CPSC Staff Statement on University of Cincinnati Report
"Toxicity Review for Bis(2-ethylhexyl) Adipate (DEHA)"[1]

October 2018

The U.S. Consumer Product Safety Commission (CPSC) contracted with the University of Cincinnati to conduct toxicology assessments for six dialkyl o-phthalate (o-DAP) substitutes: acetyl tri-n-butyl citrate (ATBC); bis(2-ethylhexyl) adipate (DEHA); di-2-ethylhexyl terephthalate (DEHT); 1,2-cyclohexanedicarboxylic acid, dinonyl ester, branched and linear (DINX); trioctyltrimellitate (TOTM); and 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate (TPIB). The reports will be used to inform staff's assessment of products that may contain these compounds and is the first step in the risk assessment process.

CPSC staff assesses a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). The FHSA is risk-based. To be considered a "hazardous substance" under the FHSA, a consumer product must satisfy a two-part definition. First, it must be "toxic" under the FHSA, or present one of the other hazards enumerated in the statute. Second, it must have the potential to cause "substantial personal injury or substantial illness during or as a proximate result of any customary or reasonably foreseeable handling or use." Therefore, exposure and risk must be considered in addition to toxicity when assessing potential hazards of products under the FHSA.

The first step in the risk assessment process is hazard identification, which consists of a review of the available toxicity data for the chemical. If it is concluded that a substance may be "toxic", then a quantitative assessment of exposure and risk is performed to evaluate whether a specified product may be considered a "hazardous substance".

The toxicity review for DEHA follows.

[1] This statement was prepared by the CPSC staff, and the attached report was produced by the University of Cincinnati for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

TOXICITY REVIEW FOR BIS(2-ETHYLHEXYL)ADIPATE (DEHA)

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1 Introduction

This report summarizes available data on the identity, physicochemical properties, manufacture, supply, use, toxicity, and exposure associated with bis(2-ethylhexyl)adipate (DEHA). It is an update of a previous contractor report to CPSC (Versar, 2010).

Literature searches for physico-chemical, toxicological, exposure, and risk information were performed in November 2017 using the CAS number and synonyms (see Appendix 1 for the full list of search terms), and using the following databases:

- EPA SRS
- PUBMED
- RTECS
- TSCATS (included in TOXLINE)
- TOXNET databases, including
    - TOXLINE
    - CCRIS
    - DART/ETIC
    - GENE-TOX
    - HSDB

Searches of the PubMed and Toxline databases covered all dates through the date of the search (November, 2017). However, studies dated up to 2007 were screened out of the library during the screening process using the Endnote files, as the current report supplements and updates a staff report prepared in 2010 (Versar, 2010). Other databases and websites were also used to identify additional key information, particularly authoritative reviews. Searches for authoritative reviews addressing general toxicity and physicochemical information were conducted with the following databases using the CAS number for DEHA and synonyms. These sites included:

- ANSES Information on Chemicals (https://www.anses.fr/en)
- ChemIDPlus (https://chem.nlm.nih.gov/chemidplus/)
- ECHA Information on Chemicals (https://echa.europa.eu/information-on-chemicals)
- EFSA (https://www.efsa.europa.eu/)
- EPA (https://www.epa.gov/)
- EPA chemistry dashboard (https://comptox.epa.gov/dashboard)
- EPA IRIS (https://www.epa.gov/iris)
- FDA (https://www.fda.gov/)
- Google

- Health Canada (https://www.canada.ca/en/health-canada.html)
- IARC (https://www.iarc.fr/)
- INCHEM (http://www.inchem.org/)
- JECFA (http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/)
- NICNAS (https://www.nicnas.gov.au/)
- NTP (https://ntp.niehs.nih.gov/)
- OECD (http://www.oecd.org/)
- WHO (http://www.who.int/en/)

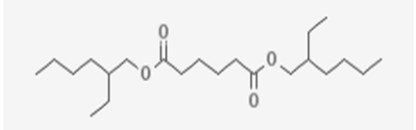
Two new DEHA toxicology studies were identified in the literature searches. These were an evaluation of ovarian toxicity, female fertility and developmental toxicity in rats (Wato et al., 2009) and a developmental toxicity study in rabbits (Anonymous, 2014, as cited by ECHA, 2018). Other new studies that were found in the primary literature included studies on toxicokinetics, exposure and mechanism of action, as well as reviews. Several of the key toxicity studies were unpublished and not available as the primary studies. Therefore, these studies were evaluated based on authoritative reviews and data compilations, including SCENIHR (2008), Danish EPA (2010), OECD (2012), ANSES (2015), ECHA (2018), and Eastman Chemical (2010).

Several additional review publications have been published since the previous CPSC assessment (Versar, 2010). Reviews and posted data from ECHA (2018) provided useful new information.

2 Physico-Chemical Characteristics

DEHA is an ester of 2-ethylhexanol and adipic acid. Physical-chemical properties for this compound are highlighted in Table 1.

Table 1: Physicochemical Properties and Identification Information for Di(2-ethylhexyl) Adipate

Chemical Name	Di(2-ethylhexyl) adipate
Synonyms	Hexanedioic acid, 1,6-bis(2-ethylhexyl) ester; Bis(2-ethylhexyl) hexanedioate; Di-(2-ethylhexyl) adipate; Dioctyl adipate; Hexanedioic acid, bis(2-ethylhexyl) ester; Adipic acid, bis(2-ethylhexyl) ester; Bis(2-ethylhexyl) adipate; DEHA; Di(2-ethylhexyl) adipate; Di(2-ethylhexyl) adipate; Di-2-ethylhexyl adipate; Hexanedioic acid, dioctyl ester; Octyl adipate
CAS Number	103-23-1
Structure	

	(EPA Chemistry Dashboard)
Chemical Formula	$C_{22}H_{42}O_4$
Molecular Weight	370.574 g/mol
Physical State	Liquid (MSDS Eastman Chemical, 2014)
Color	Colorless (MSDS Eastman Chemical, 2014)
Melting Point	-67.8°C
Boiling Point	417°C
Vapor Pressure	8.50E-07 mm Hg @ 20°C
Water Solubility	<0.005 mg/L @ 22°C (OECD SIDS)
Log Kow	6.83
Flashpoint	175°C (median; EPA Chemistry Dashboard)
Source	ChemID*plus* (unless otherwise stated)

K_{ow} is the octanol-water partition coefficient. See Appendix 2 for more detail.

DEHA is also known as dioctyl adipate (DOA), hexanedioic acid, and bis(2-ethylhexyl) hexanedioate in some documents cited below.

The vapor pressure for DEHA indicates that in the atmosphere it may exist in both the gas and particle phases. It will be removed from the air via dry and wet deposition or via degradation primarily taking place through reactions with hydroxyl radicals. Direct photolysis is also a possible degradation route, because of functional groups on the molecule that absorb UV-light (HSDB, 2008).

The water solubility of DEHA, based on a slow stir and saturator column methods, is estimated to be <0.005 mg/L (OECD SIDS). This estimate is considerably lower than the Ksol reported by HSDB (0.78 mg/L), which was likely determined by the vigorous shaking method, which can produce an emulsion rather than a solution. The lower Ksol estimate is more consistent with the solubility of other structural analogs and the high log Kow (predicted value based on structure; Table 1). DEHA has a relatively high K_{oc} value, indicating that it will sorb to organic carbon (Remberger et al., 2005). This, combined with its low vapor pressure, explains why DEHA is considered to be immobile when released to soil (HSDB, 2008). In the water environment, DEHA will sorb to particles and end up in the sediment, thus its transport via water is expected to be limited (HSDB, 2008). However, DEHA, like all adipates, is able to undergo hydrolysis, increasing its water solubility (HSDB, 2008). The BCF for DEHA is low, at 27 L/kg. In general, adipates, including DEHA, are fairly reactive substances, which readily degrade both in the environment and in organisms (Remberger et al., 2005).

3 Manufacture, Supply, and Use

Manufacture and Supply

DEHA is an EPA High Production Volume chemical, indicating an annual production volume or importation volume above 1 million pounds in the U.S. (HPVIS, 2008).

Use

DEHA is a commonly used plasticizer in lubricants, glue, scotch-tape, and sealants (Remberger et al. 2005). In particular, it is used extensively as a plasticizer in flexible polyvinyl chloride (PVC) and food contact films (Silva et al. 2013). It is also used in wire cable tubing, footwear, vinyl flooring, stationery, wood veneers, coated fabrics, gloves, artificial leather, carpet backing, and possibly toys (NICNAS, 2011; Bui et al., 2016). Unlike other adipates permitted for use as acidity regulator food additives, the U.S. FDA regulation allows DEHA only as an indirect food additive as a component of adhesives (FDA, 1999; HSDB, 2008).

As early as 2002, DEHA's presence was detected in children's soft PVC articles (Chen, 2002). In that study, the Consumer Product Safety Commission's Directorate for Laboratory Sciences purchased 41 children's products from retail stores, one of which was analytically identified as containing DEHA (Chen, 2002). However, a more recent study (Dreyfus, 2010, as cited by CPSC, 2014) did not find DEHA in any toys or childcare items. DEHA can also be found in a variety of home and office products, such as vinyl flooring, carpet backing, wood veneer, and coated fabrics (SCENIHR, 2008).

4 Toxicokinetics

Absorption

DEHA is readily absorbed in mice, rats and monkeys (ECHA, 2018). B6C3F1 mice (4/sex/dose) gavaged with a dose of 50, 500 or 5000 mg/kg ^{14}C-labeled DEHA rapidly absorbed DEHA (or its metabolites) from their GI tracts. At the low and mid doses, approximately 91% of the administered dose was eliminated in urine within 24 hours. Approximately 7-8% of the administered dose was eliminated in the feces. At the highest dose level (5000 mg/kg), approximately 75% of the administered dose was eliminated in the urine, and 4% in the feces. Although these studies indicate total oral absorption of radio-labeled ^{14}C-DEHA is ≥ 90%, these values do not indicate the systemic bioavailability of the parent DEHA itself by the oral route. That is, the amount of radiolabel absorbed is not informative as to whether the radioactivity is in the form of the parent or metabolite, and thus not informative as to the amount of parent in the blood.

DEHA has a $LogP_{O:W}$ of approximately 9, predicting low percutaneous absorption. $LogP_{O:W}$ describes the partitioning of a chemical between an aqueous phase (e.g., vehicle) and a lipid phase (e.g., stratum corneum), assuming skin permeability is directly proportional this partition coefficient. This prediction of low percutaneous absorption was supported by a study that evaluated human bioavailability of DEHA *in vitro* under conditions mimicking occlusive skin application. Doses of 5 or 100 mg DEHA, as a component of a roll-on deodorant, were applied to samples of human breast tissue. After 24 hours of continuous application, the total amount of DEHA residing in the skin depot, as well as the amount found in skin washes and the upper and lower diffusion chambers, was measured (Zhou et al., 2013). Only a small fraction (< 0.05%) of applied DHEA was found to have passed through the skin samples, with an additional 28% (low dose) to 34% (high dose) remaining within the skin samples. This finding is consistent with the prediction of low skin penetration and high retention within the skin. No experimental distinction was made between stratum corneum and deeper skin layers. It is noteworthy that mass balance analysis showed only 56 to 81% of the initial amount of DEHA applied was accounted for at the experimental conclusion (Zhou et al., 2013; ECHA, 2018).

