U.S. Army Public Health Center

# Wildlife Toxicity Assessment for Dimethyl Phthalate (DMP)

## No. HEF-042019-003

**Toxicology Directorate, Health Effects Division**

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Dimethyl phthalate (DMP or 1,2-benzenedicarboxylic acid dimethyl ester), is a component of many commercial products such as paints, adhesives, personal care products, insect repellents, and non-energetic plasticizers for military applications. This wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of DMP on wildlife. Evaluating the toxicity of DMP 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).

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WILDLIFE TOXICITY ASSESSMENT FOR DIMETHYL PHTHALATE (DMP)

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

# Wildlife Toxicity Assessment for Dimethyl Phthalate

CAS No. 131-11-3 April 2020

## 1. INTRODUCTION

Dimethyl phthalate (DMP), also known as 1,2-benzenedicarboxylic acid dimethyl ester, is a component of many consumer and commercial products such as paints, adhesives, personal care products (Wormuth et al., 2006), insect repellents (Debboun et al., 2005), and non-energetic plasticizers for military applications (MIDAS 2003 in Mirecki et al., 2006). Relatively low molecular weight phthalates, such as DMP, diethyl phthalate, and dibutyl phthalate, tend to be used as solvents and in adhesives, waxes, inks, cosmetics, insecticides, and pharmaceuticals (Schettler 2006). Due to the widespread use of DMP, all populations of people, domestic animals, and wildlife regularly encounter exposure to phthalates (Schettler 2006). This Wildlife Toxicity Assessment (WTA) summarizes current knowledge of the toxicological impacts of DMP on wildlife. Evaluating the toxicity of DMP will contribute to the derivation of toxicity reference values (TRVs) for use as screening-level benchmarks 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, currently under revision) documents the protocol for the performance of this WTA.

## 2. TOXICITY PROFILE

### 2.1 Literature Review

A deliberately comprehensive and broad electronic search of the relevant biomedical, toxicological, and ecological literature included detailed review of the following self-standing or matrixed literature database hubs: BIOSIS®; DTIC On-Line Multisearch; TOXNET®; PubMed®/MEDLINE® and PubChem® (NIH Library of Medicine); Thomson-Reuters Web of Science®; EmBase™; Scopus®; ToxNet; UpToDate®; and EBSCO®-HOST:CINAHL® Plus with full-text option that contained the following searched databases: Academic Search Complete; Health Source–Academic Edition; Military and Government Collection; Worldcat®; MEDLINE®; Primary Search®; Academic Search Premier; Psych-Extra; Psych-Articles; Psych-Info. ® AGRICOLA® (acquired by the National Agricultural Library and cooperating institutions) was searched for additional literature on animal studies. The following databases were also searched: Environmental Science Database; Nutrition and Food Sciences Database; and Google Scholar™, which was interrogated for the grey literature of citations not found in sourced primary literature databases.

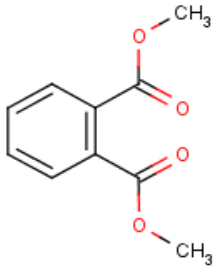
Literature searches were first conducted on July 26–27, 2010 to identify primary reports of studies and reviews on the toxicology of dimethyl phthalate. Separate searches were conducted again in March 2018 and finally, responses to reviewer comments were addressed between March and April 2019 and a systematic literature search was repeated with TOXLINE® (a

database of the National Library of Medicine's TOXNET system of clustered databases for general toxicology as well as specific searches for birds, reptiles, amphibians, and wildlife). Each database was searched by systematic nested Boolean search modifiers (OR, AND, NOT); search strings using specific search headings (defined below) were used to reveal relevant articles from the search strategy and on using the wild-card (*) search operator. Keywords that included dimethyl phthalate or its Chemical Abstracts Service (CAS) number (131-11-3) and toxicity, ecotoxicology, wild, wildlife, avian, bird, frog, amphibian, reptile, or environment were targeted. Appendix A documents the results of the nested and non-nested Boolean search strategy. The titles and abstracts (where provided) of articles identified in each systematic search strategy were reviewed for relevance. Potentially relevant articles focused on the toxicological effects on terrestrial vertebrates or environmental fate of disinfectants and dis-infection by product (DBP). All potentially relevant articles were acquired as electronic files or by visiting the University of California, Davis libraries, and interrogating the libraries of the Johns Hopkins University and School of Medicine (i.e., the Welch Medical Library and the Sheridan Libraries of Johns Hopkins University). In addition, standard review articles provided additional information that were not identified during the initial primary literature searches from the above databases.

## 2.2 Environmental Fate and Transport

DMP is used as a plasticizer for nitrocellulose and polyvinyl chloride (PVC) and cellulose acetate polymers (European Chemicals Bureau 2006; Hazardous Substances Databank, HSDB 2015; Godwin 2010). DMP has also been used in resins, rubber, and solid rocket propellants; in lacquers, plastic products, coating agents, safety glass, and in molding powders (U.S. Environmental Protection Agency 2010). DMP has also been used in automotive machine parts, electrical wiring insulation, mining and construction industries, fiberglass fabrication, paints, and as a plasticizer in children's toys (National Industrial Chemicals Notification and Assessment Scheme 2014). In addition to its former use as an insect repellent, DMP has found utility as a solvent for cosmetic products, creams, perfumes, as well as in candles, hair sprays, and shampoos (Godwin 2010). Further, DMP has been used as a solvent in fragrance bases for many household cleaning products (National Industrial Chemicals Notification and Assessment Scheme 2014). Consequently, with the industrial manufacture of a diverse array of products containing DMP, these processes might result in its release to the environment through various waste streams. Its former use as an insect repellent directly released DMP to the environment (HSDB 2015). DMP (CAS No. 131-11-3) is defined as a Low Molecular Weight Phthalate Ester (LMWPE); for chemical properties see Table I.

