducible, obtained in a nonstandard test system, or qualified to some degree by the authors.

Lastly, carcinogenic responses that were related or may have been related to DEHP exposure were modeled to estimate benchmark doses corresponding to a 10% increase in neoplasm incidence (BMD_{10}). For the similar target sites, the BMD_{10} levels based on the hepatocellular response were lower in males in the perinatal and postweaning study compared to the postweaning-only study (383 mg/kg/day and 434 mg/kg/day, respectively) and in females (123 mg/kg/day and 384 mg/kg/day, respectively). Conversely, BMD_{10} levels were lower for the pancreatic acinar neoplasm response in males with postweaning-only exposure compared with perinatal and postweaning exposure (31 mg/kg/day versus 86 mg/kg/day, respectively). The BMD_{10} for the neoplastic responses in the uterus in females was lower in the postweaning-only study compared to the perinatal and postweaning study (324 mg/kg/day versus 594 mg/kg/day, respectively). The lowest BMD_{10} levels were associated with incidences of pancreatic acinar adenoma or carcinoma (combined) in male rats in both studies. These data show no obvious overall increased sensitivity in carcinogenic response with perinatal and postweaning exposure compared to postweaning-only exposure, which is consistent with NTP's study of PFOA perinatal exposure.[211]

Conclusions

Under the conditions of the perinatal and postweaning feed study (Study 1), there was *clear evidence of carcinogenic activity* of di(2-ethylhexyl) phthalate (DEHP) in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominately adenomas) of the pancreas. There was *clear evidence of carcinogenic activity* of DEHP in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of hepatocellular adenoma or carcinoma (combined). The occurrence of pancreatic acinar adenoma or carcinoma (combined) was considered to be related to exposure. The occurrence of uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined) in female rats may have been related to exposure.

Under the conditions of the postweaning-only feed study (Study 2), there was *clear evidence of carcinogenic activity* of DEHP in male Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and acinar adenoma or carcinoma (combined) neoplasms (predominately adenomas) of the pancreas. The occurrence of testicular interstitial cell adenoma in male rats may have been related to exposure. There was *clear evidence of carcinogenic activity* of DEHP in female Hsd:Sprague Dawley® SD® rats based on the increased incidences of hepatocellular adenoma or carcinoma (combined) and uterine (including cervix) adenoma, adenocarcinoma, squamous cell carcinoma, or squamous cell papilloma (combined). The occurrence of pancreatic acinar adenoma or carcinoma (combined) in female rats was considered to be related to exposure.

The BMD analysis shows there was no consistent pattern indicating that perinatal and postweaning exposure was more sensitive compared to postweaning-only exposure and modeled responses were within threefold of each other. However, there was a stronger carcinogenic response in the reproductive organs (uterus and testis) in the postweaning-only exposure study compared to the perinatal and postweaning exposure study.

In both studies, exposure to DEHP resulted in increased incidences of nonneoplastic lesions in the liver (male and female), heart (male), pituitary gland (male), testis, and epididymis. In the postweaning-only study (Study 2), DEHP exposure increased nonneoplastic lesions in the pancreas (male and female), bone marrow (male and female), and uterus. Perinatal and postweaning exposure (Study 1) increased gross lesions with the reproductive tract (male), bone marrow (male), and kidney (male and female).

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Appendix A. Chemical Characterization and Dose Formulation Studies

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A.1. Procurement and Characterization of Di(2-ethylhexyl) Phthalate

Di(2-ethylhexyl) phthalate (DEHP) was obtained from Aldrich Chemical Company Inc. (St. Louis, MO) in a single lot (lot 01514TH) received in two shipments. The first shipment (10 L) was received on December 12, 2008 and used for chemical characterization. The second shipment (250 L) was received on November 4, 2009 and used for the dose formulations in the 2-year studies and chemical reanalysis. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at RTI International (Research Triangle Park, NC). Reports on analyses performed in support of the DEHP studies are on file at the National Institute of Environmental Health Sciences.

The appearance (clear liquid) and density of lot 01514TH (0.976 g/mL at 21.9°C) matched that of DEHP (0.985 g/mL at 25°C). Galbraith Laboratories (Knoxville, TN) performed the boiling point and elemental analyses of lot 01514TH. While the elemental analysis confirmed the anticipated relative ratios, the experimental boiling point (330.9°C) was considerably lower than that reported in the literature (384°C). Using a different method, the results (335°C, 760 mm Hg) from RTI International were consistent with Galbraith Laboratories. A precise molecular mass was measured using research-grade high-resolution mass spectrometry (HRMS) at the University of South Carolina Mass Spectrometry Facility (Columbia, SC). The observed mass values (390.2772) were within acceptable limits (≤5 ppm) of the calculated mass (390.2770).

The identity of lot 01514TH was confirmed using infrared (IR) spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography (GC) with MS detection. The IR spectrum was in good agreement with the structure of DEHP and with the reference spectrum from the National Institute of Advanced Industrial Science and Technology Spectral Database for Organic Compounds (SDBS No. 2266) for DEHP (Figure A-1). ^{1}H and ^{13}C NMR spectra (Figure A-2, Figure A-3) were consistent with the structure of DEHP and the prediction from the Advanced Chemistry Development Spectral Prediction Program (Version 10.02, Toronto, Ontario, Canada) for DEHP. GC/MS identified the major peak from the 10 L shipment of lot 01514TH as DEHP using fragmentation pattern analysis and comparison with the National Institute of Standards and Technology (NIST) reference spectrum (No. 311338) for DEHP (Table A-1, System A). The GC/MS spectra correlated well with the structure of DEHP.

The moisture content of lot 01514TH was determined by Karl Fisher titration. The purity was determined using ultra-performance liquid chromatography (UPLC) with photodiode array detector (PDA) and using GC with flame ionization detection (FID) (Table A-1, Systems B and C, respectively). The Karl Fisher titration yielded a water content of 0.145%. UPLC/PDA analysis demonstrated one major peak accounting for 99.7% and one minor peak accounting for 0.2% of the total integrated area. GC/FID analysis also found one major peak accounting for 99.7% and one minor peak accounting for 0.3% of the total integrated area. An additional GC/MS analysis of the test chemical was performed in an attempt to identify the minor component in the chromatographic profile (Table A-1, System A). The fragmentation of the minor impurity peak agreed with the NIST reference spectrum (No. 312137) for mono(2-ethylhexyl) phthalate.

Accelerated stability studies were conducted by the analytical chemistry laboratory using samples of lot 01514TH stored at ambient temperature (approximately 22°C), refrigerated temperature (approximately 5°C), and elevated temperature (approximately 60°C) in amber vials

sealed with foil-lined caps. After 14 days, samples were analyzed by GC/FID (Table A-1, System C). Stability of DEHP was confirmed for at least 2 weeks when stored in sealed glass vials at temperatures from 5° to 60°C.

Upon receipt of the 250 L shipment used for the 2-year studies, the bulk chemical of lot 01514TH was homogenized by shaking each of the 5 50 L plastic jugs for approximately 2 minutes and transferred to 70 4 L amber glass storage bottles, which were stored at room temperature.

Prior to using the bulk chemical from the 250 L shipment of lot 01514TH for dose formulations, the identity was confirmed using the same GC/MS system with comparison to the reference spectrum and an aliquot of the test article from the 10L shipment (Table A-1, System A). The GC/MS analysis of the 250 L shipment of lot 01514TH demonstrated one major peak accounting for 99.9% of the total integrated area. Periodic reanalysis of the bulk chemical lot 01514TH was performed prior to and during the animal studies by the laboratory using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection (Table A-1, System D), and no degradation of the test chemical was detected.

A.2. Preparation and Analysis of Dose Formulations

The base diet was meal feed purchased from Zeigler Brothers, Inc. (Gardners, PA). The perinatal and postweaning study (Study 1) utilized NIH-07 feed (2 lots milled March and April 2011) in addition to NTP-2000 feed (25 lots milled April 2011–March 2013). The postweaning-only study (Study 2) utilized NTP-2000 feed (25 lots milled December 2010–December 2012). In addition to determining the suitability of the vehicles for feeding the animals, analysis of the NTP-2000 feed extract performed by the study laboratory using liquid chromatography mass spectrometry (LC/MS) confirmed that the vehicle did not contain the test article DEHP.

Dose formulations were prepared monthly by mixing DEHP with feed (Table A-2). For the perinatal and postweaning study, formulations were prepared at concentrations of 0, 300, 1,000, 3,000, and 10,000 ppm in both NIH-07 feed (May 4, May 24, and June 15, 2011) and in NTP-2000 feed (31 formulations; June 2011–June 2013). For the postweaning-only study, formulations were prepared in NTP-2000 feed at concentrations of 0, 300, 1,000, 3,000, and 10,000 ppm (31 formulations; February 2011–February 2013). The plastic bags used by the study laboratory in the preparation and storage of blank and dosed feed were determined to have no levels of DEHP above the limit of detection of the assay (1.27 ppm).

Homogeneity studies were performed on the 25 and 10,000 ppm dose formulations in both 25-kg NIH-07 feed batch sizes and 25-kg NTP-2000 feed batch sizes by the analytical chemistry laboratory using UPLC/PDA (Table A-1, System B). Additional homogeneity studies of the 300 and 10,000 ppm dose formulations in a 72-kg NIH-07 feed batch size and 300, 3,000, and 10,000 ppm dose formulations in a 92-kg NTP-2000 feed batch sizes were performed before the animal studies by the study laboratory using HPLC/UV (Table A-1, System D). All formulations analyzed were determined to be homogenous and of appropriate concentration.

Stability studies conducted by the chemistry laboratory of the 25 ppm NIH-07 and 25 ppm NTP-2000 dose formulations confirmed the stability of DEHP after 42 days at room, refrigerated, or frozen temperatures. Stability was also confirmed under simulated dosing

conditions (room temperature, exposure to air and light for 7 days, in absence of excreta, and in presence of excreta). Control and dosed formulations were stored in individual plastic bag-lined containers at room temperature (approximately 25ºC) and were used within 42 days of preparation.

Periodic analyses of the preadministration dose formulations of DEHP were conducted by the study laboratory every 1 to 3 months to determine purity, while postadministration (animal room) samples were analyzed about every 1 to 7 months (Table A-3, Table A-4). All preadministration formulations were within 10% of the target concentrations. For the perinatal and postweaning study, all postadministration dose formulations of DEHP were within 10% of target concentrations. For the postweaning-only study (Study 2), all postadministration dose formulations of DEHP were within 10% of target concentrations except for the 1,000 ppm dose formulation prepared on July 30, 2012, collected from residual feed in the feeder that was 12.3% below the target concentration.

Table A-1. Chromatography Systems Used in the Two-year Feed Studies of Di(2-ethyhexyl) Phthalate

Chromatography	Detection System	Column	Mobile Phase
System A			
Gas chromatography	Mass selective detector	J&W DB-1 (25 m × 0.32 mm ID, 0.25 μm film thickness)	Helium, 1.65 mL/min flow rate
System B			
Ultra-performance liquid chromatography	Photodiode array detector (205 to 400 nm, extracted at 225 nm)	Waters Acquity UPLC BEH Phenyl (50 mm × 2.1 mm ID, 1.7 μm particle size), with Waters Acquity In-Line Filter (0.2 μm)	A: Methanol B: Water Gradient program: A:B 25:75 to 75:25 in 3 min, hold at 78:22 for 1 min, ramp to 100:0 in 1 min, hold at 100:0 for 1 min, reverse to 25:75 in 0.5 min, hold at 25:75 for 1.5 min 0.6 mL/min flow rate
System C			
Gas chromatography	Flame ionization detection (325°C)	J&W HP-5 (30 m × 0.32 mm ID, 0.25 μm film thickness)	Helium, 1 mL/min flow rate

Chromatography	Detection System	Column	Mobile Phase
System D			
High-performance liquid chromatography	Ultraviolet (225 nm)	Thermo Scientific Hypersil Phenyl (250 mm × 4.6 mm ID, 5 µm particle size) with Hypersil Phenyl guard (5 µm particle size)	A: Methanol B: ASTM Type I Water Gradient program: A:B 70:30 to 85:15 in 5 min, ramp to 100:0 in 4 min, hold at 100:0 for 4 min, reverse to 70:30 in 0.1 min, hold at 70:30 for 10.9 min 1.0 mL/min flow rate

ID = internal diameter; UPLC = ultra-performance liquid chromatography; ASTM = American Society for Testing and Materials.

