U.S. Army Public Health Center

Wildlife Toxicity Assessment for Diethyl Phthalate (DEP)

No: HEF-042019-007

Toxicology Directorate, Health Effects Research Division

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April 2020

APHC

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
10/26/2020	Technical Report	September 2018 - April 2020

4. TITLE AND SUBTITLE
Wildlife Toxicity Assessment for Diethyl Phthalate

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Aberdeen Proving Ground, MD 21010-5403

8. PERFORMING ORGANIZATION REPORT NUMBER
No: HEF-042019-007

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Environmental Command
Acquisition and Technology
2450 Connell Road, Bldg 2264
JBSA Fort Sam Houston TX 78234

10. SPONSOR/MONITOR'S ACRONYM(S)
AEC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution A: Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Diethyl Phthalate or DEP is used as a component in insecticide and mosquito repellents, solvents, adhesives, waxes, inks, and as a camphor substitute. DEP is also used as a plasticizer in solid rocket propellants. This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of DEP on wildlife. Evaluating the toxicity of DEP will contribute to the derivation of toxicity reference values (TRVs) for use as screening-level benchmarks for wildlife near contaminated sites. The protocol for the performance of this WTA is available in detail in Technical Guide No. 254 (Standard Practice for Wildlife Toxicity Reference Values).

15. SUBJECT TERMS
Environmental; Diethyl Phthalate, Toxicity Assessment; Toxicology

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Mark A. Williams
U	U	U	SAR	41	19b. TELEPHONE NUMBER *(Include area code)*

Acknowledgements

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When referencing this document, please use the following citation:
APHC. 2020. WTA No. HEF-042019-007 for Wildlife Toxicity Assessment for Diethyl Phthalate, Aberdeen Proving Ground, Maryland.

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WILDLIFE TOXICITY ASSESSMENT FOR DIETHYL PHTHALATE (DEP)

TABLE OF CONTENTS

Page

U.S. Army Public Health Center
Department of the Army

Wildlife Toxicity Assessment for Diethyl Phthalate (DEP)

CAS No. 84-66-2 April 2020

1. INTRODUCTION

This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of diethyl phthalate (DEP) on wildlife. The evaluation of the toxicity of DEP contributed to the derivation of toxicity reference values (TRVs) for use in assessing potential health effects for wildlife near contaminated sites. The U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) Technical Guide (TG) 254, *Standard Practice for Wildlife Toxicity Reference Values* USACHPPM, 2000 documents the protocol for the performance of this assessment. Any derived TRVs in this document are intended for use by toxicologists and risk assessors to develop environmental health risk management strategies. The TRVs developed throughout this document represent the likelihood of toxic effects in individual organisms that have population-level effects, but do not extend to demographic rates for specific populations. Relevant studies used for TRV derivation have several key features: (1) any toxic effects identified are linked to potential population-level effects; (2) exposure duration is clearly identified; (3) effect levels are reported as no-observable-adverse-effect-level (NOAEL) and lowest-observable-adverse-effect-level (LOAEL); effect dose (Ed_x); (4) exposure pathway is relevant to wildlife or exposure in the field; and (5) validity and quality of the study are appropriate for inclusion in use for TRV derivation.

DEP is widely used as a solvent and fixative for cosmetic products and as a plasticizer for cellulose-based packaging materials with broad utility in the pharmaceutical and food industries. Packaging composed of cellulose acetate plastics may contain up to 20% DEP (Kamrin and Mayor 1991). DEP is predominantly used as a plasticizer for cellulose ester plastic films and sheets (i.e., photographic, blister packaging, and tape applications) and molded and extruded articles (i.e., consumer articles that include toothbrushes, automotive components, tool handles, and toys) (EPA 1987). DEP is an ingredient in cosmetic formulations (Schettler 2006) and other personal care products (Wormuth et al*.* 2006) at concentrations ranging from ≤0.1 to 25-50% (CIRC 1985). In addition, DEP is used as a component in insecticide sprays and mosquito repellents, solvents, adhesives, waxes, inks, and as camphor substitute (EPA 1987, Schettler 2006). DEP is also used as a plasticizer in solid rocket propellants (Clausen et al*.* 2006; Mirecki et al*.,* 2006), as a wetting agent, as a dye application agent, as an ingredient in aspirin coatings, and as a diluent in polysulfide dental impression materials. Additional uses of DEP include its utility in adhesives, plasticizers, and surface lubricants that are used in food and pharmaceutical packaging (ATSDR 1995; Schettler 2006).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological, and ecological databases (e.g., BIOSIS®, Defense Technical Information Center (DTIC) On-Line Multisearch, and TOXNET®) were electronically searched in June 2018 to identify primary peer-reviewed reports of studies and reviews on the toxicology of DEP. Separate searches were conducted for general toxicology and specific searches for birds, reptiles, amphibians, and wildlife. Each database was searched using keywords that included diethyl phthalate or its Chemical Abstracts Service (CAS) number (No.) 84-66-2 and terms that included toxicity, ecotoxicology, wild, wildlife, mammal, avian, bird, frog, amphibian, or reptile. Appendix A (References) documents the details of the search strategy.

The articles identified in each search were reviewed for specific relevance. Potentially relevant articles focused on the toxicological effects of DEP on terrestrial vertebrates or its environmental fate. All potentially relevant articles were acquired as electronic files or by visiting the libraries of the University of California, Davis, and the Johns Hopkins University School of Medicine libraries. Review articles provided additional articles that were not identified during searches of the initial databases.

Studies were classified based on organism, route of exposure, and duration of exposure. For the purpose of this WTA, acute studies are defined as single or repeat exposures for less than 14 days or 10% of the life span of the organism; subchronic studies are defined as repeat exposures that are greater than 14 days and less than 10% of the life span of the organism, and chronic studies are defined as those equal to or greater than 10% of the life span of the organism. If exposure occurs during a sensitive life stage (e.g., gestation), then a classification as chronic is appropriate for related endpoints (e.g., early development, litter size) (USACHPPM 2000).

2.2 Environmental Fate and Transport

The production and use of DEP as a plasticizer, solvent for resins, and as a wetting agent may result in its release to the environment through various waste streams. The former use of DEP as an insect repellent resulted in its direct release to the environment. If released to the air, DEP will exist solely in the vapor phase in the atmosphere. If released to the soil, DEP will display high to low mobility based upon K_{OC} values of 69-1,726 depending on the soil type. If released to the water, DEP will moderately adsorb to suspended solids and sediment (HSDB 2009). Additional physical and chemical properties of DEP are summarized in Table 1.

DEP displays an atmospheric photolysis half-life of 1.8 to 18 days; however, its aqueous photo-oxidation half-life is much slower at 2.4 to 12 years (Staples et al*.* 1997). DEP has an aerobic soil half-life of 1.83 days and an anaerobic half-life of about 5 days (HSDB 2009).

Pseudomonas acidovorans 256-1 isolated from the soil and cultured in media with rotary shaking at 30 degrees Celsius (ºC) is capable of degrading 3,000 parts per million (ppm) DEP in 10.5 days (Kurare et al*.,* 1977); additionally, untreated soil from Broome County, New York,

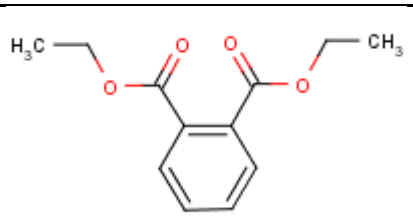
biodegrades DEP to undetectable levels after 120 days (Russell et al., 1985). A sandy clay loam soil (pH 6.25, 3.78% organic carbon content) biodegrades 100 micrograms per grams (µg/g) DEP to undetectable levels in 30 days (Cartwright et al. 2000). Further, DEP will degrade in landfills. Samples from both the acidogenic and methanogenic (at intense and stable methanogenic phases) landfill models transformed 20-25% of the added DEP during 80 days of incubation at approximately equal rates. Under methanogenic conditions, 43% of DEP degraded after 110 days. Waste samples from the pilot plant digester completely transformed DEP after 40 days and the subsequently liberated phthalic acid degraded completely to methane after 60 days (Ejlertsson et al. 1996).

DEP biodegrades in aquatic environments and does so with estimated half-lives of approximately 3 and 28 days for aerobic and anaerobic conditions, respectively. DEP also hydrolyzes slowly with an estimated half-life of 106 days at pH 8 (HSDB 2009). Staples et al., (1997) reported a much longer hydrolysis half-life of 8.8 years. Settled domestic wastewater completely degraded DEP at initial concentrations of 5 or 10 milligrams per liter (mg/L) after 7 days (Tabak et al. 1981). In addition, municipal sludge completely degraded 11,069 µg DEP/L under anaerobic conditions after 90 days (Parker et al. 1994). However, a concentration of 100 mg/L DEP in secondary sludge from a wastewater treatment facility was toxic to the diverse microbial fauna, indicating that not all DEP is degraded via primary municipal wastewater treatment. Initial concentrations of 10, 100 or 200 milligrams per liter (mg/L) left approximately 16, 26, and 32% of the initial levels undegraded after 140 days (O'Connor et al. 1989).

Microbial isolates from Japanese river water biodegraded 4.3 to 61.2% DEP after 7 days. However, river water that was collected from two locations of the Tempaku River biodegraded 88 to 100% of the DEP after 7 days (Hashizume et al. 2002). Sediment samples from the Keelung River biodegraded all DEP within 50 days in unsterilized sediments under anaerobic conditions. At the potential hydrogen (pH) level of 7, the temperature-dependent half-lives of DEP range from 15 to 21 days. Keelung River is a branch of the Danshui River and considered one of the most heavily contaminated streams in Taiwan (Chang et al. 2005). In a study from other locations in Taiwan, both aerobic and anaerobic degradation of DEP are considered. The aerobic degradation half-life in Danshui River sediment is 0.4 days; whereas, in sediment obtained from Zhonggang River, the aerobic biodegradation half-life was 5.2 days. In addition, the anaerobic degradation half-life of DEP in Danshui River sediment was 18.9 days, and in Zhonggang River sediment, the anaerobic half-life was 31.6 days. The Zhonggang River possesses almost 10-fold higher levels of phthalate contamination that were found in Danshui River (Yuan et al. 2002).

Sorption is a moderate process in the fate and transport of DEP in water columns and sediments, while photolysis and chemical hydrolysis are insignificant processes. Bacterial transformation is by far the most significant determinant of the fate of DEP in laboratory aquatic ecosystems (Lewis et al. 1984).