**Table 1. Summary of Physical-Chemical Properties of Dimethyl Phthalate**

| | |
|---|---|
| Structure |  |
| CAS No. | 131-11-3 |
| Molecular weight | 194.18 g/mol |
| Color | Colorless |
| Physical state | Oily liquid |
| Melting point | 5.5°C |
| Boiling point | 283.7°C |
| Density | 1.189 at 25°C |
| Odor | Slight aromatic odor |
| Solubility in water | 4 g/L at 25°C |
| Solubility in other solvents | Miscible with common organic solvents; e.g., ethanol, ethyl ether, ketones, esters, and chloroform. It is soluble in benzene and only slightly soluble in some mineral oils, carbon tetrachloride and practically insoluble in petroleum ether. |
| Partition coefficients: | |
| Log $K_{ow}$ | 1.60 |
| Log $K_{oc}$ | 1.74 to 2.56 in soils; > 5.2 in sediment |
| Vapor pressure at 25°C | 3.08 x $10^{-3}$ mm Hg |
| Henry's Law constant at 25°C | 1.97 x $10^{-7}$ atm-$m^3$/mole |
| Vapor density | 6.69 |
| Conversion factors | 7.93 mg/$m^3$ = approximately 1 ppm |

Source: HSDB 2015

If released to the ambient air, DMP would exist in both the vapor and particulate phases in the atmosphere. Vapor-phase DMP degrades in the atmosphere with a half-life that is estimated at 28 days (HSDB 2015), with a reported range of 9.3 to 93 days (Staples et al. 1997). Removal of particulate-phase DMP from the atmosphere is achieved by wet or dry deposition (HSDB 2015). If released to the soil, DMP has high to moderate mobility. If released into water, DMP adsorbs to suspended solids and sediment. Estimates of the hydrolysis half-life of DMP are 3.2 years under neutral conditions at 30 degrees Celsius (°C) (HSDB 2015) or 4 months at pH 8 and 30°C (Wolfe et al., 1980). In addition, the estimated half-life for direct DMP photolysis in surface waters is approximately 5 months (HSDB 2015). Kao et al., (2005) reported that approximately 15% of DMP in aquatic systems binds to suspended particulate matter.

Biodegradation half-lives of DMP in contaminated soils range from 15 to 123 days (HSDB 2015). In one aerobic study using garden soil, DMP and its primary metabolite phthalic acid were found to no longer be present by day 15 (Shanker et al. 1985). However, under anaerobic conditions, both DMP and phthalic acid were still present in the soil after 30 days (Shanker et al. 1985). Optimal conditions for aerobic degradation were found to be 37°C and pH 8.0 (Kido et al. 2007). Kido et al. (2007) found that different strains of bacteria isolated from soil varied in their abilities to degrade DMP from an observation of no degradation to complete degradation after 4 days under aerobic conditions. In another study, combinations of bacteria (*Sphingomonas paucimobilis* and *Arthrobacter* sp.) completely degraded DMP and its metabolites, monomethyl phthalate and phthalic acid, within 24 hours, with a half-life across different soil types of 3.5–30 hours (Vega and Bastide 2003). Others have reported a DMP primary metabolism half-life of about 5 days in soil, with an ultimate metabolism (e.g., including metabolites) half-life in soils that ranges from 1–40 days (Staples et al. 1997).

Experimentally determined half-lives of DMP in fresh water or sludge under aerobic or anaerobic conditions varies widely from a half-life of <1 day up to >2 months (Table 2). Major contributors to this variation include experimental conditions such as water versus (vs.) sludge, aerobic vs. anaerobic, static vs. shaken, or temperature of incubation and the deposition of microbes present in the water or sludge inoculum. Multiple studies have shown that different genera, species, or strains of bacteria have differential abilities to metabolize DMP and that metabolism occurs at different rates (e.g., Kido et al. 2007; Vega and Bastide 2003; Hashizume et al. 2002). It is likely that degradation will occur in most aqueous settings with the half-life dependent on the exact biological and environmental conditions.

**Table 2. Experimentally derived half-life values for DMP under various conditions**

| Experimental conditions | Half-life | Complete degradation | Reference |
|---|---|---|---|
| Fresh water + Aerobic | <1-10 days | | Staples et al., 1997 |
| Fresh water + Aerobic | 1-5 days[a] | 2-13 days[a] | HSDB, 2015 |
| Fresh water + Aerobic | 7 days[b] | 7 days[b] | Hashizume et al., 2002 |
| Fresh water + Aerobic | | 7 days | Tabak et al., 1997 |
| Fresh water + Anaerobic | >56 days[a,c] | | Staples et al., 1997 |
| Sludge + Aerobic | 21 hours | 5 days | Jianlong et al., 1996 |
| Sludge + Anaerobic | | 140 days | O'Connor et al., 1989 |
| Sludge + Anaerobic | >2 months[d] | | Cheung et al., 2007 |
| Sludge + Anaerobic | 16 ± 2 days | | Kleerebezem et al., 1999 |
| Sludge + Anaerobic | 38 ± 2 days | | Kleerebezem et al., 1999 |
| Sludge + Anaerobic | 17 ± 2 days | | Kleerebezem et al., 1999 |
| Sludge + Anaerobic | 24 hours | 4 days | Jianlong et al., 2000 |

Notes:
[a] Estuarine and fresh water sites (not disambiguated)
[b] Degradation ranged from 50–100% after 7 days, dependent on water source
Table 2 Notes (continued):
[c] <30% degradation after 56 days
[d] 22% degradation after 2 months under sulfate reducing conditions

## 2.3 Toxicokinetics

### 2.3.1 Absorption

Quantitative studies of the rate or quantity of DMP absorption in respiratory exposure studies that used animals were not located by literature review and represent a data gap. However, DMP was extensively absorbed by the gastrointestinal tract in rat studies (Albro and Moore 1974). In addition, DMP was readily absorbed from the gastrointestinal tract when using an *in vitro* everted gut-sac preparation from the small intestine of Sprague-Dawley rats (White et al, 1980). This study also found that DMP was extensively hydrolyzed by gut esterases to the monomethyl phthalate monoester (MMP) during absorption within the mucosal epithelium (White et al., 1980). An *in vitro* study using an everted gut-sac preparation from the Sprague-Dawley rat small intestine showed that DMP was readily absorbed from the gastrointestinal tract, following which, DMP was extensively hydrolyzed by esterase activity during absorption within the mucosal epithelium to the monoester MMP (White et al., 1980). Further*, in vitro* studies have reported dermal absorption rates of 40–50 micrograms per square centimeter per hour (µg/cm$^2$/hour) through the rat epidermis or 3 µg/cm$^2$/hour (peak rate) through pig skin (Hilton et al. 1994; as cited in National Industrial Chemicals Notification and Assessment, NICNAS Scheme 2008; Reifenrath et al. 1989; Scott et al. 1987). However, dermal absorption appeared to be highly solvent-dependent in studies conducted in rat skin models (Hilton et al. 1994; as cited in NICNAS 2008). One study comparing dermal absorption of several phthalate diesters in male F344 rats found approximately 40% of a single application of DMP to be dermally absorbed over a 7-day period under aerated occluded conditions (Elsisi et al. 1989).