Based on absorption rates from animal studies, CPSC estimated that transdermal absorption rates for DEHA in animals may be 5- to 10-fold greater than in adult human skin (Wester and Maibach, 1983 as cited by CPSC, 2014). Hence, it is assumed that adult human skin is 7-fold less permeable and infant skin 2-fold less permeable than rodent skin (Wormuth et al., 2006 as cited by CPSC, 2014). It is noteworthy, however, that this estimation is only valid if the absorption kinetics (the rate at which substances diffuses across the skin to reach the blood stream) exceed the dose rate (the mass load applied to an area of skin per time (mass/area*time) (Kissel, 2011). If the dose rate in the animal studies exceed the sorptive capacity of the skin, then

absorption will be saturated, in which case percutaneous absorption in humans could be greatly underestimated (CPSC, 2014).

Based on the physical and chemical properties of DEHA (low vapor pressure, high molecular weight, high Kow, and low water solubility), inhalation of DEHA is not likely unless liquid containing DEHA is aerosolized. No inhalation studies of DEHA toxicokinetics were identified.

Distribution

Following oral administration, there was no accumulation of DEHA or MEHA in blood, urine or any other tissue except the stomach.

Metabolism

In humans and in rats, orally administered DEHA is rapidly hydrolyzed to the monoester, mono-2-ethylhexyl adipate (MEHA) and adipic acid (AA). In homogenates prepared from tissues of male Wistar rats, the rate of formation of AA from DEHA was approximately the same for all tissues, whereas the appearance of MEHA was rapid only with pancreatic tissue, and was negligible in the intestine. These *in vitro* results are consistent with *in vivo* Wistar rat studies where animals were administered a single gavage dose (in corn oil) of 500 mg/kg. Following oral administration, there was no accumulation of DEHA or MEHA in blood, urine or any other tissue (except the stomach). The absence of MEHA, the authors concluded, suggests that MEHA is hydrolyzed more quickly than DEHA. Subsequent *in vitro* studies using homogenates of rat liver, pancreas and small intestinal tissue, confirmed that hydrolysis of the monoester (MEHA) to AA is indeed more rapid than hydrolysis of DEHA to AA. The study authors concluded that a significant pre-systemic hydrolysis of DEHA occurs in gastrointestinal tissue (Takahashi et al., 1981).

The metabolism of DEHA was investigated in six male volunteers, who each received a gelatin capsule of 46 mg deuterium-labeled DEHA formulated in corn oil. No volunteer showed any adverse effect and no significant changes in biochemical or hematological parameters were detected. Oxidative metabolites, not the parent DEHA, were identified in the plasma of the subjects. In the plasma, these metabolites consisted of 2-ethylhexanoic acid (2EHA). No effort was made to detect AA in the plasma or urine due to loss of the radiolabel. In the urine, the dominant metabolite was also 2EHA. It was present primarily in a conjugated form, and accounted for 8.5% of the dose. Oxidation products accounted for 3.5% of the administered dose (Loftus et al., 1993).

In a more recent study, Silva et al. (2013) evaluated the *in vitro* metabolism of DEHA using human liver microsomes. This study identified AA as the major metabolite, along with MEHA mono-2-ethylhydroxyhexyl adipate (MEHHA) and mono-2-ethyloxohexyl adipate (MEOHA), which were formed at concentrations of 1/10 to 1/1000 of adipic acid. The authors concluded

that first DEHA metabolite formed is the hydrolytic monoester MEHA, which is rapidly hydrolyzed. AA was the major metabolite of DEHA/MEHA (Silva et al., 2013)).

Elimination

The data indicate that there is little, if any, prolonged retention of DEHA or its metabolites in blood and tissue after oral administration in rodents or humans. In male Wistar rats, urinary excretion is the dominant route of elimination, followed by breath. The amount excreted in the feces was characterized as small. By 6 hours following oral administration of a single 500 mg/kg dose of ^{14}C-labeled DEHA, approximately 19% of the administered dose appeared in urine, with the largest fraction eliminated between 12 and 24 hours after dosing. Quantified DEHA metabolites together accounted for approximately 74% of the administered dose excreted in the urine within 24 hours of administration (Takahashi et al., 1981).

5 Hazard Information[2]

5.1 Acute Single Dose Toxicity

5.1.1 Acute Oral Toxicity

Lethality of DEHA by acute exposure is low by all routes. Smyth et al. (1951) determined a median LD_{50} value of 9110 mg/kg for DEHA from a single-dose oral range-finding study in rats, with a 14 day post-dose observation period. This report is limited by the absence of information on the rat strain or proportion of animals of each sex in the treatment groups. ECHA (2018) reported an unidentified non-GLP OECD Guideline-equivalent study (1955) in which variable numbers of male and female rats (strain unspecified) were orally administered DEHA without vehicle (method not described). This study identified a LD_{50} of about 19,100 mg/kg. It should be noted that nearly all animals in the LD_{50} (20.7 mL/kg) treatment group, as well as dose levels bounding this dose (16 mL/kg and 25 mL/kg) were female (14:1 female), and all of the 5 rats tested at the LD_{50} were female. This means that it is not clear whether the calculated LD_{50} also applies to males. NTP (1982) estimated LD_{50} values of 45,000 and 24,600 mg/kg in male and female F344 rats, respectively, that were given a single bolus gavage dose of DEHA in corn oil at levels ranging from 80 to 20,000 mg/kg (5/dose/sex) and observed for 14 days. Similar experiments in B6C3F1 mice yielded LD_{50} estimates of 15,000 mg/kg in males and 24,600 mg/kg in females (NTP, 1982). Effects on endpoints other than mortality were not reported in any of these studies.

[2] Where available, this report provides significance level p values in all sections. However, source secondary references often report only that a change was significant without reporting the p level. If no p level is reported in this text, the p level was not available in the cited secondary reference, but the significance is presumed to be statistical.

5.1.2 Acute Dermal Toxicity

A single-dose dermal range-finding LD_{50} value of 16,300 mg/kg was determined for DEHA in rabbits observed for 14 days (Smyth et al., 1951). Information on the dermal exposure conditions in this study was not available. A similar LD_{50} of > 8670 mg/kg bw was reported for rabbits by NICNAS without details (OECD, 2005 as cited by NICNAS, 2011[3]).

5.1.3 Acute Inhalation Toxicity

Acute inhalation data for DEHA were limited to one study that found no mortality among rats exposed for 8 hours to air saturated with DEHA vapor (Smyth et al., 1951). In an GLP-compliant 1998 study cited by ECHA (2018), no mortality was observed in male and female Wistar rats exposed to 5.7 mg/L DEHA aerosol (mass median aerodynamic diameter [MMAD] = 1.4 µm for 4 hours; observation continued for 14 days after the exposure. During the administration period and for 5 days post exposure, irregular and accelerated respiration was observed, as well as attempts to escape and piloerection. No changes in body weight or macroscopic pathological findings were observed at the end of the study (ECHA, 2018).

5.1.4 Irritation/Sensitization

Existing evidence from rabbit studies supports the conclusion that DEHA is minimally irritating to skin and eyes. In an unpublished study, rabbits receiving a single application of DEHA to intact or abraded skin in doses of 3600 - 8700 mg/kg under occlusive conditions for 24 hours showed dose-related transient mild skin irritation (slight erythema), but no systemic effects, as evaluated by clinical signs, body weight, food consumption, hematology and urinalysis during the following 14 days (CTFA, 1967).

A number of unpublished studies tested the dermal irritation and sensitization potential of DEHA in animals and humans; these have been evaluated in an authoritative assessment of the safety of DEHA as a cosmetic ingredient (Anonymous, 1984 as cited in Versar, 2010). In rabbits, primary dermal irritation studies of DEHA alone or in cosmetic formulations, as well as clinical patch tests of cosmetic formulations containing up to 9.0% DEHA in humans (including a 21-day cumulative irritancy test), indicated that DEHA is, at most, a weak skin irritant. The human patch tests of cosmetic products containing DEHA, as well as a study of unformulated DEHA in guinea pigs, also showed no induction of skin sensitization. Additionally, dermal phototoxicity tests of DEHA in humans and rabbits showed no phototoxic (primary irritant) or photoallergic reactions.

[3]Note: The NICNAS citation does not link to the correct chemical, and it is not clear whether this is really a second study or an alternative reporting of the Smyth et al. (1951) study.

Limited eye irritation data are available. In one study that is minimally described, a 0.1 mL of DEHA (concentration and vehicle not specified) was instilled into one eye of six albino rabbits, followed by a 72 hour observation period. No irritation was observed at any timepoint (ECHA, 2018).

Dermal sensitization potential of DEHA was evaluated in 10 male guinea pigs using the Draize test (GLP compliance unknown). On study Day 1, 0.05 mL of a 0.1% solution of DEHA in olive oil was administered by intracutaneous injection to the shaved back or side skin. Subsequently, 0.1 mL of a 0.1% DEHA solution was injected every other day for a total of 10 injections. Twenty-four hours after each injection, injection sites were examined for changes including height and color. Two weeks after the last injection, animals were challenged intradermally with 0.05 mL of the 0.1% DEHA solution. The dermal reaction 24 hours following the challenge injection was compared with an average of the original 10 induction scores. The area and height of the retest area was smaller and lower than the average induction reactions. It is concluded that DEHA is not sensitizing (ECHA, 2018, citing BUA, 1996). A separate study also concluded that DEHA is not sensitizing in rabbits. In this minimally described study, rabbits were induced with a single dermal injection of 100% DEHA (in mineral oil) and challenged two weeks later (Mallette and von Haam, 1952 as cited by ECHA, 2018).