Table 1. Summary of Physical-Chemical Properties of Diethyl Phthalate

Structure	
CAS No.	84-66-2
Molecular weight	222.24
Color	Colorless
Physical state	Oily liquid
Melting point	-40.5ºC
Boiling point	295ºC
Odor	Very slight aromatic odor
Density at 25°C	1.120
Solubility in water	1080 mg/L at 25°C:
Solubility in other solvents	Miscible with ethanol, ethyl ether, alcohols, ketones, esters, aromatic hydrocarbons; partly miscible with aliphatic solvents. Soluble in acetone, benzene, carbon tetrachloride; Practically insoluble in petroleum ether
Partition coefficients:	
Log K_{OW}	2.47
Log K_{OC}	1.83 to 3.24
Vapor pressure at 25ºC	2.1 x 10^{-3} mm Hg
Henry's Law constant at 25ºC	6.10 x 10^{-7} atm-m^3/mole
Vapor density	7.66[1]
Conversion factors	9.07 mg/m^3 = approximately 1 ppm

Legend:
degrees Celcius (ºC)
mg/L: milligrams per liter
Log Koc: octanol-water partition coefficient
Log Koc: organic carbon partition coefficient
mm Hg: millimeters of mercury
atm-m^3/mole: air to moles per cubic meter for water
mg/m^3: milligrams per cubic meter

Note:
HSDB (2009)
Source: Sandmeyer and Kirwin 1981

DEP metabolism occurs within the contents of the rat stomach, intestinal tract, and caecum without absorption by the tissues (Rowland et al. 1977). Moreover, Lake et al. (1977) measured

in vitro DEP metabolism by liver and intestinal tissues of male laboratory rats, male olive baboons (*Papio unabis*), and male albino ferrets (*Putorius putorius*). Rates of hydrolysis at 37 degrees Celcius (ºC) in intestinal tissues of the rat were 0.648–1.07 micromole per heart rate per milligram (µmole/hr/mg) of intestinal mucosal cellular protein; 4.33 µmole/hr/mg in the baboon; and 0.053 µmole/hr/mg in the ferret. Rates of hydrolysis in liver tissues were 231–237 µmole of product formed/hr/gram of liver in the rat; 516 µmole/hr/g in the baboon; and 45.9 µmole/hr/g in the ferret. The carnivorous ferret hydrolyzed DEP in intestinal and liver tissues most slowly, while the rat was the fastest to hydrolyze DEP, with the baboon displaying intermediate hydrolysis. In another study, DEP and its major metabolite monoethyl phthalate (MEP) concentrations decreased by 3 orders of magnitude within 12 hours via intravenous (IV) or oral dosing of 10 milligrams per kilograms (mg/kg) in rats (Jeong et al*.* 2018). At 24 hours post-IV administration of DEP, the largest concentrations and plasma, tissue partition coefficients were observed in kidney, liver, spleen, and adipose, respectively.

In a dermal exposure study using concentrations of 30–40 mg/kg, Elsisi et al*.* (1989) reported that rats excreted 24% of the dose in the first 24 hours (hrs) of exposure. The rate of excretion then decreased such that only 11% of the dose was excreted over the second 24-hr period and only an additional 5% was excreted from 72 hrs through 7 days post-exposure. At the end of the 7-day period, other tissues contained the following levels of the applied dose: adipose–0.3%; muscle–0.14%; skin–0.06%; skin at application site–34%; other tissues–<0.5%; and plastic cap–5%. Including the amounts found in urine and fecal specimens, 74% of the total applied dose was recovered with very little recovered from any tissue that was located away from the application site. Further, DEP is absorbed through the skin but is not retained in the body for any extended time. Indeed, DEP passes through mammalian (i.e., the rat) skin *in vitro*. After 72 hours, 36–38% of the applied dose passes through excised rat skin and into receptor fluid with 18–20% remaining at the skin surface. Approximately 34–39% of the applied DEP also remained within the skin tissue. The absorption rate remained steady throughout a 72-hour study period following an 8-hour lag period. The absorption rate was 0.094–0.103 milligrams per square centimeters per hour (mg/cm^2/h) (Mint et al*.* 1994). Based on available data, DEP metabolized in the gastro-intestinal tract and liver is readily absorbed through the dermis and not retained in the body.

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity—Acute

Smyth and Smyth (1931) did not calculate concentration resulting lethal dose of 50% (LD_{50}) values but instead reported that the lowest oral dose of DEP that could kill guinea pigs was 5,000 milligrams per kilograms (mg/kg) (body weight (BW)), and 4,000 mg/kg (BW) for rabbits. However, only one to five animals per dose level were tested. Rabbits displayed temporary distress when dosed for 8 days with 3 milliliters per kilogram (mL/kg) BW of DEP via gavage, following which, rabbits appeared perfectly normal during dosing and for 2 weeks after the final dose of DEP was administered (Blickensdorfer and Templeton 1930). Shibko and Blumenthal (1973) reported acute LD_{50} values that ranged from 9,500 to 31,000 mg/kg (BW) in rats. However, they provided no details on the test conditions or of any possible clinical or pathological findings. In rats, the LD_{50} was greater than 5.0 mL/kg (i.e., 5,600 mg/kg) following doses of 0.5, 1.0, 2.0, and 5.0 mL/kg (Consumer Product Testing 1978).

Consumer Product Testing (1978) reported results from two acute toxicity tests. In one test, one female rat died at 1.0 mL/kg (i.e., 1,120 mg/kg); in the second test, one male rat died at 5.0 mL/kg. At necropsy, one male and one female rat displayed consolidation in the lungs in Test 1, and in Test 2; one male rat had fibrous tissue encasing its heart and lungs at a dose of 0.5 mL/kg (i.e., 560 mg/kg). The report did not state whether any of the findings were treatment related. However, as one of the animals with consolidation in the lung died, it was likely that this finding was treatment related. Thus, we derived an LOAEL of 1.0 mL/kg (1,120 mg/kg) based on mortality and consolidation in the lungs.

A summary of the primary acute oral toxicity studies are summarized in Table 2.

Table 2. Summary of Acute Oral Toxicity for Diethyl Phthalate in Mammals

Test Organism	LD_{50} (mg/kg)	Test Results			Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Rats	> 5,600	560	1,120	Mortality, consolidation in lungs	Consumer Product Testing 1978
Rats	9500 to 31,000	NA	NA	Not reported	Shibko and Blumenthal 1973
Guinea Pigs	NA	NA	5,000	Mortality	Smyth and Smyth 1931
Rabbits	NA	NA	4,000	Mortality	Smyth and Smyth 1931

Legend:
LD_{50}: dose resulting in 50% mortality
mg/kg: milligram per kilogram
NOAEL: no-observed adverse effect level
LOAEL: lowest-observed adverse effect level
mg/kg/day: milligram per kilogram per day

2.3.2 Mammalian Oral Toxicity—Subchronic

In an early study, where only three or four guinea pigs received six oral doses per week for 2 weeks, it was found that all animals receiving 250 and 500 mg/kg-day showed "possible toxic action" in the liver and/or kidney, and all animals that received 1,000 mg/kg-day showed "definite toxic action" in the liver and kidney; although, the specific effects were left undefined by the original authors. Doses of DEP at 125 mg/kg-day showed no effect (Smyth and Smyth 1932). Twenty-eight days of oral gavage treatment of rats with 40, 200, and 1,000 mg/kg-day DEP did not alter female BW. However, male BW decreased at a DEP dose of 1,000 mg/kg-day (i.e., 443 grams (g) in controls vs. 417 g in treated animals). However, the difference reported was not significant. Additionally, in male rats none of the organ weights were affected following DEP treatment. Female kidney weights increased at doses of 40 and 1,000 mg/kg-day, and female adrenal weights increase when dosed with DEP at 1,000 mg/kg-day (Smyth and Smyth 1932). Frequency of urination significantly increased in male rats dosed at 1,000 mg/kg-day after 4 weeks of dosing. Activated partial thromboplastin time also increased in male rats

following DEP treatment but only at doses of 40 and 1,000 mg/kg-day. Decreases in glutamic-oxaloacetic transaminase and increased c-glutamyl transpeptidase levels were found in males when treated with DEP at 40 and 1,000 mg/kg-day. An increased albumin–globulin ratio and decreased creatinine was found in males at 1,000 mg/kg-day. Estradiol values decreased in males at a dose of 1,000 mg/kg-day. No abnormal spermatological findings appeared in DEP-treated rats. There were also no abnormalities in the estrous cycles of the rats given DEP. No dose-related changes appeared by gross pathology examination in either treated male or female rats. Additionally, no histopathological findings appeared in any of the dose groups (Shiraishi et al. 2006). It remains unclear why certain effects were found in the 40 and 1,000 mg/kg-day groups but not in the group that received 200 mg/kg-day.

Oral gavage treatment of male rats with 500 mg/kg-day DEP for 4 weeks increased salivation; however, no mortality was observed, and no effect on BW or food consumption was found. No significant differences were found for the relative weights of the thyroid, lung, heart, spleen, kidney, liver, adrenal gland, testes, and epididymis. Additionally, DEP treatment did not affect any of the hematological parameters including effects on hemoglobin, white blood cells (WBC), red blood cells (RBC), hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets. The only biochemical factor that was affected in the blood was an increase in calcium concentration. No effect was found on any analytical end-point of sperm including: average path velocity, straight-line velocity, curvilinear velocity, amplitude of lateral head displacement, beat cross frequency, and straightness, with the notable exception of linearity of sperm path, which was reduced following DEP treatment (Kwak et al. 2009).

Exposure of rats to diets containing 2% DEP for 3 weeks caused a slight but significant increase in hepatic carnitine acetyltransferase activity. Relative liver weights also increased significantly with a significant increase in liver catalase and a slight increase in liver peroxisome proliferation. No effect on body weight increase during the test period was found (Moody and Reddy 1978). Using the allometric equation recommended by the U.S. Environmental Protection Agency (EPA) (EPA 1988) the daily exposure in this study was approximately 2003–2130 mg/kg-day.

Diets that contained 0.2, 1.0, and 5.0% DEP when fed to rats for up to 8 weeks were approximately equivalent to 150, 770 and 3160 mg/kg-day in male rats of the three test groups and 150, 750 and 3710 mg/kg-day in female rats. No clinical signs or behavioral changes occurred in any group. BW gains decreased in both male and female rats that were given 5.0% DEP, and in females fed 1.0%. By contrast, a transitory reduction in BW occurred in males from day 6 to 36 at a dose of 1.0% DEP. Reduced BW accompanied reduced feed consumption.