### 2.3.2 Distribution

In terms of distribution, a review of the literature did not reveal any studies that had explored the distribution of DMP in animal models or indeed human subjects following oral or inhalation exposure. Moreover, one study found that 0.6% and 0.3% of a single dose of 157 micromole per kilogram (µmol/kg) DMP applied dermally to rats was found in muscle and adipose tissues, respectively, at 7 days following a single exposure (Elsisi et al. 1989). Approximately 19% of the original single dose was found in skin of the application area, with 0.4% found in skin outside of the application area. Less than 0.5% of the applied dose was detected in all other non-skin tissues examined, combined (i.e., brain, lung, liver, spleen, small intestine, kidney, blood, spinal cord, and testis). These results suggest that absorbed DMP was rapidly cleared from the organs tested with limited accumulation (Elsisi et al. 1989).

### 2.3.3 Metabolism

The metabolism of DMP can occur within the stomach, intestinal tract, and caecum contents without begin absorbed by the surrounding tissues (Rowland et al. 1977). Lake et al., (1977) measured *in vitro* metabolism of DMP by liver and intestinal tissues of male Sprague-Dawley rats, male olive baboons (*Papio anubis*), and male albino ferrets (*Putorius putorius*). Rates of hydrolysis at 37°C in intestinal tissues were 1.14–2.40 micromole per hour per milligram (µmole/hr/mg) of intestinal mucosal cell protein in the rat; 6.67 µmole/hr/mg in the baboon; and 0.05 µmole/hr/mg in the ferret. Rates of hydrolysis in liver tissues were 104–121 µmole of product formed/hr/gram of liver in the rat; 549 µmole/hr/g in the baboon; and 38.6 µmole/hr/g in

the ferret. Others have studied the *in vitro* metabolism of DMP in rats and found that liver homogenate almost completely degraded DMP within 1 hour, and kidney homogenate degraded 95% of the DMP after 5 hours (Kaneshima et al. 1978). MMP and phthalic acid represent the respective major and minor metabolites found in urine. *In vitro* studies suggest that enzymes found in liver homogenates and intestinal mucosal cell preparations from diverse animal species including rats, baboons, and ferrets or from human intestinal mucosal cells hydrolyze DMP to MMP (White et al. 1980; Lake et al. 1977). Skin homogenates also displayed hydrolytic activity, although less activity than liver homogenates (Kaneshima et al. 1978; Kozumbo et al. 1982; Kozumbo and Rubin 1991).

### 2.3.4 Excretion

In a dermal absorption study in rats, cumulative excretion of DMP in urine and feces at day 7 was approximately 40% of the total dose, with a constant daily excretion rate of 6–7.5% (Elsisi et al. 1989). In other published work, when adult rats received DMP via oral gavage, the urine at 24 hours contained approximately 45-mole percent of the dosed compound. Of the compound found in the urine, 8.1% was DMP, 14.4% was phthalic acid, and 77.5% was monomethyl phthalate. Both phthalic acid and monomethyl phthalate are metabolites of DMP. In intraperitoneally injected rats, only 0.6% of the injected DMP was recovered; no assessment of metabolites in the urine was performed in this study (Kozumbo and Rubin 1991). In terms of DMP elimination, no studies were found that explored toxicokinetics of the inhaled DMP in animal models.

## 2.4 Summary of Mammalian Toxicity

### 2.4.1 Mammalian Oral Toxicity—Acute/subacute

Per USACHPPM technical guidance (2000), acute exposures are defined as single 1-day exposure where subacute are repetitive exposures typically of 14 days or less. Available data suggest low acute toxicity in mammals. The lethal dose of 50 percent ($LD_{50}$) for adult female cluster of differentiation 1 (CD-1) mice dosed daily by oral gavage for 8 days was determined as ~6300 milligrams per kilograms/per day (mg/kg-day); body weights in survivors were not affected (Plasterer et al. 1985). Draize et al. (1948) designed a series of toxicity tests on several animal species and determined oral $LD_{50}$ values based on exposures of 10 animals/species/dose at 4–12 graded doses per species through a single dose and 6-day observation period. This group reported $LD_{50}$ values of 2.4 millilters per kilogram (mL/kg) (approx. 2,900 mg/kg) for Guinea pigs, 7.2 mL/kg (approx. 8,600 mg/kg) for mice, 6.9 mL/kg (approx. 8,200 mg/kg) for rats, and 4.4 mL/kg (approx. 5,300 mg/kg) for rabbits. In part of a repeated-dose study (Foster et al. 1980), groups of 12 male Sprague-Dawley rats (aged 3–4 weeks) were exposed daily for 4 days to DMP at 0 or 1,400 mg/kg-day by oral gavage, following which, general toxicological effects and responses of the testes to DMP exposure were examined. Body and testes weights of control and exposed rats showed no differences, and the toxicological end points were a no-observed adverse effect level (NOAEL) of 1,400 mg/kg-day with no lowest observed adverse effect level (LOAEL) available (Foster et al. 1980).

In a 1-week, feed-based study in 5-week old male JCL:Wistar rats dosed at 0 or 2% DMP in the chow, body, testes, and kidney weights were unaffected during the exposure period (Oishi and

Hiraga 1980). Liver weights were increased compared to controls. Testes of treated animals had decreased testosterone levels, and a concomitant decrease in serum concentrations of testosterone and dihydrotestosterone, but no changes in histology. There was no change in liver or kidney zinc levels under DMP treatment. In a single-dose oral study, $LD_{50}$ values of 5,740 (males), 4,390 (females), and 5,120 (combined) mg/kg for Sprague-Dawley albino rats following gavage treatment at five dose-levels with a mixture containing 85 percent DMP; the other constituent of the dosing compound were not reported (Union Carbide Group 1987 as cited in Versar Inc., 2011). Necropsy revealed mottled and red to pink lungs, glandular portion of stomachs white to red, red focal areas in some stomachs, a few gas-filled stomachs, and red intestines. See Table 2 for a summary of these data. The weight of evidence presented in this section is sufficient to conclude that DMP does not fit the definition of “highly toxic” via the acute or subacute oral exposure route under the Federal Hazardous Substances Act (FHSA) (Title 15 of the U.S. Code; Title 16 Code of Federal Regulations (CFR), Section 1500.3(c)(2)(i)(A)).