5.2 Repeated Dose Toxicity

A number of repeated-dose oral studies of DEHA have been conducted in rats and mice with a primary purpose of investigating peroxisome proliferation in the liver, particularly mechanisms by which it can lead to the formation of hepatocellular tumors. Most of these studies were conducted in rats exposed to DEHA in the diet for 1-4 weeks at one exposure level in the range of 1 - 2.5% (10,000 - 25,000 ppm), i.e., at dietary concentrations comparable to those tested in the NTP (1982) chronic bioassay of DEHA in rats and mice and found to be hepatocarcinogenic in mice (see Section 5.4). A few of the studies tested mice at longer exposure durations (up to 13 weeks), multiple dietary exposure levels (ranging as low as 1500 ppm) and/or gavage exposure. As discussed by Versar (2010), effects induced by DEHA in these studies are consistent with those of di(2-ethylhexyl)phthalate (DEHP) and other hepatic peroxisome proliferators in rats and mice (Cattley et al., 1998; Chevalier and Roberts, 1998; Doull et al., 1999; IARC 2000a, 2000b; Lake, 1995). These effects include liver enlargement due to hepatocellular hypertrophy and proliferation, increased replicative DNA synthesis, increased number and size of peroxisomes (ultrastructural effects), induction of peroxisomal and microsomal fatty acid-oxidizing enzymes, alterations in hepatic lipid metabolism including inhibition of cholesterolgenesis, and reduced serum/plasma cholesterol and triglyceride levels (Barber et al., 1987; Bell, 1983, 1984; Katoh et al., 1984; Kawashima et al., 1983a, 1983b; Keith et al., 1992; Lake et al., 1997; Moody and Reddy, 1978, 1982; Reddy et al., 1986; Takagi et al., 1990, 1992; Tomaszewski et al., 1986; Yanagita et al., 1987). Peroxisome proliferation is a rodent-specific effect that is of questionable relevance to hazard characterization for humans (Cattley et al., 1998; Chevalier and Roberts,

1998; Doull et al., 1999; IARC, 2000a; Klaunig et al. 2003; Lake, 1995; Melnick 2001), as discussed further in Section 5.9.

The National Toxicology Program (NTP) evaluated DEHA for systemic toxicity in 14- and 91-day oral feeding studies of Fischer 344 rats and B6C3F1 mice (NTP, 1982). In the 14-day study, male and female Fischer 344 rats and male and female B6C3F1 mice (5/sex/dose) were fed diets containing 3100 – 50,000 ppm DEHA (males) or 6300 – 100,000 ppm for 14 days. All of the rats survived, aside from one high-dose female. Reduced food consumption (by an unspecified amount) and decreased weight gain relative to controls was observed at 50,000 ppm in males and 50,000 ppm and above in females. Females treated with 6300 or 25,000 ppm also had weight gain decreased by more than 10% relative to controls. Among the mice, the only deaths were at 100,000 ppm; none of the mice survived at this dose. Weight gains decreased by more than 10% relative to controls were observed in male and female mice fed diets containing 12,500 ppm and above. The only endpoints evaluated were survival, body weight and food consumption, and the changes in body weight were often not clearly dose-related, and so no effect levels are identified from this study.

In a 91-day mouse study (NTP, 1982), mice were administered DEHA in diet at doses of 0, 1600, 3100, 6300, 12,500 or 25,000 ppm (calculated by U.S. EPA, 1992 to correspond to approximately 0, 400, 700, 1300, 2800 and 7000 mg/kg-day). Decreased weight gain was observed at several doses, but there was large variability among doses and no dose-response, and so an effect level for decreased body weight cannot be clearly established from this study.

In the NTP (1982) study, DEHA was also administered to Fisher 344 rats in the diet for 91 days at concentrations of 0, 1600, 3100, 6300, 12,500, or 25,000 ppm (calculated by U.S. EPA, 1992 to correspond to approximately 0, 100, 200, 400, 700, or 1500 mg/kg-day). Identification of an effect level is difficult, in the absence of a clear dose-response, but decreased body weight gain of 10% or more was reported for male rats at 12,500 ppm in feed and higher (~ 700 mg/kg). In females, decreased weight gain was reported at the two top doses, but were 5.7% and 8.2%, respectively. Food consumption was not decreased. Because body weight and survival were the only endpoints evaluated, these 91-day studies are not adequate to identify a clear NOAEL.

Several studies evaluated systemic endpoints as part of an evaluation of reproductive toxicity. In Fischer 344 rats exposed to 1570 mg/kg-day of DEHA (25,000 ppm) in the diet for 4 weeks, Kang et al. reported a 50% increase in relative liver weight and a 10% decrease in body weight in males. However, no effects on serum markers of hepatotoxicity (e.g., Alanine transaminase [ALT], Aspartate transaminase [AST], Gamma-glutamyl transpeptidase [GGT]), or histological effects were observed. No hepatic effects were observed at a calculated dose of 318 mg/kg-day (Kang et al., 2006, as cited by CPSC, 2014). The high dose of 1570 mg/kg-day was considered adverse, in light of the magnitude of the liver weight change and decreased body weight.

In a 28 day study, male and female Crj:CD (SD) rats (10/sex/dose) were given DEHA in corn oil by gavage at dose levels of 0, 40, 200 or 1000 mg/kg-day for at least 28 days (Miyata et al.,

2006). In addition to the reproductive endpoints described in Section 5.5, evaluations included hematology, serum biochemistry, serum hormones (thyroid stimulating hormone [TSH], T3, T4, and reproductive hormones), and weight and histopathology of reproductive organs, endocrine-related organs, and several other major organs in both sexes.

There were no treatment-related effects on body weight. Relative liver weights were significantly increased (~20%; $p<0.01$) at 1000 mg/kg-day in both sexes, but without accompanying serum chemistry or histopathology changes. In light of the peroxisome proliferative activity of DEHA, the increased liver weight at 1000 mg/kg-day is considered potentially adverse even though no histopathological findings were reported. The authors identified a NOAEL for liver effects of 200 mg/kg-day (Miyata et al., 2006). Relative kidney weights were significantly ($p<0.01$ in males and $p<0.05$ in females) increased in both sexes at the high dose, and in males at the mid dose ($p<0.01$). Increased eosinophilic bodies and hyaline droplets were seen at the high dose in males, but not in any mid-dose males. More importantly, increased kidney weights were observed in mid-dose males in the absence of increased hyaline droplets, and in female rats at the high dose. Hyaline droplets in male rats are suggestive of male rat-related alpha-2u-globulin nephropathy, but there was no specific staining for this protein, so an association with this protein could not be verified. The observation of increased kidney weight in the absence of hyaline droplets and the increased female kidney weight together suggest that at least some of the increased kidney weight is due to some cause other than alpha-2u-globulin nephropathy. Increased kidney weights are considered adverse and relevant to humans. The NOAEL for increased kidney weight was therefore 40 mg/kg-day in males and 200 mg/kg-day in females. Significantly increased relative adrenal weight was also seen in females ($p<0.05$) at the high dose, resulting in a NOAEL for endocrine effects at 200 mg/kg-day in females; the NOAEL for endocrine effects in males was 1000 mg/kg-day.

5.3 Chronic Toxicity/Carcinogenicity

F344 rats (50/sex/dose) and B6C3F1 mice (50/sex/dose) were fed a diet containing 0, 12,000 or 25,000 ppm DEHA for 103 weeks and observed for an additional 1-3 weeks following the end of exposure (NTP, 1982). Clinical signs, survival, body weight, gross pathology, and histopathology of major tissues and organs and all gross lesions were evaluated.

Based on U.S. EPA (1988) reference values for food consumption and body weight for chronic exposure in F344 rats, estimated doses of DEHA in rats were 0, 948 and 1975 mg/kg-day for the males and 1104 and 2300 mg/kg-day for the females[4] (NTP, 1982 did not report food consumption). Mean body weights of the high-dose male and female rats were reduced

[4] Based on a food factor of 0.079 for male F344 rats in a chronic study and 0.092 for female F344 rats in a chronic study. This conversion factor was used in previous CPSC reports (Versar, 2010; CPSC, 2014). Other authoritative reviews and secondary sources used other conversions. For example, the doses were reported as 600 and 1250 mg/kg-day by ECHA (2011), as 700 and 1500 mg/kg-day by U.S. EPA (1992) for its RfD, and as 697 and 1509 mg/kg-day for males and 860 and 1674 mg/kg-day for female rats by U.S. EPA (1991) for its cancer assessment.

throughout the study by more than 10% (as estimated from graphical data). At the end of the exposure period, the mean body weights of the high-dose males and females were approximately 12 and 22% lower than controls, respectively (as estimated from growth curves). No neoplastic or non-neoplastic lesions or other compound-related adverse effects were observed in dosed rats. The high dose (1975 mg/kg-day for males and 2300 mg/kg-day for females) can be considered a LOAEL based on decreased body weight. The low dose of 948 mg/kg-day for males and 1104 mg/kg-day for females can be considered a NOAEL.

The mouse study (NTP, 1982) tested dietary DEHA concentrations of 0; 12,000; and 25,000 ppm. These correspond to estimated doses of 0, 2040 and 4250 mg/kg-day for both sexes, based on U.S. EPA (1988) reference values for food consumption and body weight[5]. Mean body weights of low- and high-dose male and female mice were lower than controls throughout the study and the decreases were dose-related. In males, there was substantial variability in the control body weight throughout the study, and so it is not clear whether the trend in the low-dose group was biologically significant; the decrease at the high dose was >10%. In female mice, the decrease was >20% compared to controls at both doses. Survival at the end of study in the control, low-dose and high-dose groups was 72, 64 and 82% in males and 84, 78 and 73% in females. There were no treatment-related non-neoplastic lesions or clinical signs of toxicity at either dose. Thus, the low dose of 2040 mg/kg-day was a NOAEL in males and a LOAEL in females, based on decreased terminal body weight compared to controls.

Liver tumors (hepatocellular carcinomas and adenomas combined) were induced in both sexes. As shown in Table 2, incidences of combined hepatocellular tumors were significantly increased in high-dose male mice and low- and high-dose female mice. The increase was dose-related in males, and statistically significant in pairwise comparisons (see Table 2 for significance levels). In comparison, the historical control incidence in male mice was 22% (range 14-30%), and in female mice was 8% (range 2-20%). Time-to-tumor analysis of the data for the female mice showed that tumor development in the dosed groups was significantly shorter ($p=0.002$) relative to the control group, whereas time-to-tumor analysis in high-dose males was not significantly different. No compound-related non-neoplastic lesions were observed in the liver or other tissues.

As discussed further in the context of mode of action (MOA), the mouse liver tumors were considered related to PPAR alpha (Lake et al., 1997), and thus not relevant to humans (Felter et al., 2018).

[5] Based on a food factor of 0.17 for both sexes. This conversion factor was used in previous CPSC reports (Versar, 2010; CPSC, 2014). Doses reported by other authoritative reviews included 1715 and 3570 mg/kg-day (ECHA, 2018), 2800 and 7000 mg/kg-day (U.S. EPA 1992, in its RfD), and 2659 and 6447 mg/kg-day for male mice and 3222 and 8623 mg/kg-day for female mice (U.S. EPA, 1991, in the cancer assessment).

Table 2. Liver Tumor Incidence in DEHA Treated Mice[a]

Dose mg/kg-day (ppm in feed)	Hepatocellular Adenoma or Carcinoma	
	Males	Females
0	13/50 (26%)	3/50 (6%)
2040 (12,000)	20/49 (41%)	19/50[c] (38%)
4250 (25,000)	27/49[b] (56%)	18/49[c] (39%)

[a]NTP (1982)
[b]Significantly different from control at p=0.003
[c]Significantly different from control at p<0.001

Carcinogenicity results of chronic feeding studies of DEHA in rats and dogs were briefly reported by Hodge et al. (1966), but without sufficient documentation. No compound-related tumors were induced in rats exposed to 0, 0.1, 0.5 or 2.5% (1000, 5000 or 25,000 ppm) DEHA in the diet for 2 years. These negative results are consistent with those of the NTP (1982) rat study summarized above, which also tested DEHA in dietary concentrations up to 25,000 ppm. In the same study, no tumors were found in dogs exposed to 0, 0.07, 0.15 or 0.2% (700, 1500 or 2000 ppm) DEHA in the diet for 1 year.