Additionally, significant increases in erythrocyte count were found in male rats after 6 weeks when fed 5.0% DEP in the diet. Serum enzymes were unaffected by DEP treatment of male or female rats. Relative weights of the brain, liver, and stomach also were found to increase in both male and female rats, and relative weights of male gonads and thyroids were increased following treatment with 5.0% DEP in the diet. Male rats on diets with 1.0% DEP exhibited significantly increased relative weights of the liver (Brown et al. 1978).

Table 3. Summary of Subchronic Oral Toxicity for Diethyl Phthalate in Mammals

Test Organism	Test Duration	Test Results			Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Mice	90 days	NA	1.25	Liver histology, changes in clinical chemistry	Mapuskar et al., 2007
Rats	28 days	NA	40	Increased activated partial thromboplastin time and c-glutamyl transpeptidase levels in males. Decreased glutamic-oxaloacetic transaminase in males. Increase in female kidney weights.	Shiraishi et al., 2006
Rats	6 weeks	150 (males) 150 (females)	770 (males) 750 (females)	Males: increased relative liver weights. Females: reduced BW	Brown et al., 1978
Rat	6 weeks	2500	NA	none observed	Shibko and Blumenthal 1973
Guinea pigs	2 weeks	125	250	“possible toxic action” in livers and kidneys	Smyth and Smyth 1932
Dog	6 weeks	1250	NA	None observed	Shibko and Blumenthal 1973
Rat	GD8 - 18	900	NA	No effect on fetal testicular testosterone, implantations, or fetal mortality	Howdeshell et al., 2008
Rats	GD6 - 15	1910	3210	Decreased maternal BW; increased incidence of extra lumbar ribs	Field et al., 1993
Mice	GD6-13	4,500	NA	Maternal BW, number of viable litters, live-born animals per litter, birth weights, or pup weight gain	Hardin et al., 1987
Rats	1 week	NA	950	Increased liver weight and relative liver weight. Decreased testosterone levels in the testes and serum. No changes in body, testes, or kidney weight. No changes in zinc concentrations in the testes, liver, kidneys or serum.	Oishi and Hiraga, 1980
Rats	GD14-PND 3	750	NA	Female BW or BW gain, the number of live pups, pup weight at birth or at weaning.	Gray et al., 2000
Rats	GD12-20	1,000	NA	Birth rate, male: female ratio of pups, number of pups per litter	Hu et al., 2018
Rats	GD12-20	NA	10	BW in male pups, fetal testes effects	Hu et al., 2018
Rats	GD17-21	2.5	NA	Fetal testes effects	Spade et al., 2018
Rats	10-11 weeks	NA 1600 800 400 800	400 3200 1600 800 1600	Decreased liver weights in male pups Hematuria, decreased BWs Decreased prostate weights Increased relative liver weights in males Decreased BWs in pups, decreased viability indices	Fujii et al., 2005

Table 3 Legend (continued):
NOAEL: no-observed adverse effect level
LOAEL: lowest-observed adverse effect level
mg/kg-day: milligram per kilogram per day
GD: gestation days
PND: post-natal day

In a 90-day study, female mice were treated with 10, 25, and 50 mg/kg DEP in the diet, which was equivalent to 1.25, 3.12, and 6.25 mg/kg-day (Mapuskar et al. 2007). At the end of the 90-day study, serum acid phosphatase increased in a dose-dependent manner, with all groups showing higher levels than the controls. Serum lactate dehydrogenase was greatest at 25 and 50 mg/kg diet. Serum aspartate aminotransferase also increased in a dose-dependent manner, with all groups showing higher levels than the controls. Serum alanine aminotransferase was also significantly greater in all treatment groups; however, the levels of this enzyme at 50 mg/kg diet were significantly less than that found at 25 mg/kg diet. Furthermore, liver cholesterol was increased in all treatment groups; whereas, serum cholesterol had decreased in all treatment groups. While serum and liver triglycerides increased in a dose-dependent manner, liver glycogen increased dose-dependently. Liver histology of mice that received 10 mg DEP/kg diet showed severe intracellular vacuolations and mild loss of hepatic architecture in the hepatocytes of the centrilobular and periportal areas. Mice that received 25 and 50 mg/kg diet showed hepatocytic hypertrophy, loss of hepatic architecture and severe intracellular vacuolations along with hepatocellular degeneration in centrilobular and periportal areas. Mice that received 10 mg/kg dietary DEP showed mitochondrial and peroxisomal proliferation. Mice-fed diets 25 mg/kg DEP showed significantly increased numbers of mitochondria. Drastic increases in the number of lipid droplets appeared in the transmission electron micrographs of liver specimens examined from mice that received a dose of 50 mg/kg DEP. Hypertrophy of the nucleus, increased significantly in mice fed diets with 10 mg/kg DEP and in mice fed diets with 25 mg/kg DEP; the nuclei showed undulating membranes. A drastic shrinkage in the size of the nucleus was found in mice receiving the 50 mg/kg DEP diet (Mapuskar et al. 2007).

2.3.2.1 Mammalian Oral Toxicity—Subchronic: Reproductive Toxicity

Doses of DEP that were administered to pregnant mice via oral gavage on gestation days (GD) 6–13 provoked 4% maternal mortality at a dose of 4500 mg/kg-day. No impact on changes in maternal BW, number of viable litters, live-born animals per litter, birth weights, or pup weight gain through day 3 was found in this study (Hardin et al. 1987). Treatment of five pregnant rats with 750 mg/kg-day DEP via oral gavage during the period from GD14 to post-natal day (PND) 3 did not affect female BW or BW gain; however, two female rats died because of apparent dosing errors. The remaining three females had live pups on PND2. In addition, the three females had live pups at weaning. The number of live pups, pup weight at birth or at weaning was unaffected (Gray et al. 2000).

Feeding pregnant rats with dietary concentrations of DEP that ranged from 0.025–5.0% (i.e., 200–3210 mg/kg-day) on GD6 through GD15 caused no treatment-related mortality among the female rats. However, reduced maternal weight gain for gestation and treatment periods was found in the group that received 0.25% DEP in the diet. Reduced weight gains also

occurred in the group fed 5.0% DEP in the diet for the treatment period. The groups fed 2.5 and 5.0% DEP also exhibited reduced food consumption. These groups experienced elevated food consumption following the treatment period. Dietary DEP did not alter indices of prenatal viability like resorption incidence or live litter size (Field et al. 1993).

Oral doses of 100–900 mg/kg-day on GD8–18 did not affect the production of testosterone in fetal testes. These doses were also not maternally toxic. However, a dose of 600 mg/kg-day increased resorptions and fetal mortality—an effect that was not seen at a dose of 900 mg/kg-day. Since resorptions and fetal mortality were unaffected at 900 mg/kg-day, it was unclear whether this effect was genuinely treatment related but is unlikely due to the absence of a dose-response relationship and a lack of effects on other endpoints (Howdeshell et al. 2008).

Feeding 2% DEP to 5-week-old male rats for 1 week did not affect BW, testes weight, kidney weight, or zinc concentrations that were measured in the testes, liver, kidneys, or serum. However, increases in liver weight and relative liver weight, and testosterone levels in the testes and all serum decreased significantly. Moreover, dihydrotestosterone in the serum decreased, although not to a level that was significant (Oishi and Hiraga 1980). In addition, by using an allometric conversion for food consumption (EPA 1988), and the reported mean BW of 108 g, the daily dose of DEP was found to approximate 950 mg/kg-day.

In a preliminary range-finding study with diets containing up to 40,000 parts per million (ppm), DEP treatment caused hematuria in two male rats at 20,000 ppm DEP in the diet (Fujii et al. 2005). Additionally, hematuria was also seen in one male and one female rat when treated with 40,000 ppm DEP in the diet. BWs decreased in male and female rats at a dose of 40,000 ppm in the diet, and increased relative liver weights were seen in male rats at DEP doses of 10,000 ppm or higher in the diet. Absolute prostate weights decreased following treatment with 20,000 or 40,000 ppm DEP in the diet. Gestation length was also reduced at 40,000 ppm DEP in the diet. BW gains in pups decreased through the lactation period following treatment with 20,000 or 40,000 ppm DEP in the diet. Viability indices for pups were decreased on PND days 4 and 21 at DEP doses of 20,000 or 40,000 ppm in the diet. Finally, liver weights of male pups decreased in all treated groups (Fujii et al. 2005). Using an allometric conversion for food consumption (EPA 1988), and an estimated BW of 350 g, the daily doses were approximately 400, 800, 1600 and 3200 mg/kg-day from dietary DEP concentrations of 5000, 10,000, 20,000, and 40,000 mg/kg DEP in the diet.

A recent study wherein pregnant Sprague-Dawley rats were gavaged DEP at 0, 10, 100, 500, or 1,000 mg/kg-day during GD 12-21 found no effect on birth rates, male-to-female sex ratio, or number of pups per day (Hu et al., 2018).

2.3.2.2 Mammalian Oral Toxicity—Subchronic: Developmental Toxicity

In a study with dietary concentrations that ranged from 0.25-5.0% DEP (i.e., equivalent to 200-3210 mg/kg-day), it was found that feeding pregnant rats the treated diets on GD6 to 15 did not significantly increase the frequency of malformed fetuses per litter or the frequency of litters with malformations. However, the percentage of fetuses with variations per litter significantly increased in the group that received 3210 mg/kg-day. The most frequently observed variable was the presence of extra lumbar ribs (i.e., rudimentary; Field et al. 1993).

Treatment of pregnant female rats with 750 mg/kg-day DEP via oral gavage during the period from GD14 to PND3 did not alter the onset of puberty due to genital malformations. No effect was found on male age at puberty. In addition, DEP treatment did not affect testes weight, seminal vesicle weight, ventral prostate weight, glans penis weight, epididymal weight, or organ weights. Moreover, nipple development was absent in male rats (Gray et al., 2000).
In a study where Sprague-Dawley rats were gavaged with DEP at 0, 10, 100, 500, or 1,000 mg/kg-day during GD 12-21 found a significantly reduced BW of male pups at all doses (Hu et al. 2018). Fetal testis volume and fetal Leydig cell size, cytoplasmic size and cytoplasm: nuclear size ratio was also significantly reduced in the 10–100 mg/kg-day and higher dose groups. In another study using Sprague-Dawley rats gavaged with 2.5 mg/kg-day from GD17-21, there were no effects observed on fetal testis testosterone production or fetal testicular multinucleated germ cells (Spade et al. 2018).