### 2.4.2 Mammalian Oral Toxicity—Subchronic

In a study by Bell et al. (1978), male rats fed diets containing 0 or 0.5% DMP for 7, 14, or 21 days experienced no effect on body weight gain, relative liver weights, or serum cholesterol; however, liver lipids and cholesterol were significantly reduced. In a 4-week oral gavage study, dosing male rats with 500 mg/kg-day of DMP did not provoke any mortality or any adverse clinical signs including salivation, body weight, or altered food consumption (Kwack et al. 2009). No effects were seen for relative weights of the thyroid, lung, heart, spleen, kidney, liver, adrenal gland, testes, and epididymis. Reduced hemoglobin was observed, but no effects were found for white or red blood cells frequency/numbers, the hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, or platelets. However, increased serological concentration of liver alkaline phosphatase (ALP) were found and was the only biochemical factor that was affected in the blood. Histological analysis of major organs was not done. Toxicological end-points for the above observations included a NOAEL of 500 mg/kg-day for general and kidney toxicities with an unavailable LOAEL, and a LOAEL of 500 mg/kg-day for toxicological effects on the liver, with an unavailable NOAEL.

#### 2.4.2.1 Mammalian Oral Toxicity—Subchronic: Reproductive Toxicity

Administration of DMP to pregnant mice on gestation days (GD) 6–13 via oral gavage did not cause any maternal mortality at a dose of 3,500 mg/kg-day but did lead to a 28% mortality rate when DMP was administered at 5,000 mg/kg-day. No changes in maternal body weight, birth weights, numbers of viable litters, live-born pup numbers per litter, or pup weight gain were seen following exposure to DMP at doses of 3,500 or 5,000 mg/kg-day DMP through day 3 (Hardin et al. 1987). In a second very similar study using a single dose of 3500 mg/kg-day administered on GD 7 to 14, a mortality rate of 3% was found in pregnant female mice. No other effects were found for body weight, and all pregnant females delivered pups. No effects were found in terms of the average number of live or dead pups per litter, or the average pup body weight on days 1 or 3 (Plasterer et al. 1985). From both studies, toxicological end-points for the above reported observations resulted in a NOAEL of 3,500 mg/kg-day and a LOAEL of 5,000 mg/kg-day for general maternal toxicological effects; a NOAEL of 5,000 mg/kg-day was derived for developmental toxicity effects. A LOAEL was not determined from this data set (Hardin et al. 1987; Plasterer et al. 1985).

Feeding pregnant rats with dietary concentrations of DMP ranging from 200 to 3,570 mg/kg-day on GD 6 to 15 caused no treatment-related mortality among the female rats (Field et al., 1993). The 5.0% DMP in the diet treatment group had decreased maternal body weight on GD 9, but no reproductive effects were attributable to DMP treatment; although, markedly increased maternal relative liver weights were observed, and one dam in this group resorbed all fetuses. In the 1.0% DMP in the diet treatment group, one female gave a litter with only a single dead fetus (Field et al. 1993). Others found that on treating five pregnant female rats with 750 mg/kg-day DMP via oral gavage during the period from GD 14 to post-natal day (PND), three had no subsequent effect on female body weight or body weight gain. The remaining four females had live pups on PND 2 and at weaning. Additionally, the number of live pups, pup weight at birth, and pup weight at weaning was unaffected (Gray et al. 2000). In a recent study, timed pregnant Sprague-Dawley® rats were dosed via oral gavage at 900-mg/kg-day DMP from GD 17–21. Similar to previous reports, no differences in litter size, fetal loss, resorptions, or maternal weight were reported, and there was no induction of multinucleated germ cells or *ex vivo* testosterone production in fetal testis (Spade et al. 2018).

Kwack et al. (2009) reported no effects on any sperm analytical endpoints including average path velocity, straight-line velocity, curvilinear velocity, amplitude of the lateral head displacement, beat cross frequency, straightness, and linearity. DMP did not adversely affect the testes when approximately 1,390 mg/kg-day was administered daily via oral gavage for 10 days to 3-week-old rats (Gray and Butterworth 1980). Additionally, oral DMP concentrations of 1,400 mg/kg-day that were administered for 4 days to young male rats did not affect testes weight, zinc content, or testicular pathology (Foster et al. 1980). No studies that explored single- or multi-generational reproductive toxicity in laboratory animals exposed to DMP were located in the primary literature, but the studies that are presented here indicate a low likelihood of any reproductive toxicity caused by DMP.

#### 2.4.2.2 Mammalian Oral Toxicity—Subchronic: Developmental Toxicity

Treatment of pregnant female rats with 750-mg/kg-day DMP via oral gavage from GD 14 to PND 3 did not impact the age of puberty. No effect from exposure was seen in the context of changes to the weights of the testes, seminal vesicles, ventral prostate, glans penis, epididymis, or other organs; no effect on nipple development was seen in male rats (Gray et al. 2000). When 500 mg/kg-day of DMP was administered to pregnant female rats on GD 12 to 19, male fetal development and the anogenital distance were unaffected, and gene expression patterns within the developing testes were unchanged (Liu et al. 2005). Therefore it is unlikely that DMP results in developmental toxicity, but there is a dearth of available data.

### 2.4.3 Mammalian Oral Toxicity—Chronic

In the only chronic repeated-dose toxicity study identified from the literature, Lehman (1955 in the Cosmetic Ingredient Review Committee, CIRC 1985) reported on the exposure of female rats (unspecified strain) receiving a diet that contained 2, 4, and 8% DMP over 2 years. Dietary concentrations of 4 and 8% DMP caused slight but significant decreased body weight gain in female rats. Chronic nephritis was found in rats that were fed 8% DMP in the diet. None of these dietary concentrations increased mortality rates. Assuming 250-gram (g) body weights of the

rats and the allometric equation for food intake recommended by the U.S. Environmental Protection Agency (U.S. Environmental Protection Agency, 1988), these dietary concentrations converted to approximately 180, 360 and 720 mg/kg-day.