In other carcinogenesis studies conducted by Hodge et al. (1966), C3H/AnF mice (50/sex/dose) were exposed to DEHA by dermal application or subcutaneous injection. In the dermal portion of this study, weekly application of 0.1 or 10 mg of DEHA in acetone to a clipped area of back skin under non-occlusive conditions for life caused no gross or histological evidence of tumor formation at the application site. In the subcutaneous portion of the study, a single 10 mg dose of DEHA caused no injection site tumors following lifetime observation. The author of this report considers these studies to be minimally informative with regard to carcinogenicity in mice, because tumors were evaluated only at the application site (dermal) or injection site (subcutaneous exposure).

5.4 Reproductive Toxicity

DEHA has been suspected of having effects on the male reproductive system because it shares similarities in chemical structure and metabolism with DEHP, a well-documented inducer of testicular toxicity and antiandrogenic effects in rats and other laboratory animals (SCENIHR, 2007; IARC, 2000b). Young animals are much more sensitive to DEHP testicular toxicity than adults, and male rats have been shown to be particularly susceptible to antiandrogenic effects of DEHP when exposed during the perinatal period (NTP-CERHR, 2005). In contrast to DEHP, however, DEHA does not induce any adverse reproductive effects in male rats exposed perinatally, or exposed beginning as young adults (5-11 weeks) for 4, 13 or 103 weeks (Dalgaard et al., 2002, 2003; Kang et al., 2006; Miyata et al., 2006; Nabae et al., 2006, NTP, 1982).

A GLP-compliant (OECD Guideline 415) 1-generation reproductive toxicity study is available (CEFIC, 1988; ICI, 1988b, as described by ECHA, 2018; U.S. EPA, 1992; OECD, 2000). In this study, male and female Wistar rats (30 females and 15 males/dose) were administered DEHA at 0, 300, 1800, or 12,000 ppm in the diet. The males were exposed for 10 weeks premating and during mating, and the females were exposed for 10 weeks prior to mating, through mating and gestation, until the end of lactation (postnatal day; PND22). The offspring were reared to PND 36. Based on the companion developmental toxicity study, doses were 0, 28, 170 or 1080 mg/kg-day (U.S. EPA, 1992; ECHA, 2018). Histopathology evaluation for rats in the study was limited to the reproductive tissues and abnormal tissues.

In the study, there were no clinical signs of toxicity or changes in body weight or feed consumption during the premating period, and no effects on male or female fertility were observed. Adverse effects were limited to changes in body weight and liver weight at the high dose. Maternal body weight gain during gestation was described as being "marginally" reduced at the high dose, with the changes being statistically significant for a few treatment intervals. Litter size was slightly, but not significantly, reduced at the high dose, but the number of live born pups was not affected. This small change in litter size was considered incidental by the author. Mean pup weight was unaffected on PND1, but pup weight gain and total litter weight were reduced throughout the whole of the post-partum phase at the high dose. ECHA (2018) considered this decrease to be secondary to the decreased maternal weight gain. Although it is possible that there were effects on lactation (maternal weight was not recorded during lactation), the pup weight changes could be due to a direct effect, given that there was no effect on pup weight on PND1. Sporadic whole litter losses (total of 4) were noted in all exposed groups and not the controls, but were not considered treatment-related, because the incidence was low and not dose-related.

Postmortem examinations of the parental animals, conducted in males at the end of the mating period and females after weaning of the offspring, showed increased absolute and relative liver weights in both sexes at 1080 mg/kg-day. No exposure-related histopathological changes occurred in the reproductive tissues of the parental males and females (including those that failed to breed successfully), and no exposure-related gross pathologic changes occurred in the offspring. The high-dose of 1080 mg/kg-day was a systemic LOAEL, based on reduced maternal body weight gain during gestation, increased liver weight (considered to be associated with

peroxisome proliferation), and reduced pup weight gain that may have been secondary to the maternal effect. The maternal and developmental NOAEL was 170 mg/kg-day and is the basis of the U.S. EPA (1992) oral RfD together with the ICI (1988a) developmental toxicity study. ECHA (2018) also derived its DNEL of 170 mg/kg-day for the general population based on this study. The reproductive NOAEL was 1080 mg/kg-day.

In an enhanced screening assay (OECD Guideline 407), both male and female reproductive endpoints were assessed in 8-week-old Crj:CD (SD) rats (10/sex/dose) given DEHA in corn oil by gavage at dose levels of 0, 40, 200 or 1000 mg/kg-day for at least 28 (Miyata et al., 2006). Systemic effects in this study were described in Section 5.3. Males were sacrificed on day 29 and females were sacrificed in the diestrus stage on days 30-34. Evaluations included estrus cycling in females (assessed daily from day 22 until the day of sacrifice), sperm morphology and number in males, serum hormones (thyroid stimulating hormone [TSH], T3, T4, testosterone, follicle-stimulating hormone [FSH], luteinizing hormone [LH] and estradiol) and weight and histopathology of reproductive organs and endocrine-related organs, and several other major organs in both sexes. There was no effect on body weight. Reproductive effects were not observed in male rats. Ovarian follicle atresia (absence or disappearance by degeneration) was observed in 4/10 females at 1000 mg/kg-day (compared to 0/10, 0/10 and 0/9 female rats at 0, 40 and 200 mg/kg-day). Two of the four rats with ovarian follicular atresia had a prolonged estrus cycle (estrous stage durations of 4 and 10 days). Although the sample size was relatively small in this study, and there were no effects on hormone levels, these effects are treatment-related, in light of the clean background and clear difference from the background data. In addition, the prolonged estrous stage was associated with histopathological changes in the ovary. Thus, results suggest a NOAEL of 200 mg/kg-day and LOAEL of 1000 mg/kg-day for reproductive toxicity in female rats. A NOAEL of 1000 mg/kg-day and no LOAEL was identified for male reproductive toxicity in rats.

Wato et al. (2009) evaluated potential ovarian toxicity of DEHA. In a set of repeat-dose toxicity studies, DEHA was administered by gavage for 2 or 4 weeks to 6 week old Crl:CD(SD) female rats (10/dose) at doses of 0, 200, 1000 or 2000 mg/kg-day. A significant ($p<0.01$ to 0.05) decrease in relative ovary weight was observed at 2000 mg/kg-day; this effect was considered attributable to decreased corpus luteum formation. Increased large follicle atresia was observed at 1000 mg/kg-day and above following 2 and 4 weeks of dosing. General toxicological effects in treated dams included significant ($p<0.01$ to <0.001) increases in relative liver and kidney weights following 2- and 4-week dosings at doses of $\geq$1000 mg/kg-day. Red staining around the perineum was reported at 2000 mg/kg-day in the 2-week study and at 1000 mg/kg-day and above in the 4-week study, but was not additionally discussed by the study authors. A significant ($p < 0.05$) decrease was observed in the mean length of the estrous cycle at the 200 mg/kg-day dose group in the 4-week study. This effect was not dose related, however, as no decrease was observed in the 1000 and 2000 mg/kg-day dose groups following either 2 or 4 weeks of dosing. The study authors identified a NOAEL 200 mg/kg based on ovarian effects following both 2 and 4 weeks of dosing.

Wato et al. (2009) also conducted a separate female fertility and developmental toxicity study. In this study, Crl:CD(SD) female rats were gavage dosed with 0, 200, 1000 or 2000 mg/kg-day for 2 weeks before mating, throughout mating and until gestation day (GD) 7. Reproductive effects identified by Wato et al. included a significant increase in mean estrus length (≥1000 mg/kg-day, $p<0.05$), an increase in the post-implantation loss rate (1000 mg/kg-day, $p<0.05$), a decrease in the number of live embryos ($p<0.05$), and an increase in the pre-implantation loss ($p<0.01$) at 2000 mg/kg-day. Large follicle atresia, decreased corpus luteum formation and increased follicular cysts were also observed at doses of 1000 mg/kg-day and above. General maternal dose-related effects included staining around perineum (≥ 1000 mg/kg-day) and a significant decrease in body weight and body weight gain prior to the mating period (2000 mg/kg-day, $p< 0.05$), but not during gestation. Based on these data, the study authors identified a NOAEL of 1000 mg/kg for general toxicity in dams, and a NOAEL of 200 mg/kg-day for reproductive functions of dams and early embryonic development.

In other subchronic and chronic studies, no histopathological effects were observed in the reproductive organs (testes, seminal vesicles, prostate, ovary or uterus) of male or female F344 rats or B6C3F1 mice exposed to DEHA in the diet as part of general systemic toxicity studies at concentrations as high as 25,000 ppm for 13 or 103 weeks (NTP, 1982). The corresponding doses were ~1500 mg/kg-day in rats and ~7000 mg/kg-day in mice for the subchronic study, and ~2000 mg/kg-day in rats and ~4250 mg/kg-day in mice for the chronic study.

Nabae et al. (2006) and Kang et al. (2006) both investigated the testicular toxicity of DEHA in greater detail. In each study, 11-week-old male F344 rats (6/dose) were exposed to DEHA in the diet at concentrations of 0, 6000 or 25,000 ppm for 4 weeks. Nabae et al. (2006) reported average intakes of 0, 318 and 1570 mg/kg-day. Evaluations included body weight, spermatogenesis (sperm number, motility and morphology abnormalities), and relative weight and histopathology of the testes, epididymes, prostate and seminal vesicles. Both studies reported significantly reduced terminal body weight (>10%, $p<0.01$) at 1570 mg/kg-day. Significantly increased relative testes weight was also reported by Nabae et al. (2006) at 1570 mg/kg-day ($p<0.05$; 9.3% higher than controls).The author did not considered this change adverse because relative testes weight was increased rather than decreased (possibly secondary to reduced body weight) and not accompanied by abnormal spermatogenesis or testicular histopathology findings. Additionally, this effect was not induced by the same DEHA exposure in the Kang et al. (2006) study. Additional experiments by Kang et al. (2006) to evaluate the interaction of testicular and liver toxicity showed that similar DEHA exposures did not cause testicular toxicity in rats that were pretreated with thioacetamide to induce liver damage. In contrast, DEHP (25,000 ppm for 4 weeks) caused testicular toxicity (e.g., seminiferous tubule atrophy and degeneration) that was enhanced by liver damage induced by thioacetamide. Additional experiments by Nabae et al. (2006) demonstrated that DEHA exposures did not cause testicular toxicity in rats that were pretreated with five consecutive weekly subcutaneous injections of folic acid to induce chronic

renal dysfunction[6]. This was in contrast to rats treated with DEHP (25,000 ppm for 4 weeks), which caused testicular toxicity (e.g., decreased testicular weights, seminiferous tubule atrophy and diminished sperm counts) that was enhanced under conditions of renal dysfunction induced by folic acid. The high dose of 1570 mg/kg-day was a NOAEL for male reproductive toxicity of DEHA in these studies.