2.3.3 Mammalian Oral Toxicity—Chronic

A range-finding study in which mice were provided 0–5% DEP in the diets for 8 weeks identified no deaths or clinical signs of toxicity following treatment. The percentage weight gain for both male and female mice increased at a dietary concentration of 0.5% only, and the weight gain significantly decreased at a dietary DEP concentration of 5.0% (Reel et al. 1984; Lamb et al. 1987). In the subsequent definitive two-generation reproduction study, dietary concentrations of DEP were 0, 0.25, 1.25, and 2.5% for a 7-day premating period then for 119 days. These dietary concentrations corresponded to approximately 440, 2,200, and 4,400 mg/kg-day, respectively. All pairs produced at least one litter without affecting the number of litters per pair, number of viable born pups, the gender of the viable born pups, or the live pup birth weight. At 0.25% dietary DEP, the number of live pups was significantly elevated as compared to all other groups, the number of male pups per litter was significantly increased as compared to 2.5% dietary DEP, and the number of females per litter was significantly increased as compared the controls and 2.5% dietary DEP (Reel et al. 1984, Lamb et al. 1987).

Decreased BWs were seen at weaning for weanlings that were derived from parents that received 2.5% DEP in the diet (Reel et al*.* 1984, Lamb et al*.* 1987). Pups (first filial generation (F_1)) from controls and mice that were exposed to 2.5% DEP were continued in the study to breed an F_2 generation, in which, the male and female mice were paired from the same treatment groups for 7 days when they had reached sexual maturity at an age of 74 days (n = 20/group/sex).

The F_2 litters were examined for litter size, survival, sex ratios, and pup weights (Lamb et al. 1987), and the F_1 animals were then necropsied. In the F_1 generation that received 2.5% DEP, mating behavior was unaffected since no effects appeared in the number of live male or female mice per litter, or in the proportion of viable born pups or live pup weights. However, the number of pups born alive per litter decreased in the group that received 2.5% DEP in the diet. No effect was seen in terms of the frequency of motile sperm or the frequency of abnormal sperm. In F_1 males that were fed 2.5% DEP in the diet, no effect was seen for BW or in the weights of the liver, brain, pituitary gland, or right epididymis. In addition, decreased weights were seen for the right testis, left testis plus left epididymis, and seminal vesicles; however, the observed decreases were not statistically significant. By contrast, weights of the prostate were significantly increased. Decreases in sperm concentration were seen in the treated groups.

Prostate and relative liver weights increased in F_1 males of the treated group; whereas, BWs decreased. In female mice from the treated group, BWs had decreased, and relative liver weights had increased. In addition, pituitary weights of F_1 female mice were decreased in the treated group (Reel et al. 1984; Lamb et al. 1987).

Liver weight, BW, relative liver weight, daily water consumption, and serum glucose levels in rats were unaffected following 120 days treatment with 50 ppm DEP in the drinking water (Sonde et al. 2000). However, liver aspartate amino transferase and alanine amino transferase levels were significantly reduced, and levels of serum aspartate amino transferase and alanine amino transferase were increased significantly. In addition, while serum succinate dehydrogenase was unaffected, levels of liver succinate dehydrogenase were significantly increased. Liver and serum acid phosphatase levels were also significantly increased. By contrast, while serum levels of alkaline phosphatase increased significantly, levels of liver alkaline phosphatase remained unaltered during treatment with DEP. Further, levels of both liver glycogen and serum triglyceride also increased, although the levels of liver triglycerides decreased significantly. Serum cholesterol levels were also 18-fold higher in DEP-treated rats, and liver cholesterol was also increased (Sonde et al. 2000). Using an allometric conversion for water consumption (EPA 1988) and an estimated BW of 350g, the daily dose of DEP in drinking water was approximately 35 mg/day.

In a two-generation study, reproductive effects were seen in rats that were fed DEP in their diet (Fujii et al. 2005). The dietary concentrations of 600, 3,000, and 15,000 mg/kg DEP diet were equivalent to daily exposure levels of 40, 197, and 1016 mg/kg-day in F_0 males; 51, 255, 1297 mg/kg-day in F_0 females; 46, 222, 1150 mg/kg-day in F_1 males; and 56, 267, 1375 mg/kg-day in F_1 females respectively. Increased parental female body weights were seen at 600 ppm DEP in the diet. BW change was greatest in F_0 females during premating exposure to 600 ppm DEP in the diet. BW gains were significantly higher at a dose of 15,000 ppm DEP in the diet for F_1 males during the first week of exposure. Additionally, F_0 females gained significantly more weight during lactation when exposed to 15,000 ppm DEP in the diet.

No compound-related clinical signs were seen in either sex in either generation (Fujii et al. 2005). F_0 female animals exhibited reduced feeding from the end of the premating exposure period through lactation at 3,000 ppm DEP in the diet. F_1 males and females also showed increased feeding at a dose of 15,000 DEP in the diet during treatment week 1. Dietary DEP did not affect copulation, fertility or gestation indices, or the number of implants, the delivery index, or the numbers of pups. DEP also did not affect the estrous cycle in either generation. However, gestation length in the F_1 generation was significantly shorter by 0.3 days at a DEP dose of 15,000 ppm in the diet. No clinical signs were seen in F_1 or F_2 pups. Significantly reduced BWs were seen at 15,000 ppm DEP in the diet on PND4-21 for F_1 female pups. Moreover, significantly reduced BWs were seen for F_1 and F_2 males and F_2 females on PND21 at a DEP dose of 15,000 ppm in the diet (Fujii et al, 2005).

Sex ratio, viability index, and anogenital distance (AGD) were unaffected by DEP exposure. However, pinna detachment and eye opening were delayed in both sexes in the F_1 and F_2 generations at a dose of 15,000 ppm DEP in the diet. By contrast, DEP did not affect surface righting, negative geotaxis or mid-air righting reflexes. Sperm counts and motility were unaffected, but a significant increase in deformed sperm for F_0 males was seen at a dietary

dose of 3,000 ppm DEP and in F_1 males at doses of 3,000 and 15,000 ppm DEP. No gross lesions were seen in F_0 or F_1 pups at necropsy, and no histopathological findings were apparent in the reproductive and endocrine organs or in the livers of both males and females for F_0 and F_1 parental animals. Increases in thyroid weight were seen in F_1 males at 3,000 ppm DEP in the diet. Additionally, increased relative liver weights of F_0 and F_1 were seen at a dietary dose of 15,000 ppm DEP. Absolute weights of the adrenal gland and epididymis in F_0 males had decreased; absolute and relative kidney weights in F_1 females had increased following exposure to 15,000 ppm DEP in the diet.

In addition, the cytochrome P450 (CYP450) isozymes CYP1A1/2 and CYP2B1 were unaffected in F_0 males; however, both CYP4A1 and CYP3A2 had increased in the liver of F_0 males at 15,000 ppm DEP in the diet. Finally, serum progesterone was unaffected in F_0 males, and serum testosterone decreased significantly in F_0 males at doses of 3,000 and 15,000 ppm DEP in the diet (Fujii et al*.* 2005). No histopathological effects observed in the F_1 and F_2 pups (Fujii et al*.,* 2005). In addition, absolute adrenal gland weights were decreased in F_1 females following treatment with 3,000 and 15,000 ppm DEP, and relative uterine weight decreased in F_2 females. At 15,000 ppm DEP, relative liver weights increased in both males and females for F_1 and F_2 weanlings.

Absolute and relative thymus weights were also increased at a dose of 15,000 ppm DEP in both males and females of the F_1 and F_2 generations. Absolute spleen weights in F_1 males and absolute and relative spleen weights in F_2 males were also decreased following exposure to 15,000 ppm DEP in the diet. Absolute adrenal gland weights were significantly decreased in both males and females of the F_1 and F_2 generations at 15,000 ppm DEP in the diet. Absolute prostate weights were decreased in F_1 males at 15,000 ppm DEP. Absolute uterine weights in F_1 females, and absolute and relative uterine weights in F_2 females had decreased following treatment with 15,000 ppm DEP. Changes were also seen for the weights of the brain, kidney, thyroid, pituitary and seminal vesicles for F_1 and F_2 pups at 15,000 ppm DEP in the diet.

Taking into account the differential effects from DEP exposure on the different generations, the NOAEL for parental animals was 600 ppm, and the LOAEL was 3,000 ppm DEP in the diet. For reproductive effects and development of the F_1 and F_2 pups, the NOAEL was also 600 ppm, and the LOAEL was 3,000 ppm DEP in the diet (Fujii et al*.* 2005).