Table A-2. Preparation and Storage of Dose Formulations in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

Preparation
Stock solutions of di(2-ethylhexyl) phthalate (DEHP) were created by weighing an appropriate amount of lot 01514TH and adding it to a volumetric flask. Acetone was used to bring the solution to volume. Flasks of stocks solution were sealed and shaken until the chemical was dissolved (at least 10 inversions). An initial formulation premix was created by weighing an appropriate amount of feed (NIH-07 or NTP-2000) into a mixing bowl. A portion of the stock DEHP solution was slowly poured onto the feed and then stirred for 2 minutes at a low setting using a Hobart mixer. The mixer was stopped, and the remaining stock solution was poured onto the feed. The stock container was rinsed with acetone twice and the rinses were poured onto the feed. The premix feed was stirred under a nitrogen stream with a flow rate of 10 liters per minute for approximately an hour to encourage cyclonic flow and to ensure complete evaporation of the acetone. The formulation blends were prepared by adding half of the required blank feed to a twin shell blender and then evenly covering with the premix. The sides were "rinsed" twice with the remaining blank feed and added to the blender. The final formulation was mixed in the blender for 15 minutes. The dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number
01514TH
Maximum Storage Time
42 days
Storage Conditions
Stored in sealed plastic bag-lined container at ~25ºC
Study Laboratory
Battelle (Columbus, OH)

Table A-3. Results of Analyses of Dose Formulations Administered to Rats in the Perinatal and Postweaning Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
May 4, 2011	May 5, 2011	0	BLOQ	NA
		300	309 ± 5	3.0
		1,000	1,020 ± 20	2.0
		3,000	3,170 ± 70	5.7
		10,000	10,200 ± 100	2.0
June 6, 2011	June 6, 2011	0	BLOQ	NA
		300	291.5	−2.8
		1,000	959.5	−4.1
		3,000	2,885	−3.8
		10,000	9,790	−2.1
August 17, 2011	August 19, 2011	0	BLOQ	NA
		300	292.5	−2.5
		1,000	982.5	−1.8
		3,000	2,930	−2.3
		10,000	9,805	−2.0
October 31, 2011	November 3, 2011	0	BLOQ	NA
		300	287.5	−4.2
		1,000	922	−7.8
		3,000	2,875	−4.2
		10,000	9,560	−4.4
January 12, 2012	January 13, 2012	0	BLOQ	NA
		300	305	1.7
		1,000	993	−0.7
		3,000	2,990	−0.3
		10,000	10,100	1.0
March 26, 2012	March 29, 2012	0	BLOQ	NA
		300	307.5	2.5
		1,000	1,020	2.0
		3,000	3,045	1.5
		10,000	10,350	3.5
June 6, 2012	June 7, 2012	0	BLOQ	NA
		300	295	−1.7
		1,000	983	−1.7
		3,000	2,990	−0.3

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
		10,000	9,850	−1.5
July 30, 2012	August 1, 2012	0	BLOQ	NA
		300	300.5	0.2
		1,000	997.5	−0.3
		3,000	3,040	1.3
		10,000	10,200	2.0
October 8, 2012	October 8, 2012	0	BLOQ	NA
		300	314.5	4.8
		1,000	998	−0.2
		3,000	2,860	−4.7
		10,000	9,895	−1.1
December 18, 2012	December 18, 2012	0	BLOQ	NA
		300	292	−2.7
		1,000	971	−2.9
		3,000	3,015	0.5
		10,000	9,845	−1.6
March 4, 2013	March 5, 2013	0	BLOQ	NA
		300	302 ± 2	0.7
		1,000	998 ± 3	−0.2
		3,000	3,000 ± 40	0.0
		10,000	10,000 ± 200	0.0
April 22, 2013	April 22, 2013	0	BLOQ	NA
		300	299 ± 3	−0.3
		1,000	995 ± 2	−0.5
		3,000	2,970 ± 10	−1.0
		10,000	9,840 ± 30	−1.6
Animal Room Samples				
May 4, 2011	June 13, 2011 (feeder)	0	BLOQ	NA
		300	279 ± 1	−6.9
		1,000	939 ± 3	−6.1
		3,000	2,870 ± 30	−4.5
		10,000	9,480 ± 50	−5.2
	June 13, 2011 (bucket)	0	BLOQ	NA
		300	294 ± 1	−2.1
		1,000	957 ± 24	−4.3

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
		3,000	2,920 ± 130	−2.7
		10,000	9,560 ± 120	−4.4
June 6, 2011	July 19, 2011 (feeder)	0	BLOQ	NA
		300	295 ± 2	−1.8
		1,000	960 ± 1	−4.0
		3,000	2,880 ± 20	−4.0
		10,000	10,000 ± 0	0.0
	July 19, 2011 (bucket)	0	BLOQ	NA
		300	302 ± 1	0.5
		1,000	1,000 ± 10	0.3
		3,000	3,000 ± 30	0.1
		10,000	9,770 ± 120	−0.3
January 12, 2012	February 23, 2012 (feeder)	0	BLOQ	NA
		300	298 ± 1	−0.6
		1,000	958 ± 5	−4.2
		3,000	2,750 ± 30	−8.3
		10,000	9,270 ± 120	−7.3
	February 23, 2012 (bucket)	0	BLOQ	NA
		300	297 ± 3	−0.9
		1,000	980 ± 6	−2.0
		3,000	2,900 ± 30	−3.4
		10,000	9,360 ± 130	−6.4
July 30, 2012	September 5, 2012 (feeder)	0	BLOQ	NA
		300	288 ± 1	−4.1
		1,000	1,020 ± 60	2.2
		3,000	2,840 ± 60	−5.3
		10,000	9,190 ± 110	−8.1
	September 5, 2012 (bucket)	0	BLOQ	NA
		300	288 ± 2	−4.1
		1,000	1,000 ± 80	0.0
		3,000	2,830 ± 130	−5.7
		10,000	9,920 ± 280	−0.8
March 4, 2013	April 14, 2013 (feeder)	0	BLOQ	NA
		300	281 ±2	−6.2
		1,000	909 ± 4	−9.1

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
		3,000	2,740 ± 20	−8.6
		10,000	9,510 ± 20	−4.9
	April 14, 2013 (bucket)	0	BLOQ	NA
		300	289± 0	−3.7
		1,000	NS	NA
		3,000	2,910 ± 70	−3.0
		10,000	10,200 ± 200	2.0

BLOQ = below the limit of quantification; NA = not applicable; NS = no sample collected.
[a]Preadministration samples are an average of triplicate analysis on two sample collections from the same preparation date.
Animal room samples are an average and standard deviation of triplicate analysis of a single sample.

Table A-4. Results of Analyses of Dose Formulations Administered to Rats in the Postweaning-only Two-year Feed Study of Di(2-ethylhexyl) Phthalate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
February 2, 2011	February 5, 2011	0	BLOQ	NA
		300	295 ± 5	−1.7
		1,000	987 ± 38	−1.3
		3,000	3,040 ± 10	1.3
		10,000	10,300 ± 300	3.0
March 28, 2011	March 28, 2011	0	BLOQ	NA
		300	300 ± 3	0.0
		1,000	991 ± 3	−0.9
		3,000	2,990 ± 10	−0.3
		10,000	9,880 ± 190	−1.2
June 6, 2011	June 6, 2011	0	BLOQ	NA
		300	291.5	−2.8
		1,000	959.5	−4.1
		3,000	2,885	−3.8
		10,000	9,790	−2.1
August 17, 2011	August 19, 2011	0	BLOQ	NA
		300	292.5	−2.5
		1,000	982.5	−1.8
		3,000	2,930	−2.3
		10,000	9,805	−2.0
October 31, 2011	November 3, 2011	0	BLOQ	NA
		300	287.5	−4.2
		1,000	922	−7.8
		3,000	2,875	−4.2
		10,000	9,560	−4.4
January 12, 2012	January 13, 2012	0	BLOQ	NA
		300	305	1.7
		1,000	993	−0.7
		3,000	2,990	−0.3
		10,000	10,100	1.0
March 26, 2012	March 29, 2012	0	BLOQ	NA
		300	307.5	2.5
		1,000	1,020	2.0
		3,000	3,045	1.5

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
		10,000	10,350	3.5
June 6, 2012	June 7, 2012	0	BLOQ	NA
		300	295	−1.7
		1,000	983	−1.7
		3,000	2,990	−0.3
		10,000	9,850	−1.5
July 30, 2012	August 1, 2012	0	BLOQ	NA
		300	300.5	0.2
		1,000	997.5	−0.3
		3,000	3,040	1.3
		10,000	10,200	2.0
October 8, 2012	October 8, 2012	0	BLOQ	NA
		300	314.5	4.8
		1,000	998	−0.2
		3,000	2,860	−4.7
		10,000	9,895	−1.1
December 18, 2012	December 18, 2012	0	BLOQ	NA
		300	292	−2.7
		1,000	971	−2.9
		3,000	3,015	0.5
		10,000	9,845	−1.6
Animal Room Samples				
February 2, 2011	March 17, 2011 (feeder)	0	BLOQ	NA
		300	293 ± 6	−2.2
		1,000	933 ± 4	−6.7
		3,000	2,830 ± 30	−5.6
		10,000	9,430 ± 280	−5.7
	March 17, 2011 (bucket)	0	BLOQ	NA
		300	294 ± 6	−1.9
		1,000	972 ± 11	−2.8
		3,000	2,890 ± 40	−3.6
		10,000	9,460 ± 140	−5.4
June 6, 2011	July 19, 2011 (feeder)	0	BLOQ	NA
		300	283 ± 3	−5.7
		1,000	973 ± 3	−2.7

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)[a]	Difference from Target (%)
		3,000	2,950 ± 10	−1.7
		10,000	9,780 ± 10	−2.2
	July 19, 2011 (bucket)	0	BLOQ	NA
		300	302 ± 1	0.5
		1,000	1,000 ± 10	0.3
		3,000	3,000 ± 30	0.1
		10,000	9,770 ± 120	−0.3
January 12, 2012	February 23, 2012 (feeder)	0	BLOQ	NA
		300	299 ± 4	−0.2
		1,000	925 ± 1	−7.5
		3,000	2,790 ± 30	−6.9
		10,000	9,260 ± 110	−7.4
	February 23, 2012 (bucket)	0	BLOQ	NA
		300	297 ± 3	−0.9
		1,000	980 ± 6	−2.0
		3,000	2,900 ± 30	−3.4
		10,000	9,360 ± 130	−6.4
July 30, 2012	September 5, 2012 (feeder)	0	BLOQ	NA
		300	282 ± 4	−6.1
		1,000	877 ± 35	−12.3
		3,000	2,850 ± 10	−5.l
		10,000	9,200 ± 250	−8.0
	September 5, 2012 (bucket)	0	BLOQ	NA
		300	288 ± 2	−4.1
		1,000	1,000 ± 80	0.0
		3,000	2,830 ± 130	−5.7
		10,000	9,920 ± 280	−0.8

BLOQ = below the limit of quantification; NA = not applicable.
[a]Preadministration samples are an average of triplicate analysis on two sample collections from the same preparation date.
Animal room samples are an average and standard deviation of triplicate analysis of a single sample.