No multi-generation reproductive toxicity study of DEHA was located.

5.5 Prenatal, Perinatal, and Postnatal Toxicity

Dalgaard et al. (2002, 2003) conducted two studies in Wistar rats to investigate the developmental effects of prenatal and postnatal DEHA exposures; a smaller dose range-finding study (8 dams/dose) and a main study (20 dams/dose). In the range-finding study, dams were administered DEHA by gavage at dose levels of 0, 800 or 1200 mg/kg-day from GD 7 to PND 17. Evaluations included maternal clinical signs and body weight during the dosing period, pregnancy length, number and size of litters, sex distribution, body weight of pups at birth and on PND 3, postnatal survival through PND 21, anogenital distance on PND 3 and areola/nipple retention on PND 13 in male pups, and weights of testes, epididymides, ventral prostate and seminal vesicles in male pups on PND 21. Statistically significant effects included decreased maternal body weight gain during GD 7-21 ($p<0.05$), increased pregnancy length ($p<0.01$), and increased percentage of perinatal loss (defined as (number of implantations – live pups at weaning)/number of implantations) at 1200 mg/kg-day ($p<0.05$). Body weights of male and female pups were significantly decreased at birth at 1200 mg/kg-day ($p<0.05$) and on PND 3 (only PND evaluated) at ≥800 mg/kg-day ($p< 0.01$). The study found no antiandrogenic effects, but identified a LOAEL of 800 mg/kg-day and no NOAEL for developmental toxicity in rats based on decreased pup body weight. Maternal effects were reported only at 1200 mg/kg-day.

In the main study of perinatally Wistar exposed rats, dams (20/dose) were administered DEHA by gavage in peanut oil at dose levels of 0, 200, 400 or 800 mg/kg-day from GD 7 to PND 17 (Dalgaard et al., 2002, 2003). Evaluations included the endpoints assessed in the range finding study, as well as additional endpoints for onset of sexual maturation in both sexes, levels of reproductive hormones in males, sperm quality, weight and histopathology of male reproductive organs, and other organ weights. For analyses of sexual maturation, hormones and sperm quality, one male and one female from each litter were retained until adulthood. Statistically significant effects included increased gestation length at 800 mg/kg-day ($p<0.01$), decreased body weight of male and female pups at birth ($p<0.05$) and on postnatal day 3 ($p<0.01$) at 800 mg/kg-day, and a dose-related decrease in pup survival at ≥400 mg/kg-day ($p<0.01$). No androgenic endpoints were affected. Relative liver weight was significantly increased in male offspring on PND 21 at 800 mg/kg-day ($p<0.05$) but not as adults. The only statistically significant ($p<0.05$) changes in adult male offspring were decreased body and adrenal weights at 800 mg/kg-day. The study

[6] These experiments were conducted to investigate the potential for an interaction with folate, due either to impaired clearance due to an effect of folic acid on renal function, or a reproductive effect of folic acid via modification of zinc absorption.

identified a NOAEL of 200 mg/kg-day and LOAEL of 400 mg/kg-day for developmental toxicity in rats based on the increased postnatal deaths. The maternal NOAEL was 400 mg/kg-day, based on increased gestation length at a LOAEL of 800 mg/kg-day.

In an unpublished GLP-compliant developmental toxicity study, Wistar-derived female rats (24/dose) were fed diets[7] containing 0, 300, 1800 or 12,000 ppm DEHA on GD 1-22 (ICI, 1988a, as cited by Versar, 2010; ECHA, 2018). Average intake of DEHA was reported to be 0, 28, 170 or 1080 mg/kg-day. Maternal evaluations included clinical observations, body weight and food consumption throughout the study, and gross pathology following sacrifice on GD 22. Developmental endpoints evaluated included gravid uterus, litter and fetal weights, and numbers of corpora lutea, implantations (early and late intra-uterine deaths) and live fetuses. All fetuses were examined for gender, cleft palate, and external, visceral, skeletal and macroscopic brain abnormalities. Maternal effects occurred at 1080 mg/kg-day and consisted of statistically significant reductions in food consumption and body weight gain (-13%) throughout gestation. Fetal effects were observed at ≥170 mg/kg-day, and included several minor skeletal defects (e.g., partially ossified parietals of the skull) and variations indicative of slightly reduced ossification (e.g., partially ossified transverse process of the 7th cervical vertebrae) and two visceral variations involving the ureters (kinked ureter, slightly dilated ureter). The authors considered the ureter variations, as well as the reduced ossification as indicated by the minor skeletal defects and variations, to be the result of slight fetotoxicity, but ECHA (2018) considered the changes non-adverse. Based on the authors' interpretation of the results, this study identified a NOAEL of 28 mg/kg-day and LOAEL of 170 mg/kg-day for prenatal developmental toxicity in rats. EPA (1992), however, considered the developmental changes at 170 mg/kg-day to be non-adverse and classified 170 mg/kg-day as a NOAEL, and 1080 mg/kg-day as the LOAEL.

In another unpublished GLP-compliant (OECD Guideline 414, except that dosing was in diet instead of by gavage) developmental toxicity study, groups of 21-27 pregnant New Zealand White rabbits were treated with DEHA in the diet on days 6 to 29 post-coitum, at target doses of 0, 40, 80 or 160 mg/kg-day (Anonymous, 2014, as cited by ECHA, 2018). The mean actual measured intake was 0, 36, 70, and 145 mg/kg-day, although there was substantial inter-individual variability. The doses were based on a range-finding assay in which pregnant rabbits were administered DEHA in the diet at 100-1000 mg/kg-day and 300 on post-coitum days 7-29, in which severe toxicity was observed at 300 mg/kg-day. There was no maternal toxicity, based on the absence of an effect on clinical observations, body weight changes, food/water consumption, mortality and effects on ovaries and uterus. There were no toxicologically relevant effects on litter size, sex ratio, fetal body weight; or external, visceral, or skeletal malformations or variations. Thus, the high dose of 145 mg/kg-day was a maternal and developmental NOAEL.

DEHA and DEHP have the metabolite 2-ethylhexanol (2-EH) in common. Several studies used DEHA to investigate the hypothesis that 2-EH is responsible for some of the male reproductive effects of DEHP. In particular, if 2-EH causes these effects of DEHP, DEHA could hypothetically augment DEHP-induced changes in male reproductive endpoints when the two

[7] Administration in diet was a deviation for test guidelines, which recommend gavage dosing unless otherwise justified.

compounds are administered in combination, even though DEHA does not produce these effects on its own. In these studies, rats were administered either DEHP (300 or 700 mg/kg-day) or DEHP (750 mg/kg-day) in combination with DEHA (400 mg/kg-day) by gavage from GD 7 to PND 17 (Borch et al., 2004, 2005; Jarfelt et al., 2005, as cited by Versar, 2010). Exposure to DEHA alone was not tested. Examination of fetal, prepubertal and adult male offspring found that anti-androgenic and testicular effects of DEHP were not modulated by coadministering DEHA with DEHP. Endpoints evaluated in these studies included weight and histopathology of reproductive organs, testicular apoptosis, anogenital distance and nipple retention, sperm number and motility, and reproductive hormones

In a dominant lethal study, Singh et al. (1975) administered a single dose of DEHA by intraperitoneal (ip.) injection to male Harlan/ICR albino Swiss mice (n=10) at dose levels of 0.5, 1.0, 5.0 or 10.0 mL/kg immediately prior to an 8 week mating period. (The study did not provide the DEHA concentration; it is presumed to be neat.) The study reported that a single ip. injection at the highest dose tested significantly ($p<0.05$) reduced the percentage of pregnancies throughout the 8-week mating period; no effects were observed at any of the lower doses. An increased number of early fetal deaths ($p<0.1$) was observed at the two highest dose levels throughout the 8 week mating period which showed a significant relationship with dose ($p<0.01$) (Singh et al., 1975). A dose level of 922 mg/kg was identified as a NOAEL (OECD, 2000).

5.6 Genotoxicity

DEHA was negative or marginal in a variety of *in vitro* and *in vivo* genotoxicity assays.

When tested *in vitro*, DEHA did not induce gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 (Seed, 1982; Simmon et al., 1977; Zeiger et al., 1985), or in mouse lymphoma L5178Y cells in the presence or absence of exogenous metabolic activation (McGregor et al., 1988). Additionally, urine from rats that were administered daily gavage doses of 2000 mg/kg-day DEHA for 15 days was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 with or without metabolic activation (DiVincenzo et al., 1985). DEHA did not induce sister chromatid exchanges, micronuclei or chromosomal aberrations in cultured rat hepatocytes without exogenous metabolic activation (Galloway et al., 1987; Reisenbichler and Eckl, 1993). When tested in cultured Chinese hamster ovary (CHO) cells, DEHA did not induce sister chromatid exchanges with or without metabolic activation, although chromosomal aberrations were induced in the absence but not presence of metabolic activation (Galloway et al., 1987). SCENIHR (2016) noted that the CHO chromosome aberration assay was limited in that it did not report on cytotoxicity. DEHA was inactive in a BALB/c-3T3 cell transformation assay (Matthews et al., 1993). DEHA did not induce unscheduled DNA synthesis in primary rat hepatocytes incubated with DEHA (unpublished CMA studies, 1982d, as cited by OECD, 2000).

In *in vivo* tests, micronuclei were not induced in bone marrow cells from mice that were administered DEHA doses as high as 2000 mg/kg-day for 3 days by ip. injection (Shelby et al., 1993). There were also no chromosomal aberrations in bone marrow cells of mice administered

a single unspecified ip. dose (Shelby and Witt, 1995). Feeding or injection of DEHA did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Woodruff et al., 1985). Results from a dominant lethal assay in male mice administered a single high dose of DEHA (10 mL/kg) by ip. injection (Singh et al., 1975) have been characterized as positive (Versar, 2010), "slightly positive" (SCENIHR, 2016), and negative (OECD, 2000). Interpretation of this study is complicated because the authors did not report standard endpoints of pre- and post-implantation loss, even though corpora lutea were counted. Unscheduled DNA synthesis was stimulated in hepatocytes from rats administered a single 3.78 mmol/kg (1401 mg/kg) dose of DEHA by gavage (Busser and Lutz, 1987) but not from mice gavaged with a single 1000 or 2000 mg/kg dose of DEHA (Miyagawa et al., 1995).

5.7 Mechanistic Studies

DEHA was evaluated by the U.S. EPA Endocrine Disrupter Screening Program (EDSP) in a suite of 18 ToxCast estrogen receptor (ER) high-throughput screening assays. The suite includes assays that measure ER receptor binding, receptor dimerization, receptor DNA binding, gene transactivation, transcriptional expression, and cell proliferation. Cumulative suite accuracy was 93% for the 40 *in vitro* reference chemicals, and 84% to 95% for 43 *in vivo* reference chemicals with independently verified results in two or more guideline-like uterotrophic studies. Based on the cumulative results of the 18 assay suite, DEHA was identified as inactive for direct estrogen receptor activity (metabolites and other potential pathways of estrogenic activity were not evaluated) (Browne et al., 2015).