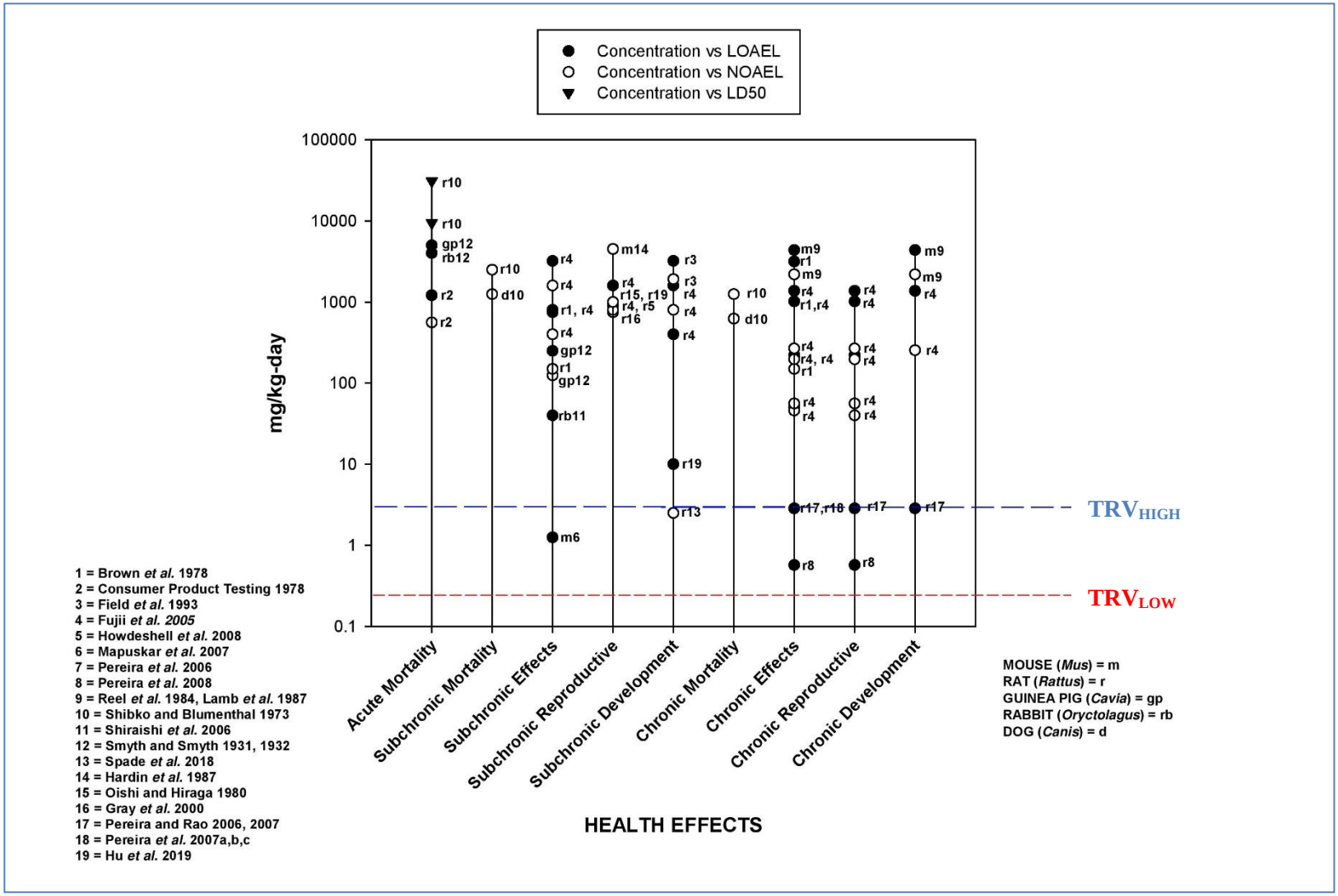


Figure 1. Diethyl Phthalate: Health Effects to Mammals

Table 4. Summary of Chronic Oral Toxicity for Diethyl Phthalate in Mammals

Test Organism	Test Duration	Test Results			Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Mice	18 weeks	2,188	4,375	Parental body weights in reproduction study	Reel et al., 1984, Lamb et al., 1987
Mice	2 generations	2,188	4,375	Decreased weanling weights	Reel et al., 1984, Lamb et al., 1987
Rats	16 weeks	150 males NA females	3160 males 150 females	Increased organ weights	Brown et al., 1978
Rats	150 days	NA	2.85	Liver hepatocyte hyperpigmentation and vaculoation; Alterations in liver and serum enzyme levels; reduced weanling weights and male weanling liver weights; increased female weanling liver weights; litter size	Pereira and Rao, 2006; Pereira and Rao, 2007
Rats	5 months	NA	0.57	Altered liver and serum enzyme activity levels, increased relative liver weights, altered liver cholesterol and glycogen levels and histology	Pereira et al., 2006
Rats	3 generations	NA	2.85	Altered liver and serum enzyme activity levels, granular deposits and vacuolation in liver	Pereira et al., 2007a
Rats	2 generations	NA	2.85	Reduced litter size; adrenal cortex vacuolations and degeneration in the zona fasciculata in males; thyroid follicle shrinkage, loss of thyroglobulin, fibrosis of the interfollicular epithelium	Pereira et al., 2007b,c
Male rats	150 days	NA	0.57	Decreased testes, epididymal, and body weights; reduced serum testosterone	Pereira et al., 2008
Rats	104 weeks	1250	NA	Not stated	Shibko and Blumenthal 1973

Table 4. Summary of Chronic Oral Toxicity for Diethyl Phthalate in Mammals (continued)

Test Organism	Test Duration	Test Results			Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Dogs	52 weeks	625	NA	Not stated	Shibko and Blumenthal 1973
Rats	2 years	40 – 46	197 – 222	Deformed sperm in F_0 and F_1	Fujii et al., 2005
		46	222	Increased thyroids in F_1 males	
		NA	51	Increased body weights and body weight gain in F_0 females	
		56	267	decreased absolute adrenal gland weights in F_1 females, decreased relative uterine weight in F_2	
		197	1,016	Decreased adrenal and epididymis weights in F_0	
		197 – 267	1,016 – 1,375	Increased relative liver weights both sexes in F_0 and F_1	
		255 – 267	1,297 – 1,375	Reduced male and female pup body weights in F_1 and F_2, numerous organ weight changes in F_1 and F_2 weanlings, delayed pinna detachment and eye opening in F_1 and F_2	
		267	1,375	Shorter gestation in F_1, age at vaginal opening in F_1, increased absolute and relative kidney weights in F_1 females	

Legend:
NOAEL: no-observed adverse effect level
LOAEL: lowest-observed adverse effect level
mg/kg-day: milligram per kilogram per day

Hayashi et al. (2010) presented the impacts of DEP when mice were exposed to a pesticide mixture for 32 days. To mimic the levels of pesticides and DEP that were measured in rice, the investigators exposed mice to a diet that was supplemented with etofenprox at a dose of 0.002 ppm, 3-t-butylphenol at a dose of 0.002 ppm, and DEP at a dose of 0.7 ppm as a concerted mixture, such that animals were exposed to all three chemicals. The stated doses of dietary DEP were equivalent to approximately 0.3 mg/kg-day (EPA 1988). Mice that received rice supplemented with similar levels of test chemicals as added to the test diet had reduced testes weights and delayed delivery from 58.2 to 65.3 days. When fed the treated diet, weights of the liver, heart, kidney, and testes were unaffected; however, the first delivery was delayed, and two of nine treated females failed to become pregnant (Hayashi et al. 2010). In a second study when only males received diets with the same test article concentrations, treated males had decreased sperm counts and decreased plasma testosterone levels, although testes weights remained unaffected (Hayashi et al. 2010). Because of treatment with a chemical mixture, it is impossible to determine if these effects are due to one or both of the chemicals.

Dietary DEP at a concentration of 50 ppm (equivalent to 2.85 mg/kg-day BW) for 150 days did not affect relative liver weight in female rats; however, the combination with Aroclor 1260 at equal parts for a dietary concentration of 50 ppm increased the relative liver weight (Pereira and Rao 2006). When animals were treated with DEP alone or in combination with Aroclor 1260, authors noted increased levels of liver and serum acid phosphatase. DEP alone significantly decreased the levels of liver and serum-borne alkaline phosphatase; whereas, the combination failed to affect liver alkaline phosphatase and yet increased levels of this enzyme in serum (Pereira and Rao 2006). Furthermore, DEP alone significantly increased both liver and serum aspartate aminotransferase; however, the combination of DEP and Aroclor 1260 significantly reduced the levels of both liver and serum aspartate aminotransferase. Additionally, DEP alone significantly increased serum and liver levels of alanine amino transferase; however, although combined treatment with DEP and Aroclor 1260 increased liver levels of alanine amino transferase, the levels of this enzyme were decreased in the serum. DEP alone and when combined with Aroclor 1260 increased the levels of liver and serum lactate dehydrogenase. By contrast, treatment with DEP alone had no effect on the levels of liver or serum-borne succinate dehydrogenase; however, combined treatment of DEP with Aroclor 1260 increased the levels of both liver and serum-borne succinate dehydrogenase (Pereira and Rao 2006).

Liver and serum cholesterol levels were increased following treatment with DEP alone; however, combined treatment of animals with DEP and Aroclor 1260 only increased the levels of liver cholesterol (Pereira and Rao 2006). The combined treatment regimen also increased liver glycogen; however, treatment with DEP alone had no effect. Further, while treatment with DEP alone increased serum-borne levels of glucose, combined treatment of animals with DEP and Aroclor 1260 decreased serum levels of glucose. Exposure to DEP alone or when in combination with Aroclor 1260 also increased the levels of both liver and serum triglycerides, and increased liver lipid peroxidation. Liver acid phosphatase activity in rats treated with DEP alone or in combination with Aroclor 1260 was 2.5-fold lower than that of serum-borne acid phosphatase. Additionally, liver glutathione and glutathione reductase levels had decreased following DEP exposure when used alone or when combined with Aroclor 1260. Rat liver histology following combined treatment showed mild hepatomegaly, hepatocyte hyper-pigmentation, and presence of fatty deposits. Rat livers following dietary treatment with DEP alone showed hepatocyte hyper-pigmentation and vacuolation. Since the impacts of treatment on enzyme activities and levels differed between DEP and Aroclor 1260, it is likely that both test articles operated through different mechanisms (Pereira and Rao 2006).

In a similar study, male rats that were exposed to multiple dietary concentrations of DEP for 5 months did not always display a typical dose-dependent pattern in the context of selected enzyme activities (Pereira et al. 2006). Dietary DEP concentrations of 10, 25, and 50 ppm corresponded to 0.57, 1.425, and 2.85 mg/kg-day. Acid phosphatase activity in both the liver and serum were at their highest in rats fed diets with 10 ppm DEP. In addition, in animals treated with 25 and 50 ppm DEP, serum-borne levels of acid phosphatase activity was significantly higher than controls but significantly lower than 10 ppm DEP treated animals. The same pattern of enzymatic activity was found for liver and serum lactate dehydrogenase and serum alanine aminotransferase (Pereira et al. 2006). Liver alanine aminotransferase was elevated following exposure to all dietary concentrations of DEP. Only aspartate aminotransferase showed a true dose-dependent response in this study, with enzyme activities increasing with increasing dietary concentration of DEP. The activity of liver and serum-borne

aspartate amino-transferase was significantly higher than that of controls for all treatments. Additionally, animals exposed to 50-ppm DEP exhibited significantly increased aspartate aminotransferase activity as compared to other treatment groups.

The same study found that DEP treated diets (at doses of 10, 25, and 50 ppm) did not alter rat behavior or food consumption following exposure (Pereira et al. 2006). However, relative liver weights were elevated only in rats dosed at 10 ppm. Liver cholesterol concentrations increased dose-dependently; by contrast, serum concentration of cholesterol decreased dose-dependently. In liver and serum, only the highest two dietary concentrations were significantly different from the control animals for cholesterol concentrations. Liver glycogen levels showed a significant dose-dependent increase with levels at 50 ppm significantly higher than were found for controls and when compared with groups of rats dosed at 10, and 25 ppm. All three treated groups showed significantly increased serum and liver triglyceride levels. Liver–lipid peroxidation levels increased significantly in rats that were dosed with DEP at 10, 25, and 50 ppm in the diet. Moreover, the 10-ppm group displayed significantly higher liver–lipid peroxidation levels than did the 25, and 50-ppm groups. Liver glutathione was significantly decreased at 10 and 50 ppm DEP with the 10-ppm group showing significantly lower liver glutathione levels than the 50-ppm group. Liver histology of 25- and 50-ppm treated rats showed hepatocyte granular deposits and mild vacuolations in the centrilobular and periportal areas. Electron micrographs of the 10-ppm treated rats showed increased peroxisome numbers, and a marked dose-dependent proliferation in mitochondria among all of the treated groups (Pereira et al. 2006).