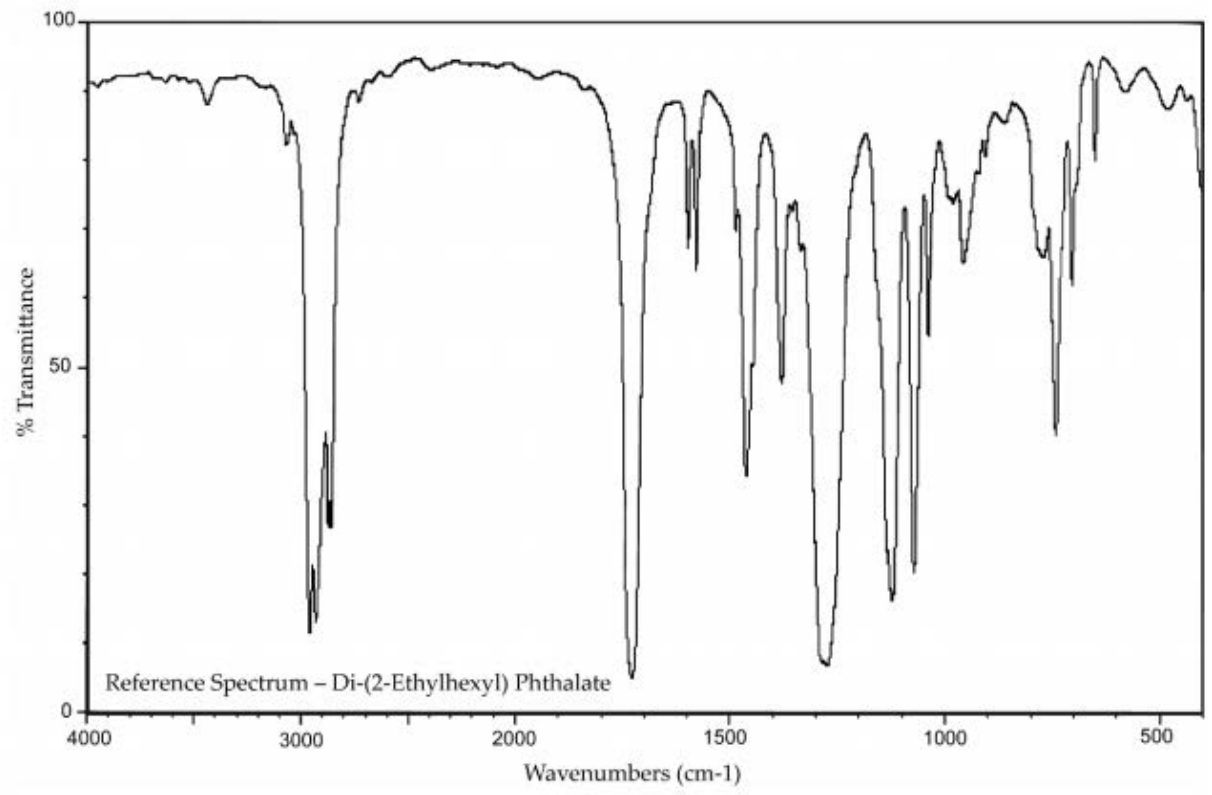

Figure A-1. Reference Infrared Absorption Spectrum of Di(2-ethylhexyl) Phthalate

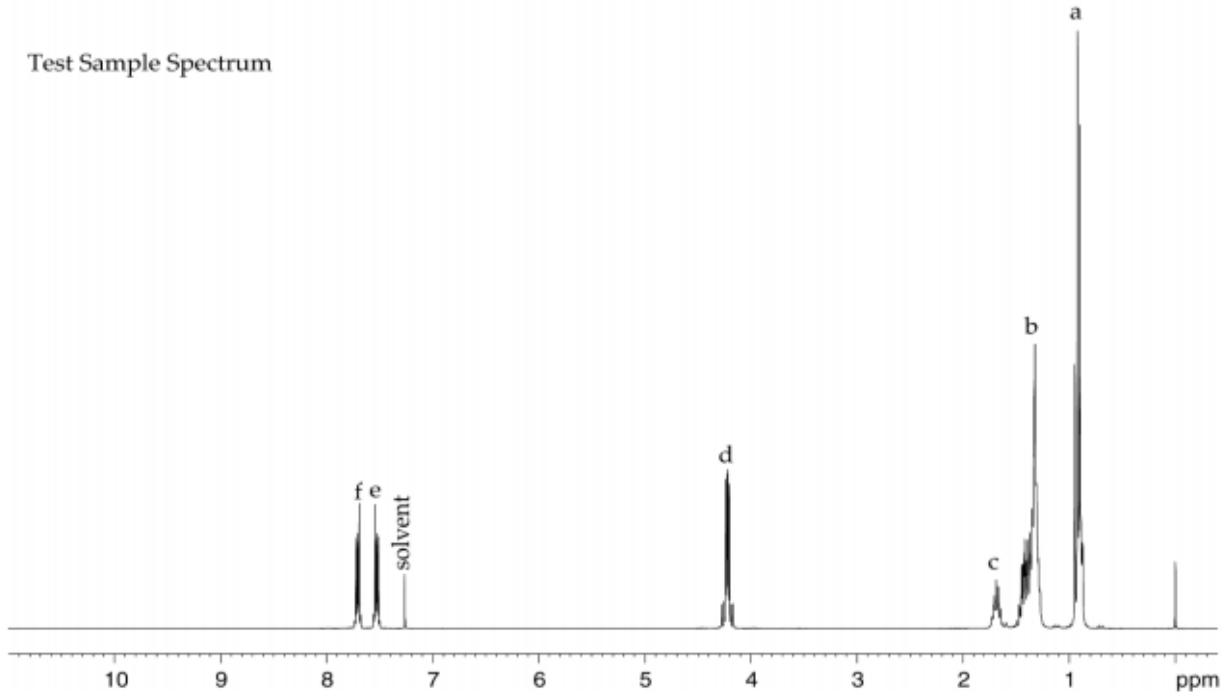

Figure A-2. Fourier Transformed ^{1}H Nuclear Magnetic Resonance Spectrum of Sample of Di(2-ethylhexyl) Phthalate (Lot 01514TH)

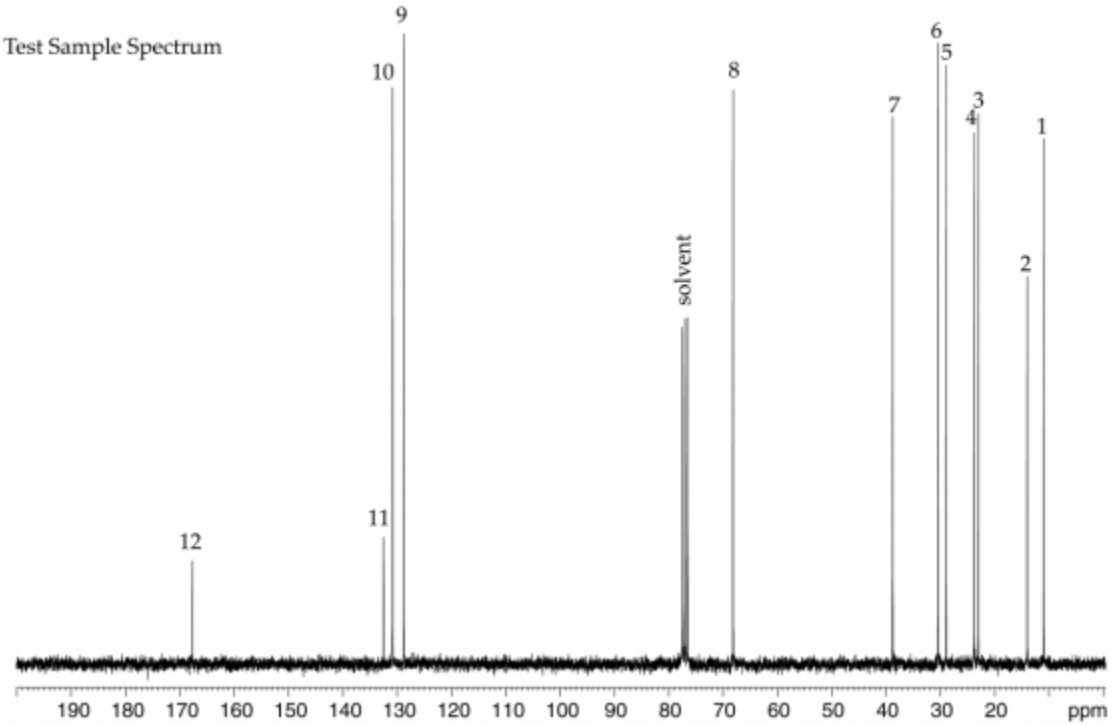

Figure A-3. Fourier Transformed ^{13}C Nuclear Magnetic Resonance Spectrum of Sample of Di(2-ethylhexyl) Phthalate (Lot 01514TH)

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 and NTP-2000 Rat Rations

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Tables

B.1. NIH-07 Feed

Table B-1. Ingredients of NIH-07 Rat Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	24.25
Wheat Middlings	10.0
Oat Hulls	0.0
Alfalfa Meal (Dehydrated, 17% Protein)	4.0
Purified Cellulose	0.0
Soybean Meal (49% Protein)	12.0
Fish Meal (60% Protein)	10.0
Corn Oil (without Preservatives)	0.0
Soy Oil (without Preservatives)	2.5
Dried Brewer's Yeast	2.0
Calcium Carbonate (USP)	0.5
Vitamin Premix[a]	0.25
Mineral Premix[b]	0.15
Calcium Phosphate, Dibasic (USP)	1.25
Sodium Chloride	0.5
Choline Chloride (70% Choline)	0.10
Dried Skim Milk	5.00
Dried Molasses	1.50
Corn Gluten Meal (60% Protein)	3.00
Methionine	0.0

USP = United States Pharmacopeia
[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NIH-07 Rat Ration

	Amount[a]	Source
Vitamins		
Vitamin A	6,062 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	5,070 IU	D-activated animal sterol
Vitamin K	3.1 mg	Menadione sodium bisulfite complex
Vitamin E	22 IU	α-Tocopheryl acetate
Niacin	33 mg	–
Folic Acid	2.4 mg	–
d-Pantothenic Acid	19.8 mg	d-Calcium pantothenate
Riboflavin	3.8 mg	–
Thiamine	11 mg	Thiamine mononitrate
B_{12}	50 µg	–
Pyridoxine	6.5 mg	Pyridoxine hydrochloride
Biotin	0.15 mg	d-Biotin
Minerals		
Iron	132 mg	Iron sulfate
Zinc	18 mg	Zinc oxide
Manganese	66 mg	Manganese oxide
Copper	4.4 mg	Copper sulfate
Iodine	2.0 mg	Calcium iodate
Cobalt	0.44 mg	Cobalt carbonate

[a]Per kg of finished diet.

Table B-3. Nutrient Composition of NIH-07 Rat Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	23.65 ± 0.070	23.6–23.7	2
Crude Fat (% by Weight)	5.15 ± 0.212	5.0–5.3	2
Crude Fiber (% by Weight)	3.29 ± 0.042	3.26–3.32	2
Ash (% by Weight)	6.015 ± 0.092	5.95–6.08	2
Amino Acids (% of Total Diet)			
Arginine	1.380 ± 0.06	1.3–1.49	10
Cystine	0.322 ± 0.031	0.274–0.372	10
Glycine	1.150 ± 0.070	1.06–1.31	10
Histidine	0.518 ± 0.024	0.497–0.553	10
Isoleucine	0.984 ± 0.024	0.952–1.03	10
Leucine	2.018 ± 0.067	1.93–2.13	10

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Lysine	1.243 ± 0.051	1.13–1.32	10
Methionine	0.488 ± 0.016	0.468–0.515	10
Phenylalanine	1.097 ± 0.022	1.07–1.12	10
Threonine	0.918 ± 0.031	0.883–0.961	10
Tryptophan	0.277 ± 0.020	0.265–0.326	10
Tyrosine	0.860 ± 0.037	0.785–0.894	10
Valine	1.134 ± 0.025	1.11–1.17	10
Essential Fatty Acids (% of Total Diet)			
Linoleic	2.30 ± 0.219	1.99–2.59	10
Linolenic	0.25 ± 0.275	0.217–0.296	10
Vitamins			
Vitamin A (IU/kg)	6,020 ± 65.05	5,560–6,480	2
α-Tocopherol (ppm)	6,704 ± 21,045	40.3–66,600	10
Thiamine (ppm)[a]	14.2 ± 0.566	13.8–14.6	2
Riboflavin (ppm)	14.47 ± 3.352	10.0–19.8	10
Niacin (ppm)	99.33 ± 8.235	87.0–112.0	10
Pantothenic Acid (ppm)	44.38 ± 3.806	38.2–51.1	10
Pyridoxine (ppm)[a]	12.876 ± 3.171	9.63–19.7	10
Folic Acid (ppm)	2.482 ± 0.487	1.68–3.09	10
Biotin (ppm)	0.3283 ± 0.172	0.0–0.638	10
B_{12} (ppb)	49.4 ± 6.83	41.8–61.6	10
Choline (as Chloride) (ppm)	1,821.0 ± 197.5	1,570–2,200	10
Minerals			
Calcium (%)	1.004 ± 0.008	0.998–1.01	2
Phosphorus (%)	0.910 ± 0.002	0.908–0.911	2
Potassium (%)	0.830 ± 0.036	0.769–0.88	10
Chloride (%)	0.652 ± 0.106	0.441–0.8	10
Sodium (%)	0.378 ± 0.46	0.318–0.469	10
Magnesium (%)	0.187 ± 0.014	0.17–0.218	10
Iron (ppm)	385.1 ± 54.9	276.0–469.0	10
Manganese (ppm)	90.81 ± 7.566	80.7–104.0	10
Zinc (ppm)	64.15 ± 10.07	52.4–89.2	10
Copper (ppm)	14.13 ± 2.57	11.9–21.1	10
Iodine (ppm)	1.811 ± 0.992	0.54–3.45	10
Chromium (ppm)	3.946 ± 0.036	3.89–4.0	8
Cobalt (ppm)	0.5155 ± 0.267	0.01–0.963	10

[a]As hydrochloride.