The "gold standard" for identifying potential estrogen receptor agonists is the OECD-validated Uterotrophic Bioassay (OECD Test Guideline [TG] 440) (Kleinstreuer et al., 2015). This short-term *in vivo* assay is part of the U.S. EPA endocrine disruptor screening program (EDSP) for evaluating the potential for chemicals to elicit estrogenic activity (OECD, 2007; Kleinstreuer et al., 2015). The endpoint measured is an increase in uterine weight caused by ER-mediated water imbibition and cellular proliferation in the uterine tissue. In this assay, 20-day-old immature female rats were administered DEHA (or a solvent control) subcutaneously on three consecutive days. The highest dose level was 1000 mg/kg-day, which was the maximum tolerated dose as determined from preliminary tests. DEHA binding to human ERα, and ERα– mediated gene transactivation were also evaluated. DEHA tested negative for estrogenic activity in all assays. These data are supported by *in vivo* receptor-mediated gene activation studies in transgenic mice where DEHA at doses from 30 to 100 mg/kg-day did not induce ER-mediated gene expression in any tissue (ter Veld et al., 2008). Likewise, a follow-up study showed that DEHA (100 mg/kg-day) was unable to elicit ER-mediated gene activation in fetuses of pregnant female mice (ter Veld et al., 2008). Taken together this battery of short-term *in vivo* studies, in addition to *in vitro* receptor based bioassay results, demonstrate that neither DEHA nor its metabolites, could mediate estrogenic activity either directly (i.e. by binding to the ER) or indirectly (i.e., by inhibiting enzymes that metabolize estrogen, or induce estrogen productions). Therefore,

together these assays effectively rule out both direct and indirect roles for either DEHA or its metabolites as estrogenic mediators.

Consistent with these findings, Miyata et al. (2006) showed the DEHA has no binding activity with the estrogen receptor, as detected in a yeast two-hybrid assay. It is noteworthy, however, that DEHA is rapidly hydrolyzed to multiple metabolites, especially following oral absorption, and these metabolites largely remain to be evaluated for reproductive toxicity. Miyata et al. speculated that ovarian effects observed in reproductive studies may be attributable to effects on the hypothalamic-pituitary-gonad axis. No mechanistic studies were identified that have investigated this possibility.

5.8 Mode of Action (MOA)

In rodents, peroxisome proliferation is a well-studied MOA for tumor formation. Peroxisome proliferators, such as DEHP, cause liver-related changes that include increased liver to body weight ratios due to hepatocellular hypertrophy and proliferation, increased replicative DNA synthesis, increased number and size of peroxisomes (ultrastructural effects) and induction of peroxisomal and microsomal fatty acid-oxidizing enzymes, among other changes. Overall, the weight of evidence from a large number of studies supports the existence a PPARα-dependent MOA for liver tumor formation in rodent models (Corton et al., 2014; Felter et al., 2018). As described by Felter et al. (2018), the key events for this MOA are: 1) activation of PPARα, 2) alteration of cell growth pathways, 3) alteration in hepatocyte fate including increased cell proliferation and decreases in apoptosis, and 4) clonal expansion leading to tumors.

Several studies have shown that DEHA induces peroxisome proliferation in rats and mice. The effects induced by DEHA in rats and mice are consistent with those described for DEHP and other hepatic peroxisome proliferators, as discussed in Section 5.3 of this assessment. As part of an investigation of the reasons for the differences between rats and mice in the hepatocarcinogenic potential of DEHA, Lake et al. (1997) conducted a detailed evaluation of the hepatic effects of DEHA. They found that DEHA induced a dose-dependent increase in relative liver weight and hepatic peroxisome proliferation in both species, and the magnitude of induction peroxisome proliferation was similar in both species. An increased hepatocyte labeling index, indicative of replicative DNA synthesis, was seen in both species after 1 week of treatment. However, a marked interspecies difference was observed at longer time periods (4 and 13 weeks). In rats, DEHA did not induce a sustained increase in replicative DNA synthesis at either of these time points at doses up to 4% in the diet, while increased replicative DNA synthesis was seen in mice at 1.2% and 2.5% (the highest dose tested) in the diet. These dietary levels of DEHA are the same as those that caused significant increases in hepatocellular tumors in mice, but not rats (NTP, 1982).

The data of Lake et al. (1997) are consistent with the hypothesis that the hepatocellular tumors are related to peroxisome proliferation, and specifically are due to a PPARα (peroxisome

proliferation-activated receptor alpha) dependent MOA. Even though PPARα activation in rats leads to liver tumors for other chemicals, liver tumors do not occur in DEHA-exposed rats, although they do occur in DEHA-exposed mice (NTP, 1982). In the rats, sustained increased cell proliferation does not occur, even though the first key event is activated. Furthermore, the doses at which the sustained cell proliferation was seen in mice are consistent with the dose-response for the tumors in mice.

The importance of sustained replicative DNA synthesis applies more generally to the relevance of this MOA to the human liver, which undergoes PPARα activation, but not replicative DNA synthesis. Furthermore, in humans, activation of PPARα does not lead to increased liver to body weight ratios, oxidative enzyme induction or other responses typically associated with sustained PPARα activation observed in wild-type mice (Felter et al., 2018; Ito et al., 2012). For DEHA, this conclusion is specifically supported by a key study that evaluated transgenic mice expressing human PPARα (hPPARα). When these mice were compared to wild-type mice expressing normal mouse PPARα (mPPARα), DEHA was a much weaker activator of hPPARα than mouse PPARα. Furthermore, DEHA was reported to be much less potent than DEHP at activating hPPARα (Ito et al., 2012). Although these data support a weak PPARα-mediated MOA for DEHA-induced hepatotoxicity and liver tumors in rodents, the weight of evidence supports the conclusion that a PPARα MOA is either "not relevant" or "unlikely to be relevant" in humans (Felter et al., 2018).

5.9 Lowest Hazard Endpoints by Organ System and Exposure Duration

The primary systemic effects of DEHA are increased liver weight (related to peroxisome proliferation) and decreased body weight.

Decreased body weight was frequently observed in toxicity studies. Although there was not a fully consistent pattern of effect levels, effects occurred in the 1500 – 2000 mg/kg-day dose range, regardless of study duration; some reproductive toxicity studies reported decrements in maternal body weight at doses as low as 1000 mg/kg-day (Dalgaard et al., 2002, 2003; ICI, 1988b, as cited by ECHA, 2018; U.S. EPA, 1992; OECD, 2000). Biologically significant decreases in body weight were seen at dietary levels of 25,000 ppm in rats and 12,000 ppm in female mice (about 2000 mg/kg-day for rats and female mice) exposed for 2 years (NTP, 1982), but 12,000 ppm (1080 mg/kg-day) in rats in a reproductive toxicity study (ICI, 1988b, as cited by ECHA, 2018; U.S. EPA, 1992). In reproductive toxicity studies, decreased body weight was reported at 2000 mg/kg-day in rats gavaged for about 3 weeks, including after mating (Wato et al., 2009), and at about 1600 mg/kg-day in rats exposed in the diet for 4 weeks (Nabae et al., 2006; Kang et al., 2006).

OECD (2000) noted that hepatic hypertrophy and increased peroxisomal enzyme activity can occur in rats and mice within a week of treatment with 12,000 ppm in feed (OECD 2000, citing CMA, 1982a, 1986, 1995). In a 28-day gavage study, increased liver weight was seen at 1000

mg/kg-day. Although peroxisome proliferation can occur in humans, current scientific opinion is that it does not proceed to increased liver weight or tumors in humans.

One study (Miyata et al., 2006) reported increased relative kidney weight and eosinophilic and hyaline droplets in male rats treated with 1000 mg/kg-day by gavage for 28 days. Although the finding of increased hyaline droplets suggests a connection with male rat-related alpha-2u-globulin nephropathy, there was no specific staining for this protein. More importantly, increased kidney weights were observed in males at 200 mg/kg-day in the absence of increased hyaline droplets, and in female rats at 1000 mg/kg-day. However, kidney histopathology was not reported in any other study, and the only other report of increased kidney weight was in female rats gavaged with 1000 mg/kg-day for 2 or 4 weeks (Wato et al., 2009).

Reproductive effects were not seen in rats in a 1-generation study with doses up to 12,000 ppm in the diet (about 1000 mg/kg-day). Similarly, there were no histopathological lesions in the reproductive organs in a 2-year bioassay in rats and mice at doses up to 25,000 ppm (NTP, 1982). In females, ovarian follicle atresia and prolonged estrus cycle occurred at gavage doses of 1000 mg/kg-day (Wato et al., 2009; Miyata et al., 2006). Unlike DEHP, no DEHA-induced reproductive effects have been observed in males.

DEHA is not a teratogen. Decreased litter weight and pup weight gain was seen in a 1-generaton reproductive toxicity study in rats at about 1000 mg/kg-day (ICI, 1988b, as cited by ECHA, 2018; U.S. EPA, 1992; OECD, 2000). Other minor variations were seen in the offspring of rats treated with the same dose of DEHA in the diet (ICI, 1988a, as cited by Versar, 2010; ECHA, 2018). Increased postnatal mortality was seen in the offspring of rats gavaged with 400 mg/kg-day on GD 7 to PND 17 (Dalgaard et al., 2002, 2003). No adverse effects were seen in a developmental study in rabbits at the highest tested dose of 145 mg/kg-day (Anonymous, 2014, as cited by ECHA, 2018).

Based on the weight of evidence, DEHA is not mutagenic. It did not cause gene mutations in bacterial or mammalian cells, or chromosome aberration in *in vitro* studies (Seed, 1982; Simmon et al., 1977; Zeiger et al., 1985; McGregor et al., 1988; Galloway et al., 1987; Reisenbichler and Eckl, 1993). DEHA was also marginal or negative for induction of chromosome aberrations and micronuclei *in vivo* (Shelby et al., 1993; Shelby and Witt, 1995). Interpretation of the results of a dominant lethal assay (Singh et al., 1975) is complicated by nonstandard reporting.

DEHA increased the incidence of hepatocellular adenomas and carcinomas in mice, but not rats. These tumors have been shown to be due to a PPARα-related MOA (Lake et al., 1997), and so are considered not relevant to humans (Felter et al., 2018).