Rats fed a diet containing 5% DEP (i.e., 3160 mg/kg-day in males and 3710 mg/kg-day in females) for 16 weeks showed a more pronounced loss of weight as compared to starting weights than their pair-fed control counterparts (Brown et al*.* 1978). The rate of weight gain was lower in test animals, although the observed effect was not statistically significant until the last week of the study. Additionally, animals receiving a 5% DEP-supplemented diet consumed more food than controls (Brown et al*.* 1978). The same group also showed decreased BW gain in female rats fed 1.0% DEP, and a transitory reduction in BW in males that were also fed 1.0% DEP (i.e., 770 mg/kg-day in males and 750 mg/kg-day in females), from day 6 to 36. Reduced BW typically accompanies reduced feed consumption.

In male rats that received 5.0% DEP in the diet, erythrocyte counts remained unaltered after 16 weeks. Serum enzymes were also unaffected, and the only gross abnormality was a unilaterally small testis in one rat that received 0.2% DEP in the diet (i.e., 150 mg/kg-day when dosed in both males and females). Relative weights of the brain, kidneys, and full caecum were found to have increased in both male and female rats fed 5.0% DEP; in male rats only, the relative weights of the heart, adrenals, pituitary, and thyroid all increased following exposure to 5.0% DEP in the diet. Additionally, female rats exhibited increased relative weights of the spleen at dietary doses of 5.0% DEP. In female rats, relative liver weights and stomach weights increased at all dose levels tested. However, increases in relative stomach weights of male rats were only seen at 1.0 and 5.0% DEP in the diet. Relative weights of the small intestines in female rats were also increased in all dose groups. However, in male rats, increased relative weights of the small intestines were only seen at 5.0% DEP in the diet. Finally, increased relative weight of the empty caecum was only seen in female rats dosed at 0.2 and 5% DEP in the diet (Brown et al*.,* 1978).

When rats were fed 50 mg/kg DEP in the diet (i.e., equivalent to 2.85 mg/kg-day) for 100 days prior to mating and then through weaning, both male and female offspring appeared to display sluggish ambulatory and lethargic behavior (Pereira and Rao 2007). The litter size reduced following the above treatment regimen. Additionally, weanling weights of both male and female pups decreased. Similarly, male and female weanling liver weights also decreased. However, relative liver weights of male weanlings decreased; whereas, relative liver weights of female weanlings were increased. The histology of DEP-exposed male and female pups showed mild vacuolations as compared to their control counterparts (Pereira and Rao 2007).

In a rat three-generation study, the F_1 and F_2 generations were not exposed to treated diets until all animals were 70–100g (Pereira et al*.,* 2007a). Each generation received a diet at roughly half the concentration that was dosed in the previous generation. The progression of diets according to the respective generations was as follows: the F_0 received 50 mg/kg DEP in the diet (i.e., 2.85 mg/kg-day); the F_1 received 25 mg/kg diet (i.e., 1.425 mg/kg-day); and the F_2 received 10 mg/kg diet (0.57 mg/kg-day). However, the authors did not explain the rationale for this reduced progression in dietary exposure concentrations. Nonetheless, each generation received the treated diet for 150 days, which included a 100-day premating period, and dietary exposure to DEP through weaning (Pereira et al*.,* 2007a). There was no effect from DEP exposure in BW or feed consumption.

The F_0 and F_1 generations exhibited reduced litter sizes. In addition, the F_1 and F_2 generations displayed increased liver weights with the effect increasing with each successive generation. Liver and serum-borne alanine amino transferase, aspartate amino transferase and liver triglycerides were also significantly increased with an increasing effect evident with each successive generation. Serum-borne triglycerides were also significantly increased; however, unlike the above observations, the effect was not exaggerated across generations. Levels of serum cholesterol were significantly increased in the F_0 generation but were significantly decreased in both the F_1 and F_2 generations, with the greatest decrease seen in in the F_2 generation. Liver glutathione and glutathione reductase were both significantly decreased with the greatest effect seen by successive generations as described before. Liver histology of the F_2 generation showed that severe fatty degeneration in the hepatocytes of the centrilobular and periportal areas in DEP-treated rats, with more severe affects seen in this generation than was comparably seen in either the F_1 or F_0 generation. The F_1 generation showed that in addition to vacuolations, fatty degeneration in the hepatocytes of the centrilobular and periportal areas was more pronounced than was seen in either the F_0 or F_2 generations. Additionally, the presence of granular deposits and vacuolations were more significantly evident in the F_0 generation.

Pereira et al*.,* (2007b) also performed a two-generation study where the F_0 generation received a diet that was supplemented with 50 mg DEP/kg (2.85 mg/kg-day) and the F_1 generation received a diet that contained 25 mg/kg (1.425 mg/kg-day). No deaths were reported in either generation. BWs were lower in the DEP treated F_1 generation, and absolute and relative liver weights were increased in the DEP-treated F_1 generation. On treated diets, the F_0 generation produced reduced litter sizes.

Clinical biochemistry analysis revealed that liver and serum alanine amino transferase was increased in both the F_0 and F_1 generations (Pereira et al*.,* 2007b). Liver and serum aspartate amino transferase was increased in both the F_0 and F_1 generations, and the level of serum

aspartate amino transferase was greater in the F_1 as compared the F_0 generation. In addition, levels of liver cholesterol were increased in the F_0 and F_1 generation and serum cholesterol, as well as the level of liver glycogen was increased in the F_0 but not in the F_1 generation. Liver glutathione and liver glutathione reductase were decreased in both the F_0 and F_1 generations. Vacuolations were also much more predominant in the F_1 generation as compared the F_0 generation following DEP treatment (Pereira et al. 2007b).

In a similar study where two generations were included and with a similar dose reduction in dietary concentrations of DEP, Pereira et al. (2007c) focused more on histopathological effects. In this study, the F_0 generation again received a diet with 50 mg DEP/kg (i.e., 2.85 mg/kg-day) and the F_1 generation received a diet containing 25 mg/kg (i.e., 1.425 mg/kg-day).

Histopathology results for males revealed vacuolations and degeneration in the zona fasciculata region of the adrenal cortex of both generations—observations that were not seen in female rats. No significant histopathological changes were found in the zona glomerulosa and zona reticularis of the adrenal cortex and the medulla region of the adrenal gland in either treated male or female rats of the F_0 and F_1 generations (Pereira et al. 2007c). Thyroid histology of treated animals from the F_0 and F_1 generations showed that male rats displayed follicle shrinkage, loss of thyroglobulin, and fibrosis of the interfollicular epithelium. In female rats, thyroid histology revealed shrinkage with loss of thyroglobulin and fibrosis of the interfollicular epithelium in all treated rats of both the F_0 and F_1 generations.

In addition in a later study, Pereira et al. (2008) studied the effects of DEP on the testes. Feeding rats with diets that were supplemented with 10, 25, or 50 mg/kg was equivalent to doses of 0.57, 1.425, and 2.85 mg/kg-day, respectively. After 150 days, all treatment groups exhibited reduced BWs, testis weights, and epididymis weights. Although seminal vesicle and ventral prostate weights were unaffected by DEP treatment, it was noted that serum testosterone had dose-dependently decreased in all groups and that serum androstenedione was decreased in all groups although the decreased levels did not appear to be strictly dose-dependent. However, dose-dependent increases in testicular lipid peroxidation; dose-dependent decreases in testicular superoxide dismutase, glutathione peroxidase, and glutathione reductase were found in DEP treated male rats (Pereira et al. 2008).

2.4 Mammalian Toxicity—Other

2.4.1 Mammalian Toxicity—Other: Acute Intraperitoneal

Smyth and Smyth (1931) reported that the lowest intraperitoneal dose to kill guinea pigs was 1,000 mg/kg, 4,000 mg/kg for rabbits, 1,000 mg/kg for rats, and 3,000 mg/kg for mice. However, only one to five animals per dose level were tested. Rabbits injected with 2 mL/kg intraperitoneally for 8 days experienced some temporary distress, both during and after the period of administration; however, there was no paralysis or other abnormal side effect reported. In addition, more than 50% of the applied dose was excreted in the urine. By contrast, Guinea pigs injected with 1.5 mL/kg intraperitoneally for 8 days showed no permanent ill effects at any time (Blickensdorfer and Templeton 1930). The LD_{50} value in mice that had been dosed with DEP via intraperitoneal injection was 3,220 mg/kg with a 95% confidence interval (CI) of 2,860 - 3,620 mg/kg (Lawrence et al. 1975) or 2,830 mg/kg with a 95% CI of 2,430–3,290 mg/kg (Calley

et al. 1966). In a later study with rats injected intraperitoneally with a single dose of DEP, the LD_{50} value was 5.0579 mL/kg with a 95% CI of 3.7973–6.7370 mL/kg (i.e., equivalent to 5,665 mg/kg with a CI of 4,253–7,545 mg/kg; Singh et al. 1972).

A cumulative LD_{50} value was calculated at the end of each week in a study where mice received intraperitoneal injections 5 days a week for 14 weeks (Lawrence et al. 1975). After the first week, the LD_{50} was 2.26 mL/kg (i.e., 2,531 mg/kg); however, by 14 weeks of the study, the cumulative LD_{50} value decreased to 1.39 mL/kg (i.e., 1,557 mg/kg; Lawrence et al. 1975). DEP can cross the placenta and subsequently appears in fetal tissues as a small fraction of the dose administered. Intraperitoneal injections of rats with DEP at a dose of 1.0116 mL/kg on GD5 led to maternal blood concentrations of 0.1% of the applied dose on GD8, which decreased to 0.012% by GD20. In the placenta, the concentration ranged from 0.002% of the applied dose on GD11 to 0.006% on GD17 and 0.003% on GD20. DEP was undetectable in amniotic fluid but increased to 0.003% in fetal tissue on GD20. Intraperitoneal injections of rats with DEP at a dose of 1.0116 mL/kg on GD10 led to maternal blood concentrations of 0.164% of the applied dose on GD11, which decreased to 0.008% by GD20. In the placenta, the concentration ranged from 0.003 to 0.005% of the applied dose. In addition, DEP decreased from 0.016% to undetectable levels in amniotic fluid, and decreased from 0.033 to 0.002% in fetal tissue on GD20 (Singh et al. 1975).