Table B-4. Contaminant Levels in NIH-07 Rat Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.3865 ± 0.013	0.377–0.396	2
Cadmium (ppm)	0.0875 ± 0.004	0.085–0.09	2
Lead (ppm)	0.072 ± 0.004	0.069–0.074	2
Mercury (ppm)	0.013 ± 0.001	0.012–0.014	2
Selenium (ppm)	0.382 ± 0.014	0.372–0.392	2
Aflatoxins (ppb)[a]	5	–	2
Nitrate Nitrogen (ppm)[b]	11.8 ± 2.55	10.0–13.6	2
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	2
BHA (ppm)[a,c]	<1.0	–	2
BHT (ppm)[a,c]	<1.0	–	2
Aerobic Plate Count (CFU/gm)	60 ± 70.7	10–110	2
Coliform (MPN/gm)	<3.0	–	2
E. coli (MPN/gm)	<10	–	2
Salmonella (MPN/gm)	Negative	–	2
Total Nitrosamines (ppb)[d]	5.5 ± 1.768	4.2–6.7	2
N-Nitrosodimethylamine (ppb)[d]	4.5 ± 1.768	3.2–5.7	2
N-Nitrosopyrrolidine (ppb)[d]	1 ± 0.0	1.0–1.0	2
Pesticides (ppm)			
α-BHC[a]	<0.01	–	2
β-BHC[a]	<0.02	–	2
γ-BHC[a]	<0.01	–	2
δ-BHC[a]	<0.01	–	2
Heptachlor[a]	<0.01	–	2
Aldrin[a]	<0.01	–	2
Heptachlor Epoxide[a]	<0.01	–	2
DDE[a]	<0.01	–	2
DDD[a]	<0.01	–	2
DDT[a]	<0.01	–	2
HCB[a]	<0.01	–	2
Mirex[a]	<0.01	–	2
Methoxychlor[a]	<0.05	–	2
Dieldrin[a]	<0.01	–	2
Endrin[a]	<0.01	–	2
Telodrin[a]	<0.01	–	2
Chlordane[a]	<0.05	–	2
Toxaphene[a]	<0.10	–	2
Estimated PCBs[a]	<0.20	–	2
Ronnel[a]	<0.01	–	2
Ethion[a]	<0.02	–	2

	Mean ± Standard Deviation	Range	Number of Samples
Trithion[a]	<0.05	–	2
Diazinon[a]	<0.10	–	2
Methyl Chlorpyrifos	<0.02	–	2
Methyl Parathion[a]	<0.02	–	2
Ethyl Parathion[a]	<0.02	–	2
Malathion	0.081 ± 0.082	0.024–0.139	2
Endosulfan I[a]	<0.01	–	2
Endosulfan II[a]	<0.01	–	2
Endosulfane Sulfate[a]	<0.03	–	2

All samples were irradiated. BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

B.2. NTP-2000 Feed

Table B-5. Ingredients of NTP-2000 Rat Ration

Ingredients	Percent by Weight
Ground Hard Winter Wheat	22.26
Ground #2 Yellow Shelled Corn	22.18
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	5.0
Fish Meal (60% Protein)	4.0
Corn Oil (without Preservatives)	3.0
Soy Oil (without Preservatives)	3.0
Dried Brewer's Yeast	1.0
Calcium Carbonate (USP)	0.9
Vitamin Premix[a]	0.5
Mineral Premix[b]	0.5
Calcium Phosphate, Dibasic (USP)	0.4
Sodium Chloride	0.3
Choline Chloride (70% Choline)	0.26
Methionine	0.2

USP = United States Pharmacopeia.
[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table B-6. Vitamins and Minerals in NTP-2000 Rat Ration

	Amount[a]	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
d-Pantothenic Acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished diet.

Table B-7. Nutrient Composition of NTP-2000 Rat Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.78 ± 0.543	13.9–16.8	28
Crude Fat (% by Weight)	8.63 ± 0.387	8.0–9.7	28
Crude Fiber (% by Weight)	9.37 ± 0.534	7.49–10.1	28
Ash (% by Weight)	5.23 ± 1.767	4.6–14.2	28
Amino Acids (% of Total Diet)			
Arginine	0.805 ± 0.075	0.67–0.97	29
Cystine	0.220 ± 0.021	0.15–0.25	29
Glycine	0.702 ± 0.038	0.62–0.80	29
Histidine	0.342 ± 0.070	0.27–0.68	29
Isoleucine	0.549 ± 0.040	0.43–0.66	29
Leucine	1.100 ± 0.063	0.96–1.24	29
Lysine	0.700 ± 0.104	0.31–0.86	29
Methionine	0.409 ± 0.042	0.26–0.49	29
Phenylalanine	0.623 ± 0.047	0.471–0.72	29
Threonine	0.513 ± 0.041	0.43–0.61	29
Tryptophan	0.155 ± 0.027	0.11–0.2	29
Tyrosine	0.422 ± 0.066	0.28–0.54	29
Valine	0.666 ± 0.040	0.55–0.73	29
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.235	3.49–4.55	29
Linolenic	0.30 ± 0.064	0.005–0.368	29
Vitamins			
Vitamin A (IU/kg)	3,886 ± 81.3	2,030–5,290	28
α-Tocopherol (ppm)	2,456 ± 12,817	13.6–69,100	29
Thiamine (ppm)[a]	7.96 ± 0.484	3.9–11.1	28
Riboflavin (ppm)	8.17 ± 2.841	4.2–17.5	29
Niacin (ppm)	78.66 ± 8.11	66.4–98.2	29
Pantothenic Acid (ppm)	26.42 ± 11.05	17.4–81.0	29
Pyridoxine (ppm)[a]	9.75 ± 2.045	6.44–14.3	29
Folic Acid (ppm)	1.58 ± 0.43	1.15–3.27	29
Biotin (ppm)	0.323 ± 0.093	0.2–0.704	29
B_{12} (ppb)	50.41 ± 34.89	18.3–174	29
Choline (as Chloride) (ppm)	2,593 ± 633.8	1,160–3,790	29

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.905 ± 0.041	0.831–1.03	28
Phosphorus (%)	0.540 ± 0.097	0.053–0.60	28
Potassium (%)	0.668 ± 0.029	0.626–0.733	29
Chloride (%)	0.392 ± 0.044	0.3–0.517	29
Sodium (%)	0.195 ± 0.027	0.16–0.283	29
Magnesium (%)	0.217 ± 0.054	0.185–0.49	29
Iron (ppm)	191.6 ± 36.18	135–311	29
Manganese (ppm)	50.11 ± 9.42	21–73.1	29
Zinc (ppm)	57.3 ± 25.54	43.3–184	29
Copper (ppm)	7.57 ± 2.49	3.21–16.3	29
Iodine (ppm)	0.513 ± 0.221	0–0.972	29
Chromium (ppm)	1.02 ± 1.04	0.33–3.97	28
Cobalt (ppm)	0.222 ± 0.152	0.0857–0.864	27

[a]As hydrochloride.

Table B-8. Contaminant Levels in NTP-2000 Rat Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.2 ± 0.048	0.147–0.383	28
Cadmium (ppm)	0.051 ± 0.008	0.038–0.082	28
Lead (ppm)	0.144 ± 0.110	0.064–0.474	28
Mercury (ppm)	0.0115 ± 0.004	0.01–0.03	28
Selenium (ppm)	0.161 ± 0.034	0.029–0.242	28
Aflatoxins (ppb)[a]	<5.0	–	28
Nitrate Nitrogen (ppm)[b]	15.7 ± 5.98	10.0–35.1	28
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	28
BHA (ppm)[a,c]	<1.00	–	28
BHT (ppm)[a,c]	<1.00	–	28
Aerobic Plate Count (CFU/gm)	<10.0	–	28
Coliform (MPN/gm)	<3	–	28
E. coli (MPN/gm)	<10.0	–	28
Salmonella (MPN/gm)	Negative	–	28
Total Nitrosamines (ppb)[d]	10.5 ± 6.01	1.5–24.5	28
N-Ndimethylamine (ppb)[d]	2.2 ± 1.50	0–6.6	28
N-Npyrrolidine (ppb)[d]	8.3 ± 5.47	1.4–20.0	28

	Mean ± Standard Deviation	Range	Number of Samples
Pesticides (ppm)			
α-BHC[a]	<0.01	–	28
β-BHC[a]	<0.02	–	28
γ-BHC[a]	<0.01	–	28
δ-BHC[a]	<0.01	–	28
Heptachlor[a]	<0.01	–	28
Aldrin[a]	<0.01	–	28
Heptachlor Epoxide[a]	<0.01	–	28
DDE[a]	<0.01	–	28
DDD[a]	<0.01	–	28
DDT[a]	<0.01	–	28
HCB[a]	<0.01	–	28
Mirex[a]	<0.01	–	28
Methoxychlor[a]	<0.05	–	28
Dieldrin[a]	<0.01	–	28
Endrin[a]	<0.01	–	28
Telodrin[a]	<0.01	–	28
Chlordane[a]	<0.05	–	28
Toxaphene[a]	<0.10	–	28
Estimated PCBs[a]	<0.20	–	28
Ronnel[a]	<0.01	–	28
Ethion[a]	<0.02	–	28
Trithion[a]	<0.05	–	28
Diazinon[a]	<0.10	–	28
Methyl Chlorpyrifos	0.092 ± 0.075	0.2–0.315	28
Methyl Parathion[a]	<0.02	–	28
Ethyl Parathion[a]	<0.02	–	28
Malathion	0.071 ± 0.07	0.02–0.297	28
Endosulfan I[a]	<0.01	–	28
Endosulfan II[a]	<0.01	–	28
Endosulfane Sulfate[a]	<0.03	–	28

All samples were irradiated. BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.
[a]All values were below the detection limit. The detection limit is given as the mean.
[b]Sources of contamination include alfalfa, grains, and fish meal.
[c]Sources of contamination include soy oil and fish meal.
[d]All values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

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Tables

C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or exposed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals are from the same production source and weanling groups as the animals used for the studies of test compounds.

For these toxicology and carcinogenesis studies, blood samples were collected from each sentinel animal, allowed to clot, and the serum was separated. Additionally, fecal samples were collected and tested for endoparasites and *Helicobacte*r species. All samples were processed appropriately with serology and *Helicobacter* testing performed by IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri), Columbia, MO, for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed in-house by the testing laboratory.

The laboratory methods and agents for which testing was performed are tabulated in Table C-1, Table C-2 below; the times at which samples were collected during the studies are also listed.

C.2. Results

C.2.1. Perinatal and Postweaning Study (Study 1)

Rats: Positive for endoparasites, pinworms (*Syphacia* spp.). All other test results were negative.

C.2.2. Postweaning-only Study (Study 2)

Rats: Positive for endoparasites, pinworms (*Syphacia* spp.). All other test results were negative.

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Rats (Study 1)

Collection Time Points	Quarantine[a]	3.5 Weeks[b]	4 Weeks Post-Study Start[c]	6 Months	12 Months	18 Months	End of Study
Number Examined (Males/Females)	0/10	0/10	5/5	5/5	5/7[d]	5/6[e]	5/5
Method/Test							
Multiplex Fluorescent Immunoassay (MFI)							
Kilham rat virus (KRV)	–	–	–	–	–	–	–
Mycoplasma pulmonis	–	–	–	–	–	–	–
Parvo NS-1	–	–	–	–	–	–	–
Pneumonia virus of mice (PVM)	–	–	–	–	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–	–	–	–	–
Rat minute virus (RMV)	–	–	–	–	–	–	–
Rat parvo virus (RPV)	–	–	–	–	–	–	–
Rat theilovirus (RTV)	–	–	–	–	–	–	–
Sendai	–	–	–	–	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–	–	–	–	–
Toolan's H-1	–	–	–	–	–	–	–
Immunofluorescence Assay (IFA)							
Pneumocystis carinii	NT	NT	NT	–	NT	NT	NT
In-house Evaluation							
Endoparasite evaluation (evaluation of cecal content)	NT	NT	NT	NT	+	+	+
Ectoparasite evaluation (evaluation of perianal surface)	NT	NT	NT	NT	–	–	–

– = negative; + = positive; NT = not tested.

[a]Age-matched nonpregnant females.

[b]Time-mated females that did not have a litter; 3.5 weeks after arrival.

[c]F_1 sentinel animals tested 4 weeks after 2-year study start.

[d]Includes samples from two females euthanized as moribund.

[e]Includes samples from one female euthanized as moribund.

Table C-2. Methods and Results for Sentinel Animal Testing in Male and Female Rats (Study 2)

Collection Time Points	4 Weeks[a]	6 Months	12 Months	18 Months	End of Study
Number Examined (Males/Females)	5/5	5/5	5/5	5/5	5/5
Method/Test					
Multiplex Fluorescent Immunoassay (MFI)					
Kilham rat virus (KRV)	−	−	−	−	−
Mycoplasma pulmonis	−	−	−	−	−
Parvo NS-1	−	−	−	−	−
Pneumonia virus of mice (PVM)	−	−	−	−	−
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	−	−	−	−	−
Rat minute virus (RMV)	−	−	−	−	−
Rat parvo virus (RPV)	−	−	−	−	−
Rat theilovirus (RTV)	−	−	−	−	−
Sendai	−	−	−	−	−
Theiler's murine encephalomyelitis virus (TMEV)	−	−	−	−	−
Toolan's H-1	−	−	−	−	−
Immunofluorescence Assay (IFA)					
Kilham rat virus (KRV)	−	NT	NT	NT	NT
Pneumocystis carinii	NT	−	NT	NT	NT
Number Examined (Males/Females)	0/0	0/0	6/5	6/5	0/0
Method/Test					
In-house Evaluation					
Endoparasite evaluation (evaluation of cecal content)	NT	NT	+	+	NT
Ectoparasite evaluation (evaluation of perianal surface)	NT	NT	+	+	NT

− = negative; + = positive; NT = not tested.

[a]Postquarantine.

Appendix D. Genetic Toxicology

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Tables

D.1. Rodent Chromosome Aberrations Test

D.1.1. Methods

Female B6C3F1 mice (four animals per exposure group) were exposed to di(2-ethylhexyl) phthalate (DEHP) (0, 3,000, 6,000, or 12,000 ppm) in dosed feed for 14 days. The animals were subcutaneously implanted with a bromodeoxyuridine (BrdU) tablet[240] 18 hours before the scheduled harvest to allow selection of the appropriate cell population for scoring.[241; 242] Chromosomal aberrations induced by test article administration are present in maximum number at the first metaphase following exposure; they decline in number during subsequent nuclear divisions due to cell death. Two hours before sacrifice, the animals received an intraperitoneal injection of colchicine in saline. The animals were euthanized 18 hours after BrdU dosing. One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). Cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained (with a modified fluorescence-plus-Giemsa technique) and scored.

Fifty first-division metaphase cells were scored from each animal. Responses were evaluated as the percentage of aberrant metaphase cells, excluding gaps. The data were analyzed by a trend test[243] with $p \leq 0.025$ considered to be significant. Pairwise comparisons of each exposure group to the corresponding solvent control group were considered significant for $p \leq 0.025/3$ (the number of DEHP-exposed groups).

D.1.2. Results

In vivo, no significant increases were observed in chromosomal aberrations in bone marrow cells of female B6C3F1 mice following administration of DEHP (3,000–12,000 ppm) in dosed feed for 14 days (Table D-1).

Table D-1. Chromosomal Aberrations in Mice Exposed to Di(2-ethylhexyl) Phthalate in Feed for Fourteen Days[a,b]

	n	Percent Cells with Aberrations	P Value (Pairwise)
DEHP (ppm)			
0	4	1.25 ± 0.48	0.0000
3,000	4	2.75 ± 1.11	0.0649
6,000	4	0.50 ± 0.29	0.8726
12,000	4	1.50 ± 0.87	0.3807
Trend[c]		p = 0.6617	

DEHP = di(2-ethylhexyl) phthalate

[a]Data are presented as the mean frequency of aberrant cells ± standard error. Gaps were not included in the calculation of the mean frequency of chromosomally aberrant cells.

[b]Pairwise comparisons to the vehicle control group performed using a t-test ($p \leq 0.025$/number of exposed groups).

[c]Exposure-related trends evaluated using the Cochran-Armitage trend test ($p \leq 0.025$).

D.2. In Vivo Micronucleus Test

D.2.1. Methods

D.2.1.1. Bone Marrow

Female B6C3F1 mice (10 animals per exposure group) were exposed to DEHP (0, 3,000, 6,000, or 12,000 ppm) in dosed feed for 14 days. Bone marrow smears were prepared from cells obtained from the femurs as described for the chromosomal aberrations assay. Air-dried smears were fixed and stained with acridine orange; 1,000 polychromatic erythrocytes (PCEs) were scored per animal for the frequency of micronucleated cells.

The results were tabulated as the mean of the pooled results from all animals within an exposure group ± the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed for positive trend over the four exposure groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the concurrent control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. For a test to be considered positive, the trend test p value ≤ 0.025 is required, along with at least one significant exposure group ($p \leq 0.025$ divided by the number of exposed groups). Ultimately, the final call is determined by scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

D.2.1.2. Peripheral Blood

A detailed discussion of this assay is presented by MacGregor et al.[244] Two 26-week exposure protocols were used. In the first study, male and female TgAC (FVB/N) transgenic mice were exposed to DEHP (0, 1,500, 3,000, or 6,000 ppm) in dosed feed. In the second study, male and female TgAC (FVB/N) transgenic mice were exposed to DEHP (0, 100, 200, or 400 mg/kg) via dermal application. In both studies, at the end of the 26-week exposure period, peripheral blood samples were obtained. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 normochromatic erythrocytes (NCEs) and 1,000 PCEs per animal.

The results were tabulated as the mean of the pooled results from all animals within an exposure group ± the standard error of the mean. The frequency of micronucleated NCEs was analyzed as described above for the bone marrow micronucleus test.

D.2.1.3. Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and differing results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall

conclusion of chemical activity in an assay. For in vitro assays conducted with and without exogenous metabolic activation, results from each testing condition are evaluated and reported separately. The summary table in the abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

D.2.2. Results

DEHP was tested in three independent erythrocyte micronucleus tests and produced varying results (Table D-2, Table D-3, Table D-4). In an in vivo bone marrow micronucleus test, B6C3F1 female mice were exposed to DEHP (3,000–12,000 ppm) in dosed feed for 14 days; results were judged to be equivocal (Table D-2). In a second test, DEHP (1,500–6,000 ppm) induced an equivocal response in male TgAC (FVB/N) mice and a positive response in female TgAC (FVB/N) mice following exposure via dosed feed for 26 weeks (Table D-3). Another 26-week exposure test in TgAC (FVB/N) mice using dermal application of DEHP (100–400 mg/kg) produced negative results in both male and female mice (Table D-4).

Table D-2. Frequency of Micronuclei in the Bone Marrow of Female B6C3F1 Mice Exposed to Di(2-ethylhexyl) Phthalate in Feed for Fourteen Days

	Micronucleated PCEs/1,000 PCEs[a]	P Value[b]	Micronucleated NCEs/1,000 NCEs[a]	P Value[b]
n	10	10	10	10
DEHP (ppm)				
0	1.90 ± 0.43		0.70 ± 0.26	
3,000	1.20 ± 0.33	0.8958	0.90 ± 0.31	0.3085
6,000	1.10 ± 0.38	0.9281	1.50 ± 0.37	0.0440
12,000	1.50 ± 0.34	0.7538	2.00 ± 0.42	0.0061
Trend[c]	p = 0.6970		p = 0.0020	

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte; DEHP = di(2-ethylhexyl) phthalate.
[a]Data are presented as mean ± standard error.
[b]Pairwise comparisons to the vehicle control group performed using a t-test ($p \leq 0.025$/number of exposed groups).
[c]Exposure-related trends evaluated by the Cochran-Armitage trend test ($p \leq 0.025$).

Table D-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female TgAC (FVB/N) Mice Exposed to Di(2-ethylhexyl) Phthalate in Feed for Twenty-six Weeks

	Micronucleated PCEs/1,000 PCEs[a]	n	P Value[b]	Micronucleated NCEs/1,000 NCEs[a]	n	P Value[b]	PCEs (%)[a]
Male							
DEHP (ppm)							
0	2.00 ± 0.39	12		2.83 ± 0.44	12		3.46 ± 0.16
1,500	–	–		2.18 ± 0.40	11	0.7975	–
3,000	–	–		3.00 ± 0.58	13	0.4182	–
6,000	3.67 ± 0.76	9	0.0108	4.33 ± 1.17	9	0.0610	3.10 ± 0.14
Trend[c]	p = 0.0110			p = 0.0260			
Female							
DEHP (ppm)							
0	2.50 ± 0.56	10		1.40 ± 0.27	10		3.47 ± 0.63
1,500	–	–		2.31 ± 0.38	13	0.0592	–
3,000	–	–		1.50 ± 0.43	6	0.4358	–
6,000	1.27 ± 0.33	11	0.9804	3.27 ± 0.51	11	0.0027	3.18 ± 0.18
Trend[c]	p = 0.9800			p = 0.0040			

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte; DEHP = di(2-ethylhexyl) phthalate.
[a]Data are presented as mean ± standard error.
[b]Pairwise comparisons to the vehicle control group performed using a t-test ($p \leq 0.025$/number of exposed groups).
[c]Exposure-related trends evaluated by the Cochran-Armitage trend test ($p \leq 0.025$).

Table D-4. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female TgAC (FVB/N) Mice Following Dermal Application of Di(2-ethylhexyl) Phthalate for Twenty-six Weeks

	Micronucleated PCEs/1,000 PCEs[a]	n	P Value[b]	Micronucleated NCEs/1,000 NCEs[a]	n	P Value[b]	PCEs (%)[a]
Male							
DEHP (mg/kg)							
0	2.45 ± 0.51	11		3.45 ± 0.59	11		4.20 ± 0.34
100	–	–		3.17 ± 0.34	12	0.6480	–
200	–	–		3.36 ± 0.37	14	0.5522	–
400	3.86 ± 0.67	7	0.0467	2.71 ± 0.68	7	0.8056	3.21 ± 0.28
Trend[c]	p = 0.0470			p = 0.7700			
Female							
DEHP (mg/kg)							
0	2.55 ± 0.47	11		1.64 ± 0.36	11		4.86 ± 0.34
100	–	–		1.91 ± 0.41	11	0.3153	–
200	–	–		2.36 ± 0.49	11	0.1137	–
400	4.09 ± 0.56	11	0.0231	2.55 ± 0.45	11	0.0700	4.24 ± 0.19
Trend[c]	p = 0.0230			p = 0.0590			

PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte; DEHP = di(2-ethylhexyl) phthalate.
[a]Data are presented as mean ± standard error.
[b]Pairwise comparisons to the vehicle control group performed using a t-test ($p \leq 0.025$/number of exposed groups).
[c]Exposure-related trends evaluated by the Cochran-Armitage trend test ($p \leq 0.025$)

Appendix E. Mono(2-ethylhexyl) Phthalate Internal Dose Assessment

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E.1. Sample Collection

Select dams and their litters were removed on gestation day (GD) 18 to quantify mono(2-ethylhexyl) phthalate (MEHP) plasma and tissue concentrations. On GD 18, blood was collected from the retroorbital sinus of randomly selected dams (n = 5 per exposure group). Blood samples were collected in tubes containing K_3 EDTA (tripotassium ethylene diamine tetraacetic acid), centrifuged, and the plasma harvested. Amniotic fluid was collected and pooled by dam, and each dam's fetuses were collected and pooled by litter. All samples were flash frozen in liquid nitrogen and stored frozen at approximately −20°C before shipment to RTI International (Research Triangle Park, NC) for analysis.

E.2. Sample Analysis

MEHP, a metabolite of di(2-ethylhexyl) phthalate, was measured in dam plasma, amniotic fluid, and fetal homogenate using a validated analytical method. The analyte stability in each matrix was also confirmed; corresponding data are given in Table E-1.

MEHP stock solutions were prepared in methanol at 0.5 mg/mL and diluted in water to prepare working standard solutions. A working internal standard (deuterated MEHP ($[^2H_4]$MEHP), C/D/N Isotopes Inc., Pointe-Claire, Canada) solution was prepared similarly at 1 µg/mL.

Plasma calibration standards were prepared at seven concentrations (25 to 5,000 ng/mL) by spiking 25 µL of plasma with an appropriate concentration of working MEHP standards. Plasma, amniotic fluid, and fetal homogenate quality control (QC) standards were prepared similarly at 100 and 2,500 ng/mL. Fetal homogenates were prepared by homogenizing fetuses in deionized water (1 g fetus in 3 mL water, equivalent to 250 mg/mL homogenate). Matrix blanks, method blanks, and study samples were prepared similarly to the matrix standards above but using 25 µL of water in place of the MEHP working standard solution. To all samples, 25 µL of internal standard solution and 425 µL acetonitrile were added, vortexed, and centrifuged at approximately 8,000 g for 10 minutes at 4°C. The supernatant was transferred to clean vials for analysis. Study samples with responses greater than the highest calibration standard were diluted with corresponding extracted blank matrix to the validated range prior to analysis.

E.3. Instrumentation and Quantitation

All samples were analyzed by ultra-performance liquid chromatography (UPLC) tandem mass spectrometry (MS/MS) using Waters ACQUITY UPLC (Milford, MA) coupled to an Applied Biosystems 4000 QTRAP (Sciex, Framingham, MA) mass spectrometer. Chromatography was performed using Waters ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.8 µm). Mobile phases A (water) and B (acetonitrile) were run with a linear gradient from 20% B to 95% B in 4.5 minutes at a flow rate of 0.5 mL/minute. The electrospray ion source was operated in negative ion mode with a voltage of −4,500 volts and source temperature of 450°C. Transitions monitored for MEHP and $[^1H_4]$ were 277.1 → 133.8 and 281.0 → 137.8, respectively.

Calibration curves relating the response ratio of analyte to internal standard and the concentration of MEHP in a matrix were constructed using a $1/X^2$ weighted linear regression. The concentrations of MEHP in the samples were calculated using response ratio, the regression

equation, initial sample weight or volume, and dilution when applicable. The concentration was reported as ng/mL for plasma and amniotic fluid. The fetal homogenate concentration estimated in ng/mL of fetal homogenate was converted to ng/g fetus by using a conversion factor of 4 (i.e., 4 mL homogenate = 1 g of fetus).

The performance of the calibration curve was evaluated before the analysis of each sample set. A successful calibration was indicated by the following: correlation coefficient (r) ≥0.99; relative standard deviation (RSD) less than or equal to ±15% (except at the limit of quantitation [LOQ] where RSD is less than or equal to ±20%); relative error (RE) less than or equal to ±15% (except at the LOQ where RE is less than or equal to ±20%). Data from study samples were considered valid if they were bracketed by valid QC sets. In general, each sample set, method blanks, and controls were bracketed by two QC sets, which consisted of a calibration blank and two concentrations of calibration standards (QC low and QC high). A QC set passed when the measured concentration for QC standards was within 15% of its nominal value. If the QC standard failed, it was necessary to reanalyze the bracketed samples. Correlation coefficient, r, for all calibration curves was ≥0.99. All QC standards were within 15% of nominal concentrations for plasma, amniotic fluid, and fetal homogenates.

Low concentrations of MEHP were observed in plasma, amniotic fluid, and fetal homogenates used as blank matrices, some of which were attributed to background contributions from the reagents and vials used in the assay. The background contribution from the reagents/vials did not affect the quantitation of study samples because matrix calibration standards and study samples were prepared and quantified similarly. In general, the background concentrations estimated in amniotic fluid and fetal homogenates were slightly higher than those observed in plasma, although the reason is not clear.

Table E-1. Analytical Method Validation and Stability Data for Mono(2-ethylhexyl) Phthalate in Plasma, Amniotic Fluid, and Fetal Homogenate[a]

Validation Parameter	Dam Plasma	Amniotic Fluid	Fetal Homogenate
Matrix Concentration Range (ng/mL)	25–5,000	–	–
LOQ (ng/mL or ng/g)	25.0	50.0	200
LOD[b] (µg/g)	5.2	12.0	10
Correlation Coefficient (r)	≥0.998	–	–
Selectivity (%)[c]	40	53	54
Recovery (%)[d]	95.0–102.0	–	–
Precision and Accuracy[e,f]			
Intra-day % RSD	≤4.1	≤4.2	≤7.1
Intra-day % RE	≤ ± 2.9	≤ ± 4.7	≤ ± 5.6
Inter-day % RSD	≤5.2	–	–
Inter-day % RE	≤ ± 5.8	–	–
Dilution Verification (50,000 ng/mL)			
% RSD	0.8	–	–
% RE	1.7	–	–
Extracted Sample Storage Stability (% of Day 0)[f]			
Ambient	102–105	–	–
Refrigerator	97–107	–	–
Freeze-thaw (3 cycles)	103–106	–	–
Matrix Storage Stability (% of Day 0)[g]	80–113	80–115	84–113

LOQ = limit of quantitation; LOD = limit of detection; RSD = relative standard deviation; RE = relative error.

[a]Method was fully validated in Harlan Sprague Dawley rat plasma and assessed in amniotic fluid and fetal homogenate using quality control samples prepared in each matrix at three concentrations (100, 500, and 2,500 ng/g).

[b]Estimated as the standard error of LOQ.

[c]Estimated using six replicate blank matrix response relative to LOQ response.

[d]Estimated by comparing response of matrix standards to solvent standards over the concentration range 25 to 5,000 ng/mL.

[e]Precision was estimated as % RSD. Accuracy was estimated as % RE.

[f]Determined for three replicate quality controls at three levels (100, 500, and 2,500 ng/mL) for up to 3 days.

[g]Determined for three replicate quality controls at two levels (100 and 3,750 ng/mL) stored at −20°C for up to 62 days.

Appendix F. Benchmark Dose Analysis

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F.1. Methods

Benchmark doses (BMDs) were calculated using the EPA Benchmark Dose Software (BMDS), version 3.1.2.[169] The dose variable for the models was the amount of di(2-ethylhexyl) phthalate (DEHP) consumed/kg body weight/day (mg/kg/day). Numbers of animals per exposure group were poly-3-adjusted survival numbers. The response variable was the incidence of the endpoint being modeled.

All of the frequentist dichotomous models in the BMDS were used. The logistic, log-probit, and probit models were used with no parameter restrictions. Other models (dichotomous Hill, gamma, log-logistic, multistage, and Weibull) were used with default restrictions on the ranges of some of the parameters, as described in the BMDS User Guide.[170]

The benchmark response (BMR) used in the models was 0.1 (10%) extra risk, with estimated background levels. The benchmark dose lower confidence limit ($BMDL_{10}$) was calculated using a 95% confidence interval. The decision logic used to recommend one model from the fitted models was the default logic.[170]

F.2. Results

F.2.1. Hepatocellular Adenoma or Carcinoma (Combined; Male Rats)

F.2.1.1. Perinatal and Postweaning Study

All models provided an adequate fit to the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and by visual inspection of the respective plots of observed versus predicted values from the various models (Table F-1; Figure F-1). The dichotomous Hill, logistic, and probit models provided similar fits to the data. The probit model was judged to provide the best model fit based on the lowest Akaike information criterion (AIC) value. The BMDS dichotomous results for the probit and other models are available as supplemental data (Appendix H).

F.2.1.2. Postweaning-only Study

All models except for the dichotomous Hill and multistage degree 1 models provided an adequate fit to the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and by visual inspection of the respective plots of observed versus predicted values from the various models (Table F-1; Figure F-1). The multistage degree 4 and multistage degree 3 models provided similar fits to the data. The multistage degree 4 model was judged to provide the best model fit based on the lowest AIC value. The BMDS dichotomous results for the multistage degree 4 and other models are available as supplemental data (Appendix H).

F.2.2. Hepatocellular Adenoma or Carcinoma (Combined; Female Rats)

F.2.2.1. Perinatal and Postweaning Study

All models except for the logistic and probit models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and by visual inspection of the respective plots of observed versus predicted values from the various models (Table F-2; Figure F-2). The log-logistic and log-probit models provided similar fits of the data. The log-logistic model was

judged to provide the best model fit based on the lowest AIC value. The BMDS dichotomous results for the log-logistic and other models are available as supplemental data (Appendix H).

F.2.2.2. Postweaning-only Study

All models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and by visual inspection of the respective plots of observed versus predicted values from the various models (Table F-2; Figure F-2). The multistage degree 4, multistage degree 2, logistic, and probit models provided similar fits of the data. The multistage degree 4 model was judged to provide the best model fit based on the lowest AIC value. The BMDS dichotomous results for the multistage degree 4 and other models are available as supplemental data (Appendix H).

F.2.3. Pancreatic Acinar Adenoma or Carcinoma (Combined; Male Rats)

F.2.3.1. Perinatal and Postweaning Study

All models had poor goodness of fit ($p < 0.1$). In addition, all models other than dichotomous Hill, log-logistic, and log-probit had high residuals near the BMD_{10}. Of the models without high residuals near the BMD_{10}, the dichotomous Hill model had the best fit based on AIC and was the only model with chi-square p value > 0.0001 (Table F-3; Figure F-3). The dichotomous Hill model provided the best model fit based on the highest chi-square p value and lowest AIC value. The BMDS dichotomous results for the dichotomous Hill model and other models are available as supplemental data (Appendix H).

F.2.3.2. Postweaning-only Study

Only dichotomous Hill, log-logistic, and log-probit models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models (Table F-3; Figure F-3). The dichotomous Hill, log-logistic, and log-probit models provided similar fits of the data. The log-logistic model provided the best model fit based on the highest chi-square p value and lowest AIC value. The BMDS dichotomous results for the log-logistic model and other models are available as supplemental data (Appendix H).

F.2.4. Uterine (Including Cervix) Adenocarcinoma, Adenoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined; Female Rats)

F.2.4.1. Perinatal and Postweaning Study

All models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models (Table F-4; Figure F-4). The multistage degree 1, logistic, and probit models provided similar fits of the data. The logistic model provided the best model fit based on the lowest AIC value. The BMDS dichotomous results for the logistic and other models are available as supplemental data (Appendix H).

F.2.4.2. Postweaning-only Study

All models provided adequate fits of the data as assessed by a chi-square goodness-of-fit test ($p \geq 0.1$) and visual inspection of the respective plots of observed versus predicted values from the various models (Table F-4; Figure F-4). The multistage degree 1, logistic, and probit models

provided similar fits of the data. The probit model provided the best model fit based on the lowest AIC value. The BMDS dichotomous results for the probit and other models are available as supplemental data (Appendix H).

Table F-1. Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma (Combined) in Male Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

Model	Chi-square P Value[a]	AIC	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	BMDS Recommendation[b]
Perinatal and Postweaning Study (Study 1)					
Dichotomous Hill	0.55	92.9	199.5	168.2	Viable–alternate
Gamma	0.48	93.4	328.0	199.7	Viable–alternate
Log-logistic	0.48	93.4	326.1	194.6	Viable–alternate
Multistage Degree 4	0.45	93.6	356.5	198.5	Viable–alternate
Multistage Degree 3	0.45	93.6	356.5	198.5	Viable–alternate
Multistage Degree 2	0.45	93.6	356.5	198.5	Viable–alternate
Multistage Degree 1	0.38	93.6	249.9	161.3	Viable–alternate
Weibull	0.47	93.5	336.2	198.8	Viable–alternate
Logistic	0.61	92.0	413.6	335.9	Viable–alternate
Log-probit	0.52	93.2	302.0	188.6	Viable–alternate
Probit	**0.63**	**91.8**	**382.9**	**306.1**	**Viable–recommended**
Postweaning-only Study (Study 2)					
Dichotomous Hill	0.04	91.6	223.1	177.2	Questionable
Gamma	0.14	89.6	416.2	262.1	Viable–alternate
Log-logistic	0.14	89.6	428.0	259.7	Viable–alternate
Multistage Degree 4	**0.26**	**87.6**	**434.4**	**263.5**	**Viable–recommended**
Multistage Degree 3	0.26	87.61	434.4	263.5	Viable–alternate
Multistage Degree 2	0.19	88.0	375.0	248.1	Viable–alternate
Multistage Degree 1	0.07	91.0	273.0	173.8	Questionable
Weibull	0.14	89.6	436.3	263.0	Viable–alternate
Logistic	0.18	87.9	421.5	349.6	Viable–alternate
Log-probit	0.13	89.6	393.9	250.8	Viable–alternate
Probit	0.15	88.1	395.2	321.4	Viable–alternate

AIC = Akaike information criterion; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk; BMDS = Benchmark Dose Software.

[a]Chi-square p value = p value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

[b]Bold text indicates the model selected for each response.

Table F-2. Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma (Combined) in Female Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

Model	Chi-square P Value[a]	AIC	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	BMDS Recommendation[b]
Perinatal and Postweaning Study (Study 1)					
Dichotomous Hill	0.14	143.1	77.6	41.5	Viable–alternate
Gamma	0.14	142.7	153.7	105.9	Viable–alternate
Log-logistic	**0.24**	**141.5**	**122.9**	**79.7**	**Viable–recommended**
Multistage Degree 4	0.14	142.7	153.7	105.9	Viable–alternate
Multistage Degree 3	0.14	142.7	153.7	105.9	Viable–alternate
Multistage Degree 2	0.14	142.7	153.7	105.9	Viable–alternate
Multistage Degree 1	0.14	142.7	153.7	105.9	Viable–alternate
Weibull	0.14	142.7	153.7	105.9	Viable–alternate
Logistic	0.02	148.2	348.5	274.2	Questionable
Log-probit	0.27	142.1	92.0	41.5	Viable–alternate
Probit	0.03	147.6	321.7	251.3	Questionable
Postweaning-only Study (Study 2)					
Dichotomous Hill	0.34	79.9	277.3	180.7	Viable–alternate
Gamma	0.36	79.9	275.7	183.2	Viable–alternate
Log-logistic	0.34	79.9	277.3	180.7	Viable–alternate
Multistage Degree 4	**0.81**	**77.0**	**383.6**	**208.0**	**Viable–recommended**
Multistage Degree 3	0.58	79.1	348.2	205.4	Viable–alternate
Multistage Degree 2	0.69	77.5	302.0	197.2	Viable–alternate
Multistage Degree 1	0.49	78.6	217.2	147.6	Viable–alternate
Weibull	0.38	79.8	286.6	186.7	Viable–alternate
Logistic	0.69	77.9	428.7	354.6	Viable–alternate
Log-probit	0.36	80.2	338.5	168.0	Viable–alternate
Probit	0.69	77.7	392.9	321.3	Viable–alternate

AIC = Akaike information criterion; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk; BMDS = Benchmark Dose Software.

[a]Chi-square p value = p value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

[b]Bold text indicates the model selected for each response.

Table F-3. Benchmark Dose Modeling Results for Pancreatic Acinar Adenoma or Carcinoma (Combined) in Male Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

Model	Chi-square P Value[a]	AIC	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	BMDS Recommendation[b]
Perinatal and Postweaning Study (Study 1)					
Dichotomous Hill	0.08	227.8	85.9	56.8	**Questionable**
Gamma	<0.0001	257.0	52.9	37.9	Questionable
Log-logistic	<0.0001	252.2	28.2	17.7	Questionable
Multistage Degree 4	<0.0001	257.0	52.9	37.9	Questionable
Multistage Degree 3	<0.0001	257.0	52.9	37.9	Questionable
Multistage Degree 2	<0.0001	257.0	52.9	37.9	Questionable
Multistage Degree 1	<0.0001	257.0	52.9	37.9	Questionable
Weibull	<0.0001	257.0	52.9	37.9	Questionable
Logistic	<0.0001	264.6	110.6	84.1	Questionable
Log-probit	<0.0001	251.5	33.1	13.5	Questionable
Probit	<0.0001	264.6	110.9	86.3	Questionable
Postweaning-only Study (Study 2)					
Dichotomous Hill	0.11	205.1	70.2	20.4	Viable–alternate
Gamma	0.07	205.3	44.7	35.0	Questionable
Log-logistic	**0.13**	**204.6**	**31.0**	**20.2**	**Viable–recommended**
Multistage Degree 4	0.07	205.3	44.7	35.0	Questionable
Multistage Degree 3	0.07	205.3	44.7	35.0	Questionable
Multistage Degree 2	0.07	205.3	44.7	35.0	Questionable
Multistage Degree 1	0.07	205.3	44.7	35.0	Questionable
Weibull	0.07	205.3	44.7	35.0	Questionable
Logistic	0.00	218.6	122.5	100.6	Questionable
Log-probit	0.11	204.9	32.6	15.3	Viable–alternate
Probit	0.00	217.6	115.8	96.9	Questionable

AIC = Akaike information criterion; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk; BMDS = Benchmark Dose Software.

[a]Chi-square p value = p value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

[b]Bold text indicates the model selected for each response.

Table F-4. Benchmark Dose Modeling Results for Uterine (Including Cervix) Adenocarcinoma, Adenoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined) in Female Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

Model	Chi-square P Value[a]	AIC	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	BMDS Recommendation[b]
Perinatal and Postweaning Study (Study 1)					
Dichotomous Hill	0.21	107.8	224.7	169.7	Viable–alternate
Gamma	0.42	106.0	560.0	289.2	Viable–alternate
Log-logistic	0.42	106.0	558.7	276.0	Viable–alternate
Multistage Degree 4	0.41	106.0	594.6	286.7	Viable–alternate
Multistage Degree 3	0.41	106.0	594.6	286.7	Viable–alternate
Multistage Degree 2	0.41	106.0	594.6	286.7	Viable–alternate
Multistage Degree 1	0.52	104.4	520.3	275.4	Viable–alternate
Weibull	0.42	106.0	566.6	288.7	Viable–alternate
Logistic	**0.60**	**104.1**	**594.2**	**432.2**	**Viable–recommended**
Log-probit	0.43	105.9	524.7	254.6	Viable–alternate
Probit	0.60	104.10	578.3	405.4	Viable–alternate
Postweaning-only Study (Study 2)					
Dichotomous Hill	0.33	147.6	180.6	116.6	Viable–alternate
Gamma	0.25	148.4	273.3	144.1	Viable–alternate
Log-logistic	0.25	148.4	267.5	127.5	Viable–alternate
Multistage Degree 4	0.25	148.5	272.6	143.1	Viable–alternate
Multistage Degree 3	0.25	148.5	272.9	143.1	Viable–alternate
Multistage Degree 2	0.25	148.5	272.6	143.1	Viable–alternate
Multistage Degree 1	0.41	146.6	224.4	141.7	Viable–alternate
Weibull	0.25	148.4	272.6	143.9	Viable–alternate
Logistic	0.39	146.7	344.0	268.9	Viable–alternate
Log-probit	0.26	148.2	260.3	96.7	Viable–alternate
Probit	**0.41**	**146.6**	**324.1**	**249.0**	**Viable–recommended**

AIC = Akaike information criterion; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk; BMDS = Benchmark Dose Software.

[a]Chi-square p value = p value from the chi-square test for lack of fit. Values <0.1 fail to meet conventional goodness-of-fit criteria.

[b]Bold text indicates the model selected for each response.

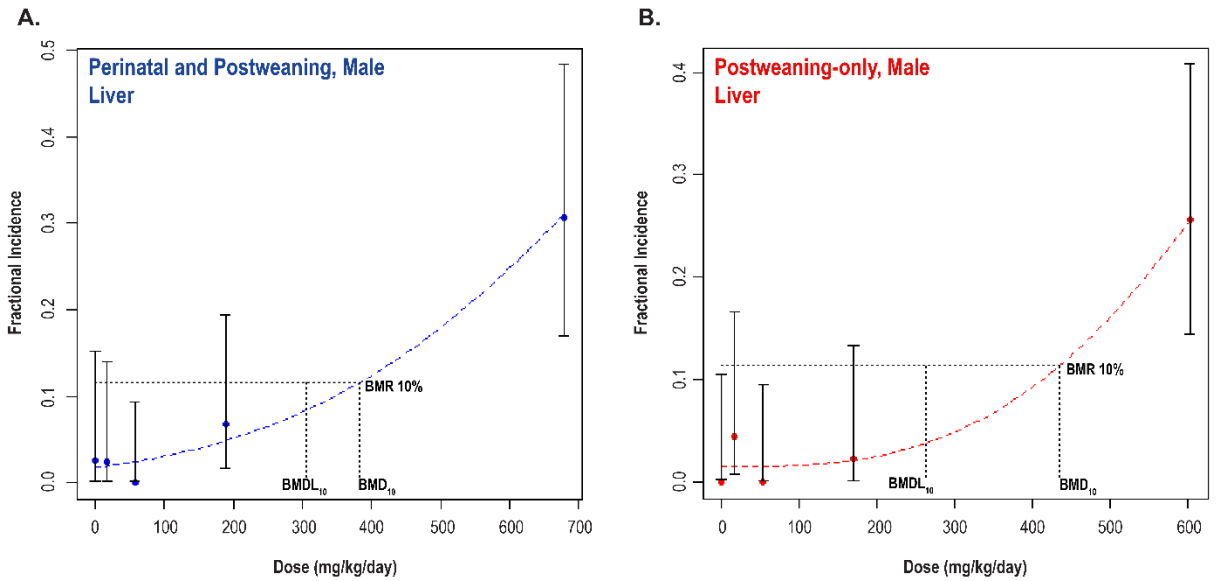

Figure F-1. Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma (Combined) in Male Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

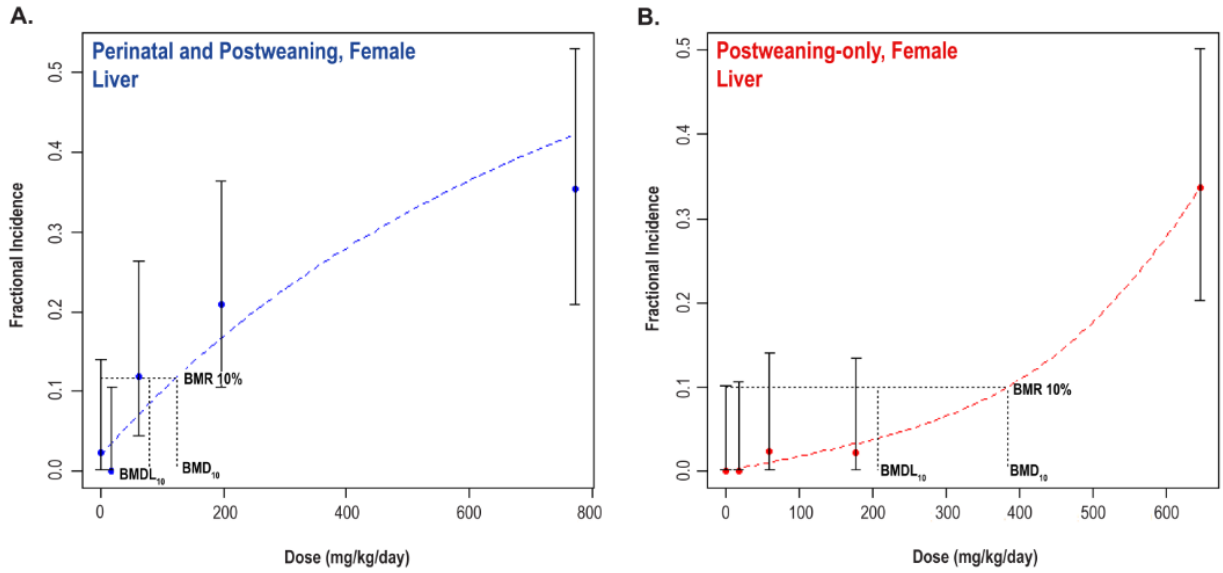

Figure F-2. Benchmark Dose Modeling Results for Hepatocellular Adenoma or Carcinoma (Combined) in Female Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

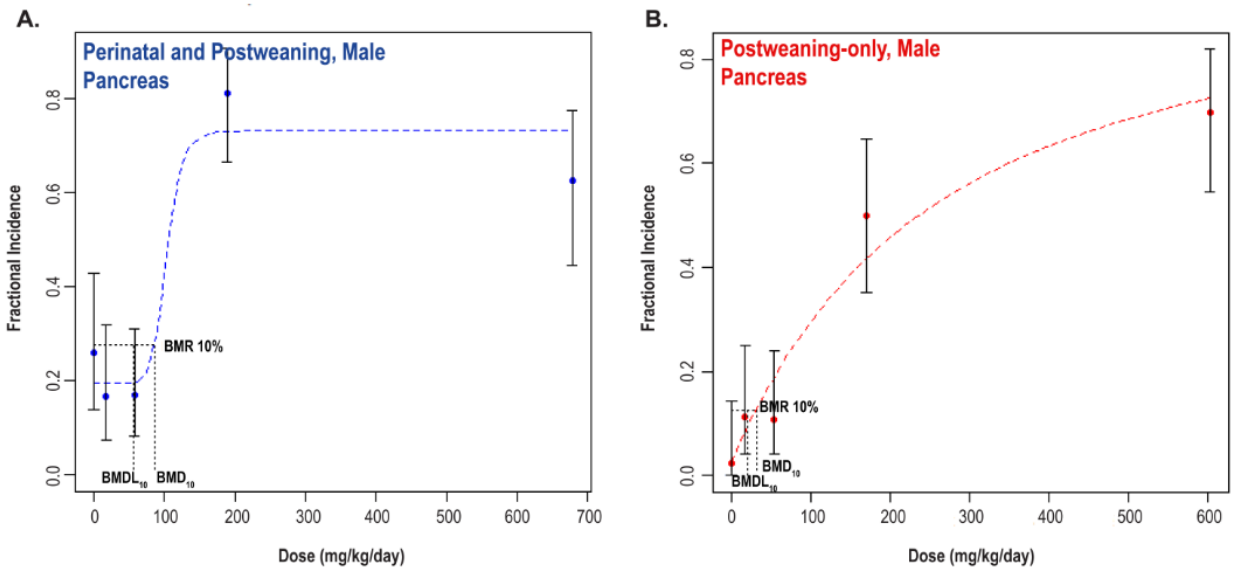

Figure F-3. Benchmark Dose Modeling Results for Pancreatic Adenoma or Carcinoma (Combined) in Male Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

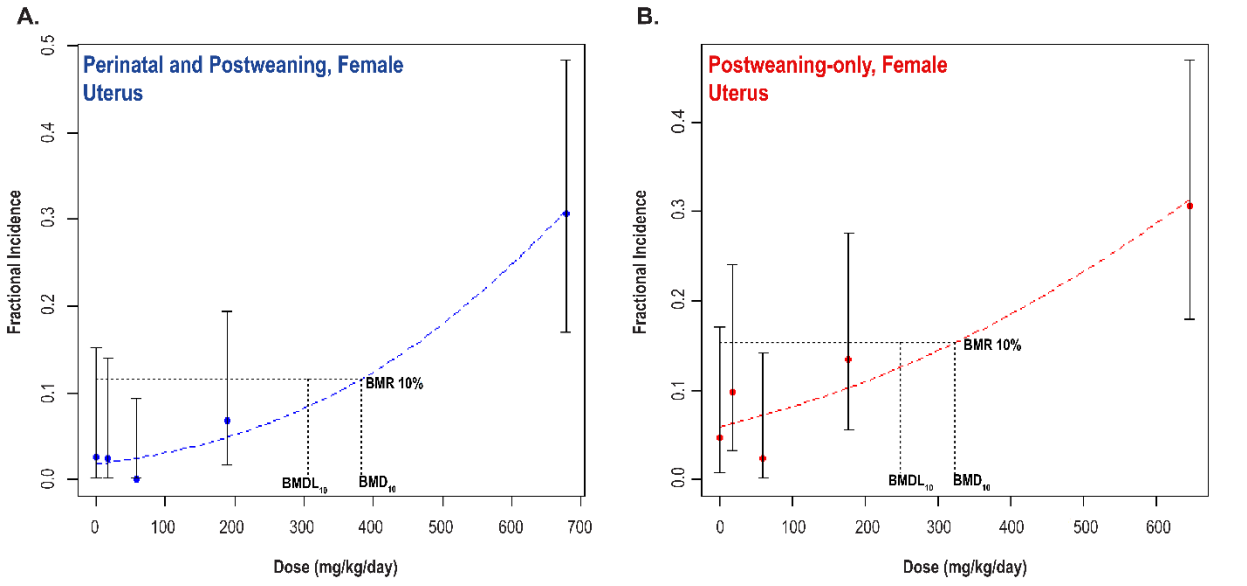

Figure F-4. Benchmark Dose Modeling Results for Uterine (Including Cervix) Adenocarcinoma, Adenoma, Squamous Cell Carcinoma, or Squamous Cell Papilloma (Combined) in Female Rats in the Two-year Feed Studies of Di(2-ethylhexyl) Phthalate

BMR = benchmark response; BMD_{10} = benchmark dose corresponding to a 10% extra risk; $BMDL_{10}$ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk.

Appendix G. Peer-review Report

Note: The peer-review report will appear in a future draft of this report.

Appendix H. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TR-601.[202]

H.1. Perinatal and Postweaning Study in Rats (Study 1)

H.1.1. Data Tables

E01 – Animal Removal Summary by Treatment Group

E02 – Animals Removed from Experiment

E03 – Growth Curves

E04 – Mean Body Weights and Survival Table

E05 – Clinical Observations Summary

E08 – Feed Water and Compound Consumption Table

Gestational Body Weights

Gestational Food Consumption

Gestational and Lactational Chemical Consumption

Lactational Body Weights

Lactational Food Consumption

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

P04 – Neoplasms by Individual Animal

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

P08 – Statistical Analysis of Primary Tumors

P09 – Non-Neoplastic Lesions by Individual Animal

P10 – Statistical Analysis of Non-Neoplastic Lesions – Litter based

P11 – Statistical Analysis of Survival Data

P14 – Individual Animal Pathology Data

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

P22 – Cause of Death Summary

P40 – Survival Curves

PA46Rs – Summary of Gross Pathology with Litter Incidence

PA48 – Summary of Tissue Concentration

PND 1 Litter Data

PND 4 Litter Size and Survival Data

Pup Body Weights

R02 – Reproductive Performance Summary

R23 – Gubernaculum Length Summary

H.1.2. Individual Animal Data

Female Individual Animal Body Weight Data All Animals

Female Individual Animal Body Weight Data All Animals – Lactation

Female Individual Animal Body Weight Data All Animals – Female Pups

Female Individual Animal Body Weight Data All Animals – Gestation

Female Individual Animal Clinical Observations

Female Individual Animal Neoplastic Pathology Data

Female Individual Animal Non-Neoplastic Pathology Data

Female Individual Animal Survival Data

Female Individual Animal Survival Data – Female Pups

Female Individual Animal Survival Data – Gestation

Female Individual Animal Survival Data – Lactation

Female Individual Animal Terminal Body Weight Data

Female Individual Animal Terminal Body Weight Data – Female Pups

Female Individual Animal Terminal Body Weight Data – Gestation

Female Individual Animal Terminal Body Weight Data – Lactation

Male Individual Animal Body Weight Data All Animals

Male Individual Animal Body Weight Data All Animals – Male Pups

Male Individual Animal Clinical Observations

Male Individual Animal Neoplastic Pathology Data

Male Individual Animal Non-Neoplastic Pathology Data
Male Individual Animal Survival Data
Male Individual Animal Survival Data - Male Pups
Male Individual Animal Terminal Body Weight Data
Male Individual Animal Terminal Body Weight Data – Male Pups
Gubernaculum_and_Urogenital_Findings_Data
Individual Animal Clinical Observations Data
Individual Animal DamID and PupID Data
Individual Animal Reproductive Performance Data
Individual Animal Tissue Concentration Data
Individual Pup Census and Litter Weight by Sex Data

H.2. Postweaning-only Study in Rats (Study 2)

H.2.1. Data Tables

E01 – Animal Removal Summary by Treatment Group
E02 – Animals Removed from Experiment
E03 – Growth Curves
E04 – Mean Body Weights and Survival Table
E05 – Clinical Observations Summary
E08 – Feed Water and Compound Consumption Table
P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site
P04 – Neoplasms by Individual Animal
P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)
P08 – Statistical Analysis of Primary Tumors
P09 – Non-Neoplastic Lesions by Individual Animal
P10 – Statistical Analysis of Non-Neoplastic Lesions
P11 – Statistical Analysis of Survival Data
P14 – Individual Animal Pathology Data
P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

P22 – Cause of Death Summary

P40 – Survival Curves

H.2.2. Individual Animal Data

Female Individual Animal Body Weight Data All Animals

Female Individual Animal Clinical Observations

Female Individual Animal Neoplastic Pathology Data

Female Individual Animal Non-Neoplastic Pathology Data

Female Individual Animal Survival Data

Female Individual Animal Terminal Body Weight Data

Male Individual Animal Body Weight Data All Animals

Male Individual Animal Clinical Observations

Male Individual Animal Neoplastic Pathology Data

Male Individual Animal Non-Neoplastic Pathology Data

Male Individual Animal Survival Data

Male Individual Animal Terminal Body Weight Data

H.3. Benchmark Dose Analysis

H.3.1. Results Summary

BMD Results Summary

H.3.2. Perinatal and Postweaning Study in Rats (Study 1)

Female Liver BMD_analysis

Female Pancreas BMD_analysis

Female Uterus BMD_analysis

Male Liver BMD_analysis

Male Pancreas BMD_analysis

Male Testes BMD_analysis

H.3.3. Postweaning-only Study in Rats (Study 2)

Female Liver BMD_analysis

Female Pancreas BMD_analysis

Female Uterus BMD_analysis

Male Liver BMD_analysis

Male Pancreas BMD_analysis

Male Testes BMD_analysis

H.4. Genetic Toxicology

H.4.1. In Vivo Peripheral Blood Micronucleus Study A04384 (Dosed Feed)

G04 – In Vivo Micronucleus Summary Data

Individual Animal In Vivo Micronucleus Data

H.4.2. In Vivo Peripheral Blood Micronucleus Study A15851 (Dermal)

G04 – In Vivo Micronucleus Summary Data

Individual Animal In Vivo Micronucleus Data

H.4.3. Rodent Chromosome Aberrations Study A15927 (Dosed Feed)

Data can be found here https://manticore.niehs.nih.gov/cebssearch/genetox/002-01969-0036-0000-6/

H.4.4. In Vivo Bone Marrow Micronucleus Study A15927 (Dosed Feed)

G04 – In Vivo Micronucleus Summary Data

Individual Animal In Vivo Micronucleus Data