5.10 Uncertainties and Data Gaps

The overall database on DEHA is extensive, including at least one of all key study types, and numerous supplemental mechanistic and specialized studies. Subchronic and chronic bioassays are available in rats and mice (NTP, 1982), although the extent of endpoint evaluation was less thorough than modern standards, particularly for the subchronic studies. Two-generation reproductive toxicity studies are not available, but a guideline-compliant unpublished 1-

generation reproductive study is available (ICI, 1988b, as cited by ECHA, 2018; U.S. EPA, 1992; OECD, 2000). This information is supplemented by specialized studies evaluating reproductive effects in males (Nabae et al., 2006; Kang et al., 2006; Dalgaard et al. 2002, 2003), females (Wato et al., 2009), or both (Miyata et al., 2006). In addition, standard guideline-compliant developmental toxicity studies are available in rats (ICI, 1988a, as cited by Versar, 2010; ECHA, 2018) and rabbits (Anonymous, 2014, as cited by ECHA, 2018).

The only significant data gap was the absence of repeated dose data via the dermal and inhalation routes.

Hazard:

Liver: The PPAR MOA is well-understood, and considered to lack human relevance. No significant uncertainties were identified.

Kidney: There is some uncertainty associated with the kidney effects of DEHA, since they were seen in only two studies (Miyata et al., 2006; Wato et al., 2009). There is also uncertainty regarding a potential association of the kidney effects with male rat-related alpha-2u-globulin nephropathy, although some similar effects were seen in female rats, or in the absence of hyaline droplets (Miyata et al., 2006).

Developmental: There is some uncertainty regarding the toxicological significance of the minor developmental variations noted in rats (ICI, 1988a, as cited by Versar, 2010; ECHA, 2018).

Cancer: The PPAR MOA is well-understood, and considered to lack human relevance. No significant uncertainties were identified.

Table 3. Summary of NOAELs/LOAELs Identified for DEHA by Organ System

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
Fischer 344 (M&F) 50/sex/dose NTP (1982)	103 weeks Diet 0, 12,000, 25,000 ppm (0, 948, 1975 mg/kg-day (M); 0, 1104, 2300 mg/kg-day (F)	Body weight	NOAEL = 948 (M), 1104 (F) LOAEL = 1975 (M), 2300 (F)	Reduced growth throughout study	Decrease estimated from graph as >10%
		Liver	NOAEL = 1975 M), 2300 (F) LOAEL = N/A		No effects reported
		Cancer	NOAEL = 1975 M), 2300 (F) LOAEL = N/A		Tumors that were noted were those seen routinely in this strain of rat, and they occurred in comparable numbers in control and dosed rats.
B6C3F1 (M&F) 50/sex/dose NTP (1982)	103 weeks Diet 0, 12,000, 25,000 ppm (0, 2040, 4250 mg/kg-day, M&F)	Body weight	NOAEL = 2040 (M), N/A (F) LOAEL = 4250 (M), 2040 (F)	Decrease compared to controls of >10%	Estimated from graph; no statistical analysis or quantitative data provided. Body weight in control males varied substantially over time.
		Liver	NOAEL = 4250 (M &F) LOAEL = N/A		No changes in liver weight or histopathology reported
		Cancer	Statistically significant increase at 4250 in males and 2040 in females; dose-related increase in males		Basis for cancer assessment (WOE and OSF) on IRIS (U.S. EPA, 1991). Increased incidence of liver tumors (combined hepatocellular adenomas and carcinomas) was observed in

[8] All effect levels as identified by the authors of this assessment. Effect levels identified by previous assessments are in the comments column. N/A = not applicable.

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
					high dose males and all treated females.
Reproductive/Developmental Toxicity					
Wistar rat (M&F) 15M+ 30F/ dose OECD Guideline 415 ICI, 1988b, as cited by ECHA, 2018; U.S. EPA, 1992; OECD, 2000	10 weeks before mating through lactation Diet 0, 300, 1800, 12,000 ppm (0, 28, 170, 1080 mg/kg-day)	Body weight	NOAEL = 170 (F) LOAEL = 1080 (F)	Decreased maternal body weight gain during gestation,	Co-principal study for the RfD on IRIS (U.S. EPA, 1992)
		Liver	NOAEL = 170 (M&F) LOAEL = 1080 (M&F)	Increased absolute and relative weight, considered associated with peroxisome proliferation	Not relevant to humans
		Developmental	NOAEL = 170 LOAEL = 1080	Decreased litter weight, and offspring weight gain	Decreased pup weight may have been secondary to decreased maternal weight gain, or may have been direct toxic effect
		Reproduction	NOAEL = 1080 (M&F) LOAEL = N/A	No effects on fertility, reproductive organs,	
Sprague-Dawley rat (M&F) 10/sex/dose Miyata et al., 2006 GLP Compliant,	28 days Gavage in corn oil 0, 40, 200, 1000 mg/kg-day	Body weight	NOAEL (M, F) = 1000 LOAEL = N/A		Study designed to investigate effects on hormones and reproductive organs in males and females. Liver weight change potentially adverse in light of peroxisome proliferative potential
		Liver	NOAEL = 200 LOAEL = 1000	Increased relative liver (M and F)	
		Kidney	NOAEL = 40 (M) LOAEL = 200 (M) NOAEL = 200 (F) LOAEL = 1000 (F)	Increased relative kidney weight. Also, increased eosinophilic and hyaline droplets	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
OECD Guideline 407 compliant		Adrenal	NOAEL = 200 (F) LOAEL = 1000 (F) NOAEL = 1000 (M) LOAEL = N/A (M)	Increased relative adrenal weight	Kidney findings are suggestive of male rat-related alpha-2u-globulin nephropathy, there was no specific staining for this protein, and increased kidney weight was seen in females and in the absence of hyaline droplets.
		Reproductive (M)	NOAEL = 200 (F) LOAEL = 1000 (F) NOAEL = 1000 (M) LOAEL= N/A (M)	No effects in males Ovarian follicle atresia and prolonged estrus cycle in females	
Sprague-Dawley rat (F) 10/dose Wato et al., 2009	2 or 4 weeks Gavage 0, 200, 1000 or 2000 mg/kg-day	Liver	NOEL = 200	Increased relative liver weight at 2 and 4 weeks	No evaluation of histopathology, but increase at 1000 was only 12%
		Kidney	NOAEL = 200 LOAEL = 1000	Increased relative kidney weight at 2 and 4 weeks	Eosinophilic change of proximal tubule were observed at 2000 mg/kg-day in the 2-week study and 1000 mg/kg-day and above in the 4-week study
		Reproductive	NOAEL = 200 LOAEL = 1000	Decrease in relative ovary weight, increased large follicle atresia	Mean estrous cycle length was reduced at 200 mg/kg-day, but not at higher doses
Sprague-Dawley (Crj:CD) rat (F)	2 weeks before mating through GD 7	Body weight	NOAEL = 1000 LOAEL = 2000	Decreased body weight and body weight gain prior to the mating period, but not during gestation	No effect on food consumption

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
10 F/dose mated to untreated males Wato et al, 2009	Gavage in Corn Oil 0, 200, 1000, 2000 mg/kg-day	Reproductive	NOAEL = 200 LOAEL = 1000	Follicle atresia in the ovary; significant increase in mean estrus cycle length; decreased corpus luteum formation and increased follicular cyst	
		Development	NOAEL = 200 LOAEL = 1000	Increased pre- and post-implantation loss, decreased number of live embryos	
Fisher 344 Rat (M) 6/dose Nabae et al., 2006	4 weeks Diet 0, 6000, 25,000 ppm (0, 318, 1570 mg/kg-day)	Body weight	NOAEL = 318 LOAEL = 1570		Study designed to investigate testicular effects Relative testes weight was increased, but not considered adverse because relative testes weight was increased rather than decreased (possibly secondary to reduced body weight) and not accompanied by abnormal spermatogenesis or testicular histopathology findings.
		Reproductive	NOAEL = 1570 LOAEL = N/A		
Fisher 344 Rat (M), 6/dose Kang et al., 2006	4 weeks Diet 0, 6000, 25,000 ppm	Body weight	NOAEL = 318 LOAEL = 1570		Study designed to investigate testicular effects Relative liver weight increased by 50% at high dose
		Reproductive	NOAEL = 1570 LOAEL = N/A		

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
	(0, 318, 1570 mg/kg-day)				No effect on testes weight
Wistar rat 8 dams/dose Dalgaard et al. 2002, 2003	GD 7 to PND 17 0, 800, or 1200 mg/kg-day Gavage in peanut oil	Maternal	NOAEL = 800 LOAEL = 1200	Decreased maternal body weight gain during GD 7-21, increased pregnancy length	
		Developmental	NOAEL = N/A LOAEL = 800	Body weight of M and F pups decreased on PND 3 (the only PND evaluated)	Increased percentage of perinatal loss at 1200 mg/kg-day. Body weight also decreased at birth at 1200 mg/kg-day. No anti-androgenic effects
Wistar Rat 20 dams/dose Dalgaard et al., 2002, 2003	GD 7 to PND 17 Gavage in peanut oil 0, 200, 400, 800, 1200 mg/kg-day 1/sex/litter retained until adulthood for measurement of sexual maturation	Maternal	NOAEL = 400 LOAEL = 800	Increased gestation length	Study designed to investigate anti-androgenic and other developmental effects of perinatal exposure; no anti-androgenic effects observed
		Developmental	NOAEL = 200 LOAEL = 400	Increased postnatal mortality	
Wistar Rat (F) 24 dams/dose	GD 1-22	Maternal effects	NOAEL = 170 LOAEL = 1080	Decreased food consumption and body	

Species (Sex), Reference	Exposure Regimen	Effect Category	Toxicological Endpoint (mg/kg-day)[8]	Toxicological Basis	Comments
ICI, 1988a, as cited by Versar, 2010; ECHA, 2018 OECD Guideline 414 and GLP compliant	Diet 0, 300, 1800 or 12000 ppm Reported by authors as 0, 28, 170, 1080 mg/kg-day			weight gain (-13%) throughout gestation	Standard teratogenicity study. Co-principal study for the RfD on IRIS (U.S. EPA, 1992). The study authors considered the low dose to be a developmental NOAEL, while EPA considered the mid dose a NOAEL, and ECHA considered the high dose a NOAEL. It is not clear how the incidence of the variations differed at the mid and high doses.
		Fetal development	NOAEL = 28-170 LOAEL = 1080	Minor skeletal variations (delayed ossification) and visceral variations (kinked ureter and slightly dilated ureter)	
New Zealand White Rabbit (F) 21 to 27/dose Anonymous (2014), as cited by ECHA (2018) OECD Guideline 414 and GLP compliant	Days 6 to 29 post-coitum Diet Concentration in diet not reported; Target doses of 0, 40, 80 and 160 mg/kg-day; actual intake was 0, 36, 70, and 145 mg/kg-day	Maternal	NOAEL = 145 LOAEL = N/A	No adverse effects.	ECHA (2018), concluded there were no adverse effects based on clinical observations, body weight changes, food/water consumption, mortality and effects on ovaries and uterus. ECHA (2018) concluded there were no toxicologically relevant effects on litter size, sex ratio, fetal body weight, external, visceral, or skeletal malformations or variations
		Developmental	NOAEL = 145 LOAEL = N/A	No adverse effects	

6 Exposure

The use of DEHA in consumer products has been described in Section 3 of this report.