Increased numbers of skeletal abnormalities were seen at all dose levels when pregnant female rats received intraperitoneal injections of DEP at doses of 0.506, 1.012, 1.686 mL/kg (i.e., equivalent to 567, 1133, and 1888 mg/kg) on GD5, 10, and 15 respectively. Specifically, 12 of 16 stained fetuses showed complete or incomplete elongated and fused ribs with one fetus exhibiting curved and elongated upper and lower jawbones in the group dosed with DEP at 1.686 mL/kg. Furthermore, at 1.012 mL/kg, 8 of 17 fetuses revealed abnormal skeletal structures, especially elongated and fused ribs and incomplete skull bone formation. Five of 19 fetuses in the low dose group had elongated and fused ribs. Additionally, all dose levels led to significant reductions in fetal BWs. Doses of 0.506 mL/kg increased the number of resorptions; however, the resorptions did not increase at DEP doses of 1.012 or 1.686 mL/kg. Non-viable fetuses were not evident at any dose tested, although the number of live fetuses decreased at 0.506 mL/kg. Additionally, no gross abnormalities were seen at any of the doses of DEP studied (Singh et al. 1972).

2.4.2 Mammalian Toxicity—Other: Enzyme Effects

In vitro exposure of rat liver microsomes to 1.35 DEP significantly inhibited UDP-glucuronyltransferase activity (Gollamudi et al. 1985). This exposure did not adversely affect cytochrome P-450 or N-acetyltransferase of the rat liver *in vitro* (Gollamudi et al. 1985). In addition, intraperitoneal injection of DEP into mice at a dose of 500 mg/kg increased hexobarbital sleeping times from 46 to 88 minutes, which indicated a measurable effect on enzymes of the P-450 group that can metabolize hexobarbital (Calley et al. 1966).

Intraperitoneal injections of DEP into mice at doses of 0.287, 0.574, or 1.435 mL/kg reduced the induction time at the 0.574 mL/kg level but increased the induction time when DEP was used at a dose of 1.435 mL/kg. Induction time is the time interval between the injection of pentobarbital and the loss of the righting reflex. Animals dosed with DEP at 0.287 and 0.574 mL/kg, but not at

1.435 mg/kg reduced pentobarbital sleeping times. Sleeping time is the time interval during which the righting reflex is absent (Lawrence et al. 1975).

2.4.3 Mammalian Toxicity—Other: Mutagenicity

DEP is not mutagenic in the Ames' test using *Salmonella typhimurium* (i.e., strains TA 98, TA 100, TA 1535, and TA 1537) with and without S9 activation at concentrations of up to 10,000 µg/plate (Florin et al. 1980; NTP 1995). However, Agarwal et al. (1985) and Kozumbo et al., (1982) found that DEP was indeed positive for mutagenicity in TA 100 and TA 1535 cultures without S9 activation; however, DEP was negative with S9 activation at concentrations of up to 1,000 µg/plate. Using cultured Chinese hamster ovary cells for the sister chromatid exchange and chromosomal aberration tests with and without S9 activation, DEP was positive only with S9 activation in the sister chromatid exchange test. DEP was also negative for mutagenic effects under other conditions (NTP 1995).

2.4.4 Mammalian Toxicity—Other: Carcinogenicity

DEP was found not to initiate or promote carcinogenesis in mice (NTP 1995). Dermal application of a single dose of 0.1 mL of DEP at one time followed by 54 weeks of acetone did not provoke any form of skin lesion. However, when DEP was followed by 54 weeks of application of 12-*O*-tetradecanoylphorbol-13-acetate (which is a known promoter), it increased the incidence of acanthosis, ulceration, exudates, and hyperkeratosis. Additionally, when 0.1 mL DEP was applied three times a week for 54 weeks as a promoter following a single application of acetone, there were no increases in the incidence of acanthosis, ulceration, or exudates; however, increases in the incidence of hyperkeratosis were found. Furthermore, no increased incidences of squamous cell papilloma or squamous cell carcinoma were found (NTP 1995).

2.4.5 Mammalian Toxicity—Other: Endocrine Effects

When tested for estrogenic activity using a recombinant yeast-screen assay with concentrations of up to 0.001 M, DEP did not show any demonstrable estrogenic effects (Harris et al. 1997). Similarly, DEP competes weakly for the estrogen receptor in uterine cytosol preparations from non-pregnant rats; thus, it is considered weakly estrogenic (Blair et al. 2000). By contrast, Parveen et al. (2008) determined that DEP displays significant estrogenic activity. DEP showed a moderate correlation with gene expression caused by natural estrogen with an estrogen-like gene expression profile when human breast cancer MCF-7 cells were incubated with 10 micrometer (µM) DEP.

Studies by Kumar et al. (2014) in carbohydrate chol-cholesterol (CHO) and MCF-7 cell-lines demonstrated that DEP interacts with, and increases the transactivation of estrogenic receptors and, thus, represents a weak estrogenic molecule with effects similar to estradiol. Additionally it was found that DEP enhanced the gene expression of *pS2* with simultaneous increased activation of mitogen-activated protein kinase (MAPK) signaling as shown by an increased extracellular signal-regulated kinases (p-ERK/ERK) ratio (Kumar et al. 2014). The effects of DEP on the estrous cycle were also determined *in vivo* by daily oral gavage dosing adult female Wistar rats (45 days old) for 40 days at doses of 50 and 100 mg/kg (BW) as compared vehicle control treated animals (n = 6 per group; Kumar et al. 2014). From the uterotrophic assay

studies of immature female rats, it was found that DEP treatment increased uterine weight and enhanced luminal epithelial cell heights in both the vaginal and uterine tissues.

2.4.6 Mammalian Toxicity—Other: Testes

Oral gavage doses of 2 grams per kilograms (g/kg) to male rats aged 6–8 weeks old on two consecutive days did not affect seminiferous tubular or Leydig cell structures. However, this treatment caused mitochondrial swelling, focal dilation, and vesiculation of the smooth endoplasmic reticulum in Leydig cells (Jones et al. 1993). Additionally, oral doses of 1600 mg/kg-day administered for 4 days to young male rats did not affect testes weight or zinc content. Additionally, when DEP was used at a dose of 1600 mg/kg-day, it did not affect testicular pathology (Foster et al. 1980). When DEP was administered at 500 mg/kg-day to pregnant female rats on GD12-19, male development was unaffected. The AGD was also unaffected and gene expression profiles within the developing testes were unaltered. However, gene expression in the testes was altered by phthalates that had no effect on the development of the testes (Liu et al. 2005).

2.4.7 Mammalian Inhalation Toxicity

No information available.

2.4.8 Mammalian Dermal Toxicity

The dermal LD_{50} for DEP in rats exceeded 10 mL/kg (i.e., 11,200 mg/kg). Doses of DEP up to 10 mL/kg did not affect mortality or gross pathological changes (Consumer Product Testing, 1978). No dermal toxicity or other adverse clinical signs occurred in mice treated dermally five times a week for 4 weeks at doses of up to 123 milligrams per mouse (mg/mouse). However, doses of 62 and 123 mg/mouse increased absolute and relative liver weights (NTP 1995). Similar findings were found in rats dosed with up to 369 mg/rat five times a week for 4 weeks. Relative liver weights increased in males and females that had been dosed with 369 mg and in females that had been dosed with 184 mg. Increases in relative kidney weights were seen in males that were dosed with 184 and 369 mg as well as in females that were dosed with 184 mg (NTP 1995). It was also reported previously that a dose of 100 microliter (µL) DEP was equivalent to 123 µg (NTP 1995); however, this determination was clearly incorrect. Indeed, a dose of 100 µL DEP is equivalent to 123 mg DEP.

In a chronic study that used doses of 35 or 100 µL/mouse (i.e., 39 and 112 mg/mouse) five times a week for 32 weeks, the mice showed significant weight loss at both dose levels (NTP 1995). When doses of 7.5, 15, and 30 µL/mouse were used for 103 weeks, it was found that neither survival nor BWs were adversely affected. The incidences of basophilic foci increased in the livers of male but not female mice at doses of 15 and 30 µL/mouse. The incidence of hepatocellular adenomas increased slightly in males and females at all dose levels. The slightly increased incidence of hepatocellular adenomas and carcinomas provides equivocal evidence of carcinogenicity in mice (NTP 1995). In a companion study using doses of 0, 100, and 300 µL in rats, survival was unaffected after 15 months; however, after 2 years, the survival of all males including controls was somewhat adversely affected with survival rates of 4/50, 6/50 and 6/51 rats in the 0, 100, and 300 µg/L treatment groups respectively. Survival of female animals was

not affected in this study. It was found that mortality in males was likely as a result of benign anterior pituitary adenomas. Additionally, no changes in the BWs of male or female animals were found. Skin at the site of application showed acanthosis in 2/50, 5/50, and 21/50 of males and 8/50, 14/49, and 23/50 of females respectively at doses of 0, 100, and 300 µg/L DEP. Fatty liver degeneration was diminished in treated animals with rates of 26/50, 8/50, and 4/51 for males and 23/50, 11/50, and 3/50 in females respectively. Neoplastic findings were not found in male or female animals, and there was no evidence of carcinogenicity (NTP 1995).

Table 5. Summary of Subchronic and Chronic Dermal Toxicity for Diethyl Phthalate in Mammals

Test Organism	Test Duration	Test Results			Study
		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effects Observed at the LOAEL	
Mice	4 weeks	1150 (males) 1350 (females)	2300 (males) 2700 (females)	Increased absolute and relative liver weights	NTP 1995
Mice	32 weeks	NA (males) NA (females)	1445 (males) 1670 (females)	Decreased body weights	NTP 1995
Mice	103 weeks	280 (males) 735 (females)	560 (males) 1470 (females)	Increased relative liver and kidney weights	NTP 1995
Rats	4 weeks	410 (males) 735 (females)	820 (males) 1470 (females)	Increased relative liver and kidney weights	NTP 1995
Rats	103 weeks	410 (males) 735 (females)	820 (males) 1470 (females)	Increased relative liver and kidney weights	NTP 1995

Legend:
NOAEL: no-observed adverse effect level
LOAEL: lowest-observed adverse effect level
mg/kg-day: milligram per kilogram per day
NTP: National Toxicology Program

Dermal treatment of rabbits with 2 mL/kg of a suspension containing 0, 5, 15, or 50% DEP (equivalent to 0, 100, 300, or 1,000 mg/kg-day) on GD6-18 did not result in any signs of maternal toxicity and no conclusive effects on fetal development. Within the dose group receiving 15% DEP, two fetuses from different litters had malformations. One showed fused and split ribs and missing lumbar and coccygeal vertebrae, and the second showed acrania, hernia umbilicalis and incurved ribs.