**Table 3. Summary of Oral Toxicity studies for Dimethyl Phthalate in Mammals used in TRV determination**

| | | Test Results | | | |
|---|---|---|---|---|---|
| Test Organism | Test duration | NOAEL (mg/kg-day) | LOAEL (mg/kg-day) | Effects Observed at the LOAEL | Reference |
| CD-1 mice (female) | 8 days | 1,750 | 3,500 | Mortality, no effect on body weights<br>$LD_{50}$ = 6,282 | Plasterer et al., 1985 |
| Guinea pigs<br>Mice<br>Rats<br>Rabbits | 6 days | NA<br>NA<br>NA<br>NA | NA<br>NA<br>NA<br>NA | $LD_{50}$ = 2,866<br>$LD_{50}$ = 8,597<br>$LD_{50}$ = 8,239<br>$LD_{50}$ = 5,254 | Draize et al., 1948[a] |
| CD-1 mice (female) | GD 6-13 | 3,500 | 5,000 | Maternal mortality (27.9%) | Hardin et al., 1987 |
| Sprague-Dawley rats (female) | GD 6-15 | 840 | 3,570 | Increased maternal relative liver weights; reduced maternal body weight | Field et al., 1993 |
| Rats (female) | 2 years | 180<br>360 | 360<br>720 | Decreased weight gain<br>Nephritis | Lehman, 1955 |

Note: Single dose, followed by 6 days of observation

### 2.4.4 Mammalian Toxicity—Other

#### 2.4.4.1 Mammalian Toxicity—Other: Intraperitoneal

The $LD_{50}$ in mice that were dosed with DMP via intraperitoneal injection was calculated to be 1,580 mg/kg with a 95% confidence interval (CI) of 980 to 1,990 mg/kg (Calley et al. 1966). In a study conducted with rats/mice that were injected with a single intraperitoneal dose of DMP, the $LD_{50}$ was 3, 375 mg/kg in rats and 3, 980 mg/kg in mice (Singh et al. 1972). Dosing pregnant female rats via intraperitoneal injection of 0.338, 0.675, or 1.125 ml/kg DMP (i.e., at equivalent doses of 404, 806, and 1343 mg/kg) on GD 5, 10, and 15 produced an increased number of resorptions at 0.338 and 1.125 ml/kg but not at the 0.675 ml/kg dose level. An increased number of dead fetuses was seen at 1.125 ml/kg, and a decreased number of live fetuses was seen at doses of 0.338 and 1.125 ml/kg (Singh et al. 1972). Increased numbers of gross and skeletal abnormalities resulted when pregnant female rats received intraperitoneal injections of DMP at 0.338, 0.675, 1.125 mL/kg (equivalent to doses of 404, 806, and 1343 mg/kg) on GD 5, 10, and 15. In the group that received 1.125 mL/kg, three fetuses were noted to lack tails or were missing both eyes with another fetus that displayed a normal tail but lacked both eyes (Singh et al. 1972). While an intraperitoneal dosing route is unlikely to be directly relevant to exposures occurring in natural populations, these data do show some evidence that DMP may

be a direct reproductive and developmental toxicant. Based on findings presented in the reproductive and developmental toxicity subsections above, there is most likely moderation of these effects via normal absorption, distribution, metabolism and excretion (ADME) processes.

#### 2.4.4.2 Mammalian Toxicity—Other: Carcinogenicity

The National Toxicology Program (NTP, 1995) studied cancer initiation and promotion potentials of DMP in mice following dermal exposure to DMP. Applying DMP one time at 0.1 ml followed by acetone treatment to test for the cancer initiating potential of DMP did not increase the development of any form of skin lesion. When applied as an initiator, DMP increased the incidence of microscopic calculi. NTP researchers concluded that DMP did not initiate skin carcinogenesis. Repeatedly applying 0.1 ml of DMP (i.e., three times per week for 54 weeks) as a promoter did not affect body weight. However, irritation and ulceration developed at application sites, although no increased incidences of squamous cell papilloma or squamous cell carcinoma were found (NTP, 1995).

#### 2.4.4.3 Mammalian Toxicity—Other: Endocrine Effects

DMP failed to occupy the human estrogen receptor (Nakai et al. 1999) but weakly competed for the estrogen receptor in uterine cytosol preparations from nonpregnant rats and was shown to be weakly estrogenic (Blair et al., 2000). Endocrine effects, such as the mild estrogenic activity of DMP, may affect reproductive and developmental trajectories in wildlife species.

### 2.4.5 Studies Relevant for Mammalian TRV Development for Ingestion Exposures

There are five major studies identified in the literature that were used to develop a TRV for ingestion exposure (Table 3). These studies spanned acute, subchronic, and chronic durations in four species across two taxonomic orders. Within these studies, there were at least 2 chronic LOAELs and 1 chronic NOAEL. These fulfill the minimum criteria to complete a TRV based on USACHPPM technical guidance (2000).

### 2.4.6 Mammalian Inhalation Toxicity

Very few studies were retrieved that report on inhalation exposures of DMP in mammals. From the available work, no mortality was found among rats inhaling a saturated vapor of DMP for 6 hours/day; however, additional details with regard the design and experimental outcomes from this study were lacking (Levinskas 1973; as cited in National Industrial Chemicals Notification and Assessment Scheme 2014). The same report described a respiratory study in cats with no mortality (e.g., NOAEL) following inhalation of a mist containing 2.0mg DMP/L and one of two cats that inhaled a mist containing 10.2 mg DMP/L died (Levinskas 1973; as cited in National Industrial Chemicals Notification and Assessment Scheme, 2014); however, the number of animals tested in this study was very small.

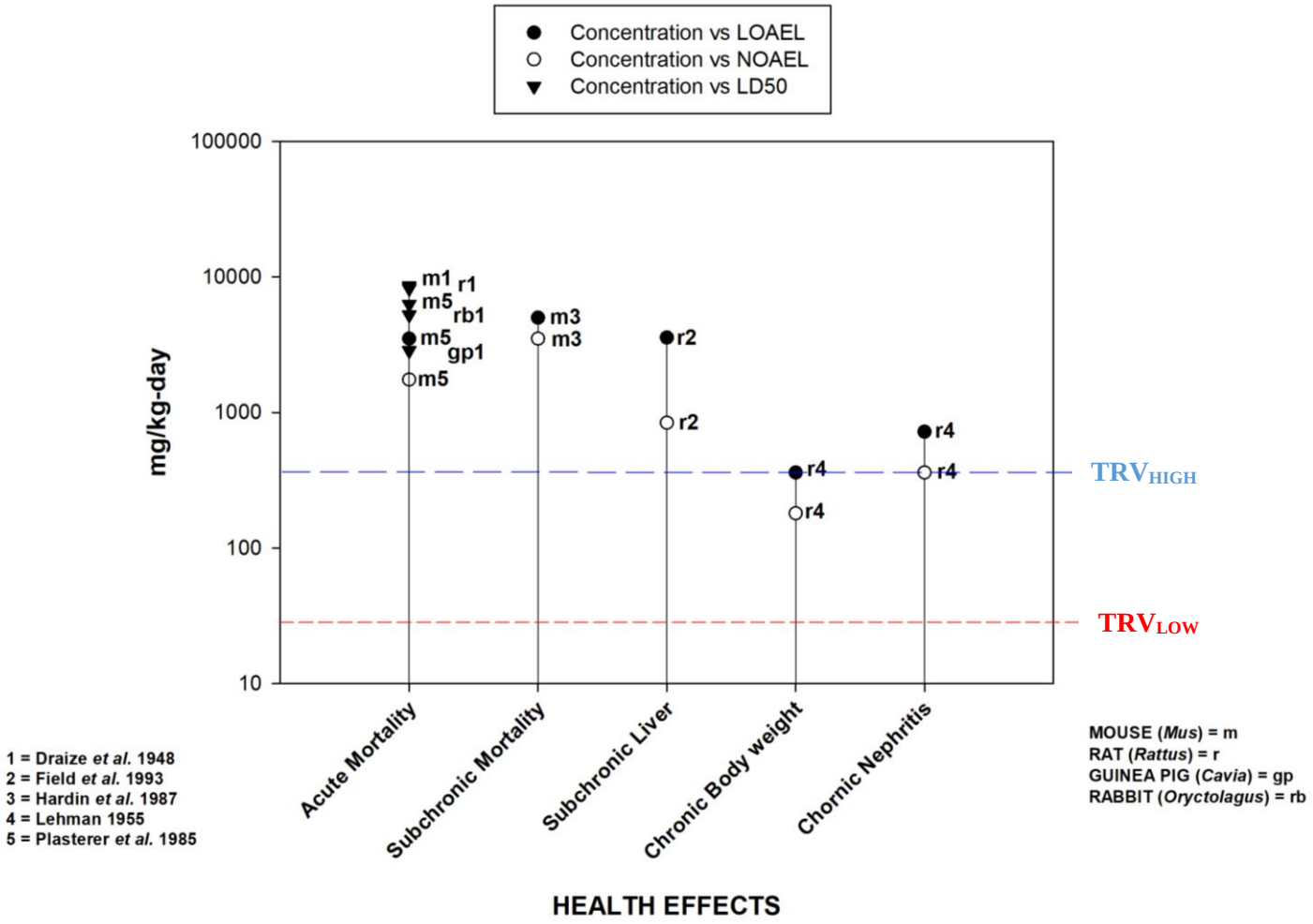



**Figure 1. Dimethyl Phthalate: Health Effects to Animals**

### 2.4.7 Mammalian Dermal Toxicity

In a 52-week study where male Swiss CD-1 mice were treated dermally with 0.1 mL of undiluted DMP 5 times per week, the incidence of skin acanthosis, ulceration, exudates, or hyperkeratosis was not markedly elevated as compared with controls (NTP 1995). Repeated application (25 total applications) of 4 mL undiluted DMP/kg to the shaven abdomen of rabbits under occluded conditions for 33 days failed to provoke significant skin irritation or histopathological findings (Dow Chemical Company 1946).

Similarly, no significant primary irritation of the skin of exposed rabbits was seen following single concentration and 90-day repeated exposures to DMP of intact or abraded skin (with the notable exception of molting areas) of the animals (Lehman 1955; Draize et al. 1948) or in guinea pigs with intact or abraded skin following dermal applications of 0.05 mL DMP (Dupont, 1970). The weight of evidence (including sufficient human data, which was not described in any detail in this WTA) of animal studies supports the conclusion that DMP fails to fit the definition of "corrosive" as outlined in the FHSA (16 CFR Section1500.3(c)(3)). Moreover, taking into account that there are currently insufficient methodological details of the approaches used and few animal studies, the available data supports the conclusion that there is "inadequate evidence" for the designation of DMP as a "primary dermal irritant" as aligned to the criteria contained in FHSA (16 CFR Section1500.3(c)(4)).

In acute toxicological studies, Draize et al. (1948) reported an acute dermal $LD_{50}$ of more than 10 mL/kg (i.e., 11,940 mg/kg using the reported density of 1190 kilograms per cubic meter ($kg/m^3$) for DMP) in rabbits. By contrast, more recent studies (European Commission, 2000) have listed dermal $LD_{50}$ values that range from >4,800 mg/kg in guinea pigs to 38,000 mg/kg in rats. In addition, pure DMP applied 10 times to the ear of rabbits had no effect, but it produced slight hyperemia and scaled skin effects after 2 days of being applied to the belly (Dow Chemical Company 1953). Thus, sufficient information was provided in the above referenced animal studies to show that all $LD_{50}$ values were greater than the dermal $LD_{50}$ range of 200–2,000 mg/kg that is required by the FHSA to conclude that a chemical is acutely toxic. Consequently, DMP also does not align to the definition of an "acutely toxic" chemical via the dermal route of exposure under the FHSA (16 CFR Section1500.3(c)(2)(i)(C)).

In a 90-day dermal toxicity study, an $LD_{50}$ of greater than 4 ml/kg (4,776 mg/kg) was reported (Draize et al. 1948). In the 90-day study, pulmonary edema was reported in addition to slight kidney damage. In surviving animals, varying degrees of nephritis were seen at the higher two concentration levels, including the highest dose of 4 ml/kg-day. Dermatitis was not evident, but mild symptoms of systemic toxicity were present at 4 ml/kg-day. In irritation studies, DMP was found not to be irritating to the skin; however, DMP was irritating to the mucus membranes (i.e., eye and penile mucosa; Draize et al. 1948). In the context of testing DMP in primary irritancy studies, DMP was not considered a primary dermal irritant.

Finally, in terms of dermal sensitization studies, only one rabbit study was available for consideration (Lehman 1955). In this work, sensitization to DMP was not reported following daily dermal applications. This study also failed to report any further detailed methodological approaches or results (Lehman 1955). Given the lack of methodological details the few studies

available inform us that there are clearly data gaps. Thus, there is insufficient evidence to designate DMP as a “strong sensitizer” as aligned to FHSA (16 CFR Section1500.3(c)(5)).

### 2.5 Summary of Avian Toxicity

Draize et al. (1948) reported that the oral $LD_{50}$ in chicks was 8.5 mL/kg (i.e., 10,149 mg/kg). Lee et al. (1974) demonstrated physical deformities (brain and spinal cord malformations, changes in embryonic vascularization, clubbed feet), neurological effects (reduced righting response, altered locomotion and coordination), and death in a series of *in vitro* and *in ovo* studies. These results differed from other *in ovo* studies that reported adverse effects not observed in Lee et al. (1974; Bower et al. 1970).

Authors have suggested that differences in observed effects may be due to the egg injection technique rather than the direct toxicity of DMP. Based upon this limited information, DMP was concluded not to be acutely toxic to birds when exposed by the oral route of administration; however, longer duration oral exposure studies with laying females are needed to address the discrepancies found in the *in ovo* studies.

### 2.6 Summary of Amphibian Toxicity

No toxicological data for the effects of DMP on amphibians were located.

### 2.7 Summary of Reptilian Toxicity

No toxicological data for the effects of DMP on reptiles were located.

## 3. RECOMMENDED TOXICITY REFERENCE VALUES

### 3.1 Toxicity Reference Values for Mammals

#### 3.1.1 Toxicity Reference Values for Mammals—Oral

Five acute toxicity tests have $LD_{50}$ values ranging from 2,866 mg/kg in Guinea pigs to 8,597 mg/kg in mice. Only one test includes a NOAEL and LOAEL. Plasterer et al. (1985) report a NOAEL for mortality of 1,750 mg/kg and a LOAEL for mortality of 3,500 mg/kg with mice. Two subchronic oral toxicity tests include a single study with mice and one study with rats. The LOAEL in the rat study (Field et al. 1993), based on increased maternal relative liver weights, is 3570 mg/kg-day following a 9-day dosing period. The NOAEL in this same study was 840 mg/kg-day leaving too great a difference between these two concentrations to consider this a reliable estimate of a NOAEL for oral toxicity. Hardin et al. (1987) reported a LOAEL that was based on maternal mortality of 5,000 mg/kg-day following a 7-day dosing period. A single chronic oral toxicity test (Lehman 1955 in the CIRC 1985), reported a LOAEL that was based on body weight to be 360 mg/kg-day and a NOAEL of 180 mg/kg-day. In this same study, the LOAEL that was based on chronic nephritis is 720 mg with a NOAEL of 360 mg/kg-day.

**Table 4. Selected Ingestion TRVs for Class Mammalia**

| TRV | Concentration | Confidence |
|---|---|---|
| $TRV_{Low}$ | 29 mg/kg | Medium |
| $TRV_{High}$ | 360 mg/kg | Medium |

No single endpoint is well-represented within this set of studies with the possible exception of mortality. Using the suggested uncertainly factors for acute, subchronic, or chronic endpoints (USACHPPM, 2000) leads to a range of potential TRV's from 29 to 360 mg/kg-day.

Specifically, the $TRV_{High}$ is derived from the lowest chronic LOAEL value for body weight effects, and the $TRV_{Low}$ is derived from an acute $LD_{50}$ value in guinea pigs with an uncertainty factor of 100, which becomes the most sensitive endpoint in this assessment (Figure 1). 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 4).

It should be noted that the above studies were thoroughly interrogated with the intention of those studies being considered for Benchmark Dose (BMD) modeling; however, it was determined that a paucity of quality dose-response data, poorly described study design, or even missing technical information (e.g., in the case of Lehman 1955), precluded BMD analysis and TRV development for DMP at this time. Further, the hazard database for DMP consisted predominantly of a very low number of quality "subchronic" and developmental toxicity studies. As mentioned above, the study by Lehman 1955 was poorly described/detailed. It was determined that additional studies have satisfactorily described the acute toxicological effects of single DMP exposures, and those studies were adequately described in this WTA. In the context of TRV development for DMP, toxicity data associated with DMP exposure were quite limited. The few identified reliable NOAEL and LOAEL values for developmental or repeated-dose systemic toxicity from DMP exposure were analyzed and described in this WTA.

### 3.1.2 Toxicity Reference Values for Mammals—Inhalation

Additional studies are required to provide sufficient data for TRV derivation.

### 3.1.3 Toxicity Reference Values for Mammals—Dermal

Draize et al. (1948) provided the only estimates of dermal toxicity in mammals, but the range of concentrations are not provided in either the acute or 90-day exposure tests. Also, these studies fail to identify $LD_{50}$ values with estimates of greater than 11,940 mg/kg in the acute study and greater than 4,776 mg/kg in the 90-day study. Therefore, these studies do not provide sufficient information to determine a TRV for dermal exposure.

## 3.2 Toxicity Reference Values for Birds

The only oral avian toxicity study was performed by Draize et al. (1948) and does not report the range of concentrations but only provides an $LD_{50}$ value of 10,149 mg/kg. The remaining avian

toxicity test involve *in vitro* or *in ovo* exposures and are not appropriate for TRV derivation. Thus, insufficient data are available an avian TRV.

### 3.3 Toxicity Reference Values for Amphibians

Not available.

### 3.4 Toxicity Reference Values for Reptiles

Not available.

### 3.5 Important Research Needs

The lack of data on the toxicity of DMP to wildlife species weakens confidence in the development of a TRV. Hence, more toxicological studies of the compound are recommended—particularly repeat-dose chronic exposure toxicological studies. In addition, no studies had investigated the effects of DMP on wild mammal or avian species, at least to the final literature review cross-check conducted on April 2, 2019. Studies were not found that investigated the impacts of DMP exposure on amphibians or reptiles—and this represents a significant data gap in TRV development. Moreover, herpetofauna are very likely to be impacted by DMP due to the ease of waterborne transport (i.e., via adsorption) in the environment. Thus, studies that focus on acute, sub-chronic, and chronic toxicity studies on wild mammalian species as well as non-mammalian wildlife such as birds, reptiles, and amphibians are thoroughly warranted and recommended.

# APPENDIX A

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# APPENDIX B

## LITERATURE REVIEW

A very broad search was conducted on July 26, 2010 using Defense Technical Information Center(DTIC) Multisearch function used the single search term, dimethyl phthalate. This search identified 1278 documents.

Additional focused searches on July 26 and 27, 2010 using DTiC's Multisearch function used the terms (* refers to the wildcard Boolean Search String operator)—

dimethyl phthalate + quail*. This search identified 3 documents.
dimethyl phthalate + mallard*. This search identified 7 documents.
dimethyl phthalate + bird*. This search identified 25 documents.
dimethyl phthalate + avian. This search identified 7 documents.
dimethyl phthalate + mouse. This search identified 32 documents.
dimethyl phthalate + mice. This search identified 32 documents.
dimethyl phthalate + rat. This search identified 41 documents.
dimethyl phthalate + rats. This search identified 41 documents.
dimethyl phthalate + mammal*. This search identified 33 documents.
dimethyl phthalate + ecotox*. This search identified 11 documents.
dimethyl phthalate + toxic*. This search identified 121 documents.
dimethyl phthalate + amphib*. This search identified 19 documents.
dimethyl phthalate + frog. This search identified 6 documents.
dimethyl phthalate + reptil*. This search identified 10 documents.

On July 27, 2010, a search of the U.S. Environmental Protection Agency's online ECOTOX database used the CAS No. 131-11-3. No references for amphibians, reptiles, or birds were identified. A single mammalian reference was found.

A search of the TOXLINE database, a database of the National Library of Medicine's TOXNET system (http://toxnet.nlm.nih.gov), on July 26, 2010 used the CAS No. 131-11-3 as the search term. A total of 2570 articles were identified. This search was refined with—

131-11-3 and ecotox* resulted in 12 hits
131-11-3 and reptil* resulted in no hits
131-11-3 and amphib* resulted in 1 hit
131-11-3 and frog resulted in no hits
131-11-3 and avian resulted in 2 hits
131-11-3 and mallard resulted in no hits
131-11-3 and quail resulted in no hits
131-11-3 and bird* resulted in 2 hits
131-11-3 and wild* resulted in 8 hits
131-11-3 and mammal* resulted in 45 hits

Searches of the BIOSIS database, on July 27, 2010, used a number of keyword combinations to capture articles that might have been missed in the broader searches. These combinations were—

dimethyl phthalate and ecotox* resulted in 3 hits
dimethyl phthalate and reptil* resulted in 0 hits
dimethyl phthalate and amphib* resulted in 1 hits
dimethyl phthalate and frog resulted in 1 hits
dimethyl phthalate and avian resulted in 0 hits
dimethyl phthalate and mallard resulted in 0 hits
dimethyl phthalate and quail resulted in 0 hits
dimethyl phthalate and bird* resulted in 3 hits
dimethyl phthalate and wildlife resulted in 6 hits
dimethyl phthalate and wild* resulted in 12 hits
dimethyl phthalate and toxic* resulted in 124 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 75 articles were reviewed.

In addition, this WTA was revised during March 19 through April 12, 2018 to an updated version and a systematic literature search was repeated with the TOXLINE database, a database of the National Library of Medicine's TOXNET system of clustered databases found at the following URL: http://toxnet.nlm.nih.gov, using the CAS No. 131-11-3 as the search term. A total of 1098 articles were identified. This search was refined with—

131-11-3 AND ecotox* resulted in 39 hits
131-11-3 AND reptil* resulted in 04 hits
131-11-3 AND amphib* resulted in 09 hits
131-11-3 AND frog resulted in 01 hits
131-11-3 AND avian resulted in 0 hits
131-11-3 AND mallard resulted in 0 hits
131-11-3 AND quail resulted in 0 hits
131-11-3 AND bird resulted in 01 hits
131-11-3 AND wild* resulted in 07 hits
131-11-3 AND mammal* resulted in 30 hits
131-11-3 AND rat resulted in 14 hits
131-11-3 AND mouse or mice resulted in 01 hits

In addition, the TOXNET system (http://toxnet.nlm.nih.gov), using dimethyl phthalate as the search term. A total of 1098 articles were identified. This search was refined with—

dimethyl phthalate AND ecotox* resulted in 41 hits
dimethyl phthalate AND reptile* resulted in 02 hits
dimethyl phthalate AND amphib* resulted in 05 hits
dimethyl phthalate AND frog resulted in 06 hits
dimethyl phthalate AND avian resulted in 05 hits
dimethyl phthalate AND mallard resulted in 09 hits

dimethyl phthalate AND quail resulted in 08 hits
dimethyl phthalate AND bird* resulted in 11 hits
dimethyl phthalate AND wildlife resulted in 08 hits
dimethyl phthalate AND rat resulted in 159 hits
dimethyl phthalate AND mouse or mice resulted in 92 hits
dimethyl phthalate AND wild* resulted in 25 hits
dimethyl phthalate AND toxic* resulted in 580 hits

Finally, responses to reviewer comments were addressed between March and April 2019 and a systematic literature search was repeated with the TOXLINE database, a database of the National Library of Medicine's TOXNET system of clustered databases found at the following URL: http://toxnet.nlm.nih.gov, using the CAS No. 131-11-3 as the search term (date of search: April 2, 2019). A total of 1130 articles were identified.

This search was refined to capture newer publications that may not have been included in the previous literature review (April 2018) using:

131-11-3 AND 2017 resulting in 36 hits
131-11-3 AND 2018 resulting in 25 hits
131-11-3 AND 2019 resulting in 16 hits

One of the studies identified in this search was appropriate for consideration (e.g., relevance, quality, content) in this WTA and has been incorporated into the assessment.

In addition, the TOXNET system was queried (http://toxnet.nlm.nih.gov), using dimethyl phthalate as the search term and results were limited to publications in English and published in 2017-2019 (date of search: April 2, 2019). A total of 65 articles were identified. No additional studies were identified in this search to be incorporated into the assessment based on relevance, quality, and content.