There are limited data on DEHA residues in products or in environmental compartments. DEHA was found in 1 of 41 children's products in a CPSC 2002 evaluation of children's soft PVC articles (Chen, 2002), but not in a more recent study (Dreyfus, 2010, as cited by CPSC, 2014). In Japan, 8.5% of products that children often mouth or hold contained DEHA (Kawakami et al., 2011, as cited by Bui et al., 2016). Bui et al. (2016) also reported that DEHA was found in new infant crib mattress covers (4.8 mg/g material, 11.1% detection frequency) and the breathing zones of sleeping infants contained an average concentration of 8.4 μg/m^3 (Boor et al., 2015; Liang and Xu, 2014; as cited by Bui et al., 2016). Liang and Xu (2014, as cited by Bui et al., 2016) estimated emissions of DEHA from crib mattress covers at different temperatures, and used the resulting data to validate an emission model for predicting concentrations in indoor air. Using the model, they estimated a concentration of 1.05 μg/m^3 in indoor air (presumably the entire well-mixed room).

Remberger et al. (2005) reported that no adipates, including DEHA, were detected in air or human breast milk (Remberger et al. 2005). DEHA metabolites were not investigated in that study. However, a more recent study found DEHA in breast milk at 2 μg/L (additional methods information not available in the secondary source) (Palm-Cousins et al., 2007, as cited by Bui et al., 2016). Other measurements reported DEHA in dust (2–10 μg/g) and indoor air (5–15 ng/m^3) (Rudel et al., 2003, as cited by Bui et al., 2016).

DEHA is used as a plasticizer in various food storage wraps and it has been shown to migrate into stored foods; thus the general population can be exposed through consumption of foods stored in plastic films (HSDB, 2008), and this can be a major source of general population exposure. For example, in a migration study by Petersen and Naamansen (1998), DEHA migration into fresh meat from food packaging was measured between 1 and 40 mg/kg depending on fat content and number of times the meat was sliced and repacked in the DEH-containing film. Higher temperatures and microwave cooking of foods can also enhance migration (Startin et al., 1982, as cited by OECD, 2000).

Data on exposure to DEHA from toys and child care articles in the U.S. are not available (CPSC, 2014). Bui et al. (2016) summarized several studies of total DEHA intake, however. Estimates included 1 μg/kg-day for children aged 15–20 months old, from the diet (Fromme et al., 2013), infant intake of 2.35 μg/kg-day from inhalation (Liang and Xiu, 2014), adult dietary intake of 0.67 μg/kg-day (Fromme et al., 2007), and 0.46 μg/kg-day from oral, inhalation and dermal exposure of an unspecified population (Stuer-Lauridsen et al., 2001).

Fromme et al. (2007) also quantified the median dietary intake of DEHA in a European population as 0.7 μg/kg-day (27 female and 23 male subjects aged 14-60 years). The median, as well as the 95th percentile daily dietary intake, did not exceed the recommended tolerable daily intake (Fromme et al., 2007). In one-week duplicate diet samples provided by three Japanese hospitals, Tsumura et al. (2003) determined a total mean daily intake of DEHA as 12.5 μg.

Dietary exposures have also been estimated for Canadian populations as 137 to 259 μg/kg-day (Page and Lacroix, 1995; Carlson and Patton, 2012).

Inhalation of indoor air in office buildings using DEHA-containing plastics is another route of human exposure (HSDB, 2008). Based upon indoor air monitoring of an office building, the representative indoor air concentration of DEHA was determined to be 2.0 ng/m^3; the source of the DEHA exposure was thought to be from plasticizer use (HSDB, 2008).

Widespread use of DEHA has made its investigation alongside phthalates in exposure and leaching studies commonplace (Cao, 2008; Fromme et al., 2007; Kueseng et al., 2007; Tsumura et al., 2003). Additionally, heavy and widespread use in food packaging and other industries has led to widespread human exposure to this chemical (Remberger et al., 2005). In this study, conducted by the Swedish Environmental Research Institute, eight adipates were screened for in air, water, sediment, sludge, biota and human breast milk. DEHA was the only adipate frequently detected in samples. That is, it was detected in the majority of the samples, compared to the seven other adipates tested, five of which were not detected at all. Two were detected in sludge.

Only limited information was located on concentrations of DEHA in environmental media. OECD (2000) summarized environmental monitoring data collected by Felder et al. (1986) and reported by Hicks and Michael (1983). The study evaluated water and sediment samples collected at 24 sites in the US. Of the 85 water samples, 6 exceeded the detection limit of 0.2 μg/L, with a maximum reported value of 1.0 μg/L from one of four replicates at one site (and the other three replicates not exceeding the detection limit). The geometric mean concentration of

DEHA in sediments was <0.8 mg/kg dry weight. Air emission in 1994 in the U.S. was estimated as 315,000 kg (U.S. EPA, 1996, as cited by Bui et al., 2016).

Occupational exposure to DEHA occurs during its production, its use as a plasticizer, and its use as a lubricant and functional fluid (IARC, 1982). Exposure can occur through dermal contact and inhalation (IARC, 1982). OECD (2000, source not provided) reported that, based on an uncited survey of manufacturers, it is estimated that only 25-50 individuals in the US are involved in the manufacturing and handling process for DEHA. However, occupational exposure also includes users of DEHA-containing products, and so is a much larger population. The NIOSH NOES Survey (NIOSH, 1983) has statistically estimated that 15,636 workers (3,628 of these are female) are potentially exposed to DEHA in the U.S. For example, the average concentration of DEHA in the air of a meat-wrapping department of a supermarket, as a result of heating polyvinyl chloride film during meat packaging operations, was estimated to be 0.014 ppm (0.2 mg/m^3) (IARC, 1982).

The U.S. EPA has set a regulatory threshold for DEHA in water and toxicological threshold for risk purposes. The U.S. EPA Maximum Contaminant Level (MCL) for DEHA in drinking water is 0.4 mg/L (U.S. EPA, 2012), and the oral reference dose (RfD) is 0.6 mg/kg-day (U.S. EPA, 1992).

Biomonitoring

No biomonitoring data was identified for DEHA.

7 Discussion

7.1 Toxicity Under FHSA

Animal data were sufficient to support the conclusion that **DEHA does not fit the designation of "acutely toxic" under the Federal Hazardous Substances Act (FHSA) (16 CFR§1500.3(c)(2)(i)(A))** following single oral or dermal exposures. The oral LD_{50} is >20,000 mg/kg in rats (NTP, 1982). The dermal LD_{50} of DEHA in rabbits is >8000 mg/kg (Smyth et al., 1951; OECD, 2005, as cited by NICNAS, 2011), but the available studies were not well-documented. No inhalation LC_{50} is available, but no mortality occurred in rats exposed to 5.7 mg/L DEHA aerosol for 4 hours (1998 study cited by ECHA, 2018). DEHA is minimally irritating to skin and eyes (CTFA, 1967; Anonymous, 1984), and not a sensitizer (ECHA, 2018). Dermal phototoxicity tests of DEHA in humans and rabbits showed no phototoxic (primary irritant) or photoallergic reactions (Anonymous, 1984 as cited in Versar, 2010).

The systemic toxicity of DEHA following repeated dosing is low. The major effects observed are decreased body weight and increased liver weight related to peroxisome proliferation. There are also sporadic reports of increased kidney weight.

Anti-androgenic effects have not been seen with DEHA. Reproductive effects were seen only in females and were limited to ovarian follicle atresia and prolonged estrus cycle (Wato et al., 2009; Miyata et al., 2006).

DEHA is not a teratogen. Decreased litter weight and pup weight gain was seen in a 1-generaton reproductive toxicity study in rats (ICI, 1988b, as cited by ECHA, 2018; U.S. EPA, 1992; OECD, 2000), and minor variations were seen in the offspring of treated rats (ICI, 1988a, as cited by Versar, 2010; ECHA, 2018). There was also a report of increased postnatal mortality was seen in the offspring of rats gavaged on GD 7 to PND 17 (Dalgaard et al., 2002, 2003).

Based on the weight of *in vitro* and *in vivo* evidence, DEHA is not mutagenic.

DEHA is a mouse hepatocarcinogen via a PPAR MOA that is well understood (Lake et al., 1997) and not considered relevant to humans (Felter et al., 2018).

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APPENDIX 1

Search Terms Used

“Bis(2-ethylhexyl) hexanedioate” OR “Di-(2-ethylhexyl) adipate” OR “Dioctyl adipate” OR “Hexanedioic acid, bis(2-ethylhexyl) ester” OR “Adipic acid, bis(2-ethylhexyl) ester” OR “Bis(2-ethylhexyl) adipate” OR “DEHA” OR "Di(2-ethylhexyl) adipate" OR "Di(2-ethylhexyl)adipate" OR "Di-2-ethylhexyl adipate" OR “Hexanedioic acid, dioctyl ester” OR "Hexanedioic acid, 1,6-bis(2-ethylhexyl) ester" OR “Octyl adipate” OR (103-23-1)

APPENDIX 2

Explanation of Physico-chemical Parameters

The organic carbon normalized solid-water partition coefficient (K_{oc}), also known as the organic carbon adsorption coefficient, is defined as the ratio of the chemical's concentration in a state of sorption (i.e. adhered to soil particles) and the solution phase (i.e. dissolved in the soil water). K_{oc} is crucial for estimating a chemical compound's mobility in soil and the prevalence of its leaching from soil. For a given amount of chemical, the smaller the K_{oc} value, the greater the concentration of the chemical in solution. Thus, chemicals with a small K_{oc} value are more likely to leach into groundwater than those with a large K_{oc} value (http://www.acdlabs.com/products/phys_chem_lab/logd/koc.html).

Henry's law, one of the gas laws formulated by William Henry, states that "at a constant temperature, the amount of a given gas dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid (http://en.wikipedia.org/wiki/Henry's_law)." Henry's Law Constants characterize the equilibrium distribution of dilute concentrations of volatile, soluble chemicals between gas and liquid phases (http://www.epa.gov/athens/learn2model/part-two/onsite/esthenry.htm).

The octanol/water partition coefficient (K_{ow}) is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system. In recent years, this coefficient has become a key parameter in studies of the environmental fate of organic chemicals. It has been found to be related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. Because of its increasing use in the estimation of these other properties, K_{ow} is considered a required property in studies of new or problematic chemicals (http://www.pirika.com/chem/TCPEE/LOGKOW/ourlogKow.htm).

The bioconcentration factor (BCF) is the concentration of a particular chemical in a tissue per concentration of chemical in water (reported as L/kg). This property characterizes the accumulation of pollutants through chemical partitioning from the aqueous phase into an organic phase, such as the gill of a fish. The scale used to determine if a BCF value is high, moderate or low will depend on the organism under investigation. The U.S. EPA generally defines a high potential BCF as being greater than 5,000; a BCF of moderate potential as between 5,000 and 100; a low potential BCF as less than 100 (http://en.wikipedia.org/wiki/Bioconcentration_factor; http://sitem.herts.ac.uk/aeru/footprint/en/Quest/ecotox.htm).