Findings from both of these fetuses were not considered definitive evidence of a teratogenic effect, largely based on the observation that two incidents of differing fetal malformations within a single dose group is within the historical incidence of spontaneous malformation rates observed in the in-house rabbit strain (Procter & Gamble Co. 1984). In addition, severe dermal irritation was seen in rabbits at 10, 15, and 26 minutes following intradermal injections of 0.2 mL of 100 mg/mL DEP (Calley et al. 1966).

2.5 Summary of Avian Toxicology

Smyth and Smyth (1931) reported that the lowest oral dose to kill chickens was 15,000 mg/kg, and the lowest intraperitoneal dose to kill chickens was 5,000 mg/kg. Injections of 0.25 mL DEP into the yolk sac produce 67% mortality as compared with 45, 53, and 31% mortality in embryos that were injected with sesame seed oil, Crisco® oil, and those that were uninoculated. Nine of 10 hatched chicks exposed to DEP *in ovo* developed normally with the tenth chick displaying congenital malformations (Bower et al. 1970).

2.6 Summary of Amphibian Toxicology

DEP was found to competitively bind to the estrogen receptor in the livers of adult African clawed frogs (*Xenopus laevis*) with an inhibitory concentration 50 (IC_{50}) of 12,483 x 10^{-9} M. The IC_{50} for estradiol was 42 x 10^{-9} M. Thus, DEP showed weak estrogenic properties (Lutz and Kloas 1999). DEP was also found to induce malformations (incomplete gut coiling, edemas, and tail/eye malformations) at 0.1 µM and increase mRNA [messenger RNA] expression of androgen-related genes (seteroid-5α-reductase 1, steroid-5β-reductase, heat shock protein 70) between 0.1-10 µM in Western clawed frog (*Silurana tropicalis*) embryos (Bissegger, et al. 2018).

2.7 Summary of Reptilian Toxicology

No toxicological data for the effects of DEP on reptiles was located.

3. RECOMMENDED TOXICITY REFERENCE VALUES (TRV)

3.1 Toxicity Reference Values for Mammals

3.1.1 Toxicity Reference Values for Mammals—Oral

No acute toxicity studies were available that identified well-defined LD_{50} values. In subchronic studies, liver histology was affected by doses of DEP as low as 1.25 mg/kg-day in mice, and serum-borne enzymes were affected at DEP doses as low as 40 mg/kg-day. Fetal testicular testosterone was unaffected at DEP doses as high as 900 mg/kg-day in subchronic bioassays, and teratological effects were not seen until 3210 mg/kg-day.

Chronic effects of DEP on liver and serum enzymes were seen at doses as low as 0.57 mg/kg-day coupled with reduced serum testosterone. Deformed sperm are present at daily doses of 222 mg/kg-day. Chronic reproductive and developmental effects were found to range from 2.85

to approximately 1300 mg/kg-day. Up to 400-fold differences in chronic reproductive and developmental effects are found in the studies described in this document. Differences in species (rat vs. mouse), strain of animal, chemical source, or other unidentified causes may be the source of this variation. Specifically, the upper limit TRV_{High} is derived from the lowest chronic LOAEL value for the reproductive and developmental endpoints of reduced litter size and reduced weaning weight (Pereira and Rao 2007). The lower limit TRV_{Low} is derived by adjusting the chronic LOAEL using an uncertainty factor of 10 (Figure 1). Using the suggested uncertainly factors to adjust a chronic LOAEL to a NOAEL-based (TRV_{Low}) value (USACHPPM 2000) leads to a range of potential TRVs from 0.285 to 2.85 mg/kg-day. The confidence level for these TRV recommendations is based on confidence in the studies used in this assessment, the range of interspecific variation, and professional judgement (Table 6).

Table 6. Selected Ingestion TRVs for Class Mammalia

TRV	Dose	Confidence
TRV_{Low}	0.285 mg/kg-day	Medium
TRV_{High}	2.85 mg/kg-day	Medium

Legend:
TRV: Toxicity Reference Value
mg/kg-day: milligram per kilogram per day

3.1.2 Toxicity Reference Values for Mammals—Inhalation

Not available at this time.

3.1.3 Toxicity Reference Values for Mammals—Dermal

Based on subchronic and chronic toxicity studies dermal NOAELs in mice and rats ranged from 280–1350 mg/kg-day, while LOAELs ranged from 560–2700 mg/kg-day. A single acute study reported the LD_{50} of DEP was greater than 11,200 mg/kg. Based on the results of these limited studies, the selected dermal TRVs are shown below (Table 7).

Table 7. Selected Dermal TRVs for Class Mammalia

TRV	Dose	Confidence
TRV_{Low}	280 mg/kg-day	Medium
TRV_{High}	560 mg/kg-day	Medium

Legend:
TRV: Toxicity Reference Value
mg/kg-day: milligram per kilogram per day

3.2 Toxicity Reference Value for Birds

TRVs for birds are unavailable at this time due to the paucity of the toxicological data.

3.3 Toxicity Reference Values for Amphibians

TRVs for amphibians are unavailable at this time due to the paucity of the toxicological data.

3.4 Toxicity Reference Values for Reptiles

No reptilian data are available at this time.

4. IMPORTANT RESEARCH NEEDS

The lack of toxicological data across clades (e.g., birds, amphibians, and reptiles) and exposure routes (e.g., dermal, and inhalation) of DEP weakens the development of a TRV for wildlife species. Hence, additional toxicological studies of the test article and its derivatives are recommended. Most studies have focused on pharmacological end-points such as enzyme effects. Thus, studies that focus on both acute and chronic toxicity studies in mammals and non-mammalian wildlife including birds, reptiles, and amphibians are particularly warranted.

APPENDIX A

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APPENDIX B

LITERATURE REVIEW

A very broad search on June 3, 2018 using DTIC's MultiSearch function used the single search term, dimethyl phthalate. This search identified 851 specific documents.

GENERAL APPROACH

Relevant biomedical, toxicological, and ecological databases (e.g., BIOSIS, Defense Technical Information Center's (DTIC) On-Line Multisearch, and TOXNET) were electronically searched on January 4 to 6, 2018 to identify primary peer-reviewed reports of studies and reviews on the toxicology of DEPSeparate searches were conducted for general toxicology and specific searches for birds, reptiles, amphibians, and wildlife. Each database was searched using keywords that included diethyl phthalate or its CAS No. 84-66-2 and terms that included toxicity, ecotoxicology, wild, wildlife, avian, bird, frog, amphibian, or reptile. Appendix A documents the details of the search strategy.

The titles of articles identified in each search were reviewed for specific relevance. Potentially relevant articles focused on the toxicological effects of DEP on terrestrial vertebrates or its environmental fate. All potentially relevant articles were acquired as electronic files or by visiting the libraries of the University of California, Davis and the Johns Hopkins University School of Medicine libraries. Review articles provided additional articles that were not identified during searches of the initial databases.

Additional focused searches on June 3, 2018 using the DTIC's MultiSearch function, which also searches records archived and found in PubMed (National Library of Medicine, National Institutes of Health used the following terms including use of the wild-card (*) search operator:

diethyl phthalate + quail*. This search identified 4 documents.
diethyl phthalate + mallard*. This search identified 6 documents.
diethyl phthalate + bird*. This search identified 43 documents.
diethyl phthalate + avian. This search identified 17 documents.
diethyl phthalate + mouse. This search identified 22 documents.
diethyl phthalate + mice. This search identified 32 documents.
diethyl phthalate + rat. This search identified 33 documents.
diethyl phthalate + rats. This search identified 33 documents.
diethyl phthalate + mammal*. This search identified 38 documents.
diethyl phthalate + ecotox*. This search identified 33 documents.
diethyl phthalate + toxic*. This search identified 313 documents.
diethyl phthalate + amphib*. This search identified 57 documents.
diethyl phthalate + frog. This search identified 30 documents.
diethyl phthalate + reptil*. This search identified 37 documents.
diethyl phthalate + wildlife. This search identified 144 documents.

1st Draft

WILDLIFE TOXICITY ASSESSMENT FOR DIETHYL PHTHALATE

On June 3, 2018, a search of the U.S. EPA's online Ecotox database used the CAS No. 84-66-2. During this search, there were 40 hits. No references for amphibians, reptiles, or birds were identified. Twenty-seven mammalian references were found—predominantly mouse and rat as the standard test species in listed studies.

A search of the TOXLINE database [a sub-database of the National Library of Medicine's TOXNET Toxicology Data Network (https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE)] on June 3, 2018 used the CAS No. 84-66-2 as the search term. A total of 1206 articles were identified. This search was refined with:

84-66-2 AND ecotox* resulted in 43 hits
84-66-2 AND reptil* resulted in no hits
84-66-2 AND amphib* resulted in 1 hit
84-66-2 AND frog resulted in 1 hit
84-66-2 AND avian resulted in 0 hits
84-66-2 AND mallard resulted in 0 hits
84-66-2 AND quail resulted in 0 hits
84-66-2 AND bird* resulted in 3 hits
84-66-2 AND wildlife resulted in 2 hits
84-66-2 AND mammal* resulted in 56 hits
84-66-2 AND toxicity resulted in 238 hits

Independent searches of the BIOSIS Citation Index (also known as Web of Science), on June 3, 2018, used a number of keyword combinations to capture articles that might have been missed in the broader searches. These combinations were:

diethyl phthalate AND ecotox* resulted in 12 hits
diethyl phthalate AND reptil* resulted in 0 hits
diethyl phthalate AND amphib* resulted in 2 hits
diethyl phthalate AND frog resulted in 2 hits
diethyl phthalate AND avian resulted in 0 hits
diethyl phthalate AND mallard resulted in 0 hits
diethyl phthalate AND quail resulted in 0 hits
diethyl phthalate AND bird* resulted in 0 hits
diethyl phthalate AND wildlife resulted in 5 hits
diethyl phthalate AND wild* resulted in 9 hits
diethyl phthalate AND toxic* resulted in 281 hits

The different searches defined above identified many of the same articles. Additional references were identified during the review of individual articles. A total of 108 articles were reviewed.

Updated searches were performed on 28 June 2019 for additional articles published between 2018-2019 using the following online references and search strings.

- DTIC
  84-66-2; Resulted in 3 hits. None were relevant to this WTA.
  Diethyl phthalate; Resulted in 10 hits. None were relevant to this WTA.

Draft

- ECOTOX
  84-66-2; Resulted in 0 hits.
  Diethyl phthalate; Resulted in 0 hits.

- TOXLINE
  84-66-2; Resulted in 86 hits. 4 were relevant to this WTA.
  Diethyl phthalate; Resulted in 86 hits. 4 were relevant to this WTA